Exploring Determinants of Execution in Early Phase Clinical Studies with Cell Therapies in Stroke

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THESIS DECLARATION

I 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.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library Search and through web search engines, unless permission has been granted by the University to restrict access for a period.

Signature:

Date: May 3, 2019
ABSTRACT

Background

Stroke is associated with a significant disease burden across the world (1). Ischaemic stroke accounts for over 80% of the total number of strokes and specifically refers to central nervous system infarction accompanied by overt symptoms (2). Cell therapies (CTs) represent a composite of different cell types being investigated in different phases of stroke, with use of different dose and delivery regimens (2). Preliminary evidence for meaningful clinical translation is now available with CTs in stroke, as early studies have demonstrated safety and a trend towards functional improvement over a longer time window of application (2).

Research Aims

This research aimed to analyse study design, regulatory policy, ethical and economic considerations, as well as to describe their impact on the quality of execution of early-phase clinical CTs studies in stroke

Methods

The thesis is a compendium of subprojects that evaluated these considerations for efficient implementation of early phase CTs studies, using a mixed methodology approach.

Results

Study design considerations: a systematic review of early phase clinical studies with CTs in ischaemic stroke indicated a trend towards improvement across varied domains of functional impairment and reasonable safety and feasibility, in patients with stroke receiving CTs (2). A high level of heterogeneity was observed, in terms of differences in cell types used and route, dose and time of administration, use of randomised control design and selection of trial endpoints. Most
studies reported temporal changes in global endpoints such as those measured by the National Institutes of Health Stroke Scale (NIHSS), Barthel Index (BI) or Modified Rankin scale (mRS).

**Regulatory considerations:** A narrative review examined different national regulatory provisions and described standardization of research terminology and access to expertise in manufacturing as the key determinants critical to the execution of early phase studies with CTs in stroke.

**Ethical considerations:** A qualitative study was undertaken to understand the perspective of stroke survivors on the research design of a proposed early phase clinical study with adult human dental pulp stem cells in chronic ischaemic stroke. The study found that patients considered outcomes such as recovery in social participation and decreased dependence on carers as most meaningful to them. Whilst improved motor function was important, the impact on cognition, memory, mood, pain and fatigue were bigger determinants of their perception of benefit. The perception of risk versus benefit was influenced by the time elapsed since stroke.

**Health economic considerations:** A systematic review reported that there is limited evidence for economic evaluation at early stage of research in CTs. Only three studies have been published to date. All studies undertook a cost utility analysis of CTs versus current standard of care using decision analytical modelling and reported that CTs could provide meaningful cost savings in terms of direct costs of disease management accrued to the government (healthcare bodies and social services).

**Discussion**

Successful clinical translation of CTs in stroke requires efficient development strategies potentially comprising the use of adaptive trial designs and the use of domain specific endpoints for efficacy evaluation (8, 9). Addressing regulatory requirements and patients’ preferences in research design can significantly improve the eventual clinical relevance of data generated within these trials (11, 12).
12). Collection of data on cost-effectiveness of their use from the early phase of research is critical, as these therapies are likely to be expensive (13).

**Conclusions**

Development of a practical framework comprising key elements of study design and regulatory policy, as well as ethical and health economic considerations that is available to different research groups can potentially accelerate clinical translation of CTs in stroke.
ACKNOWLEDGEMENTS

I would like to thank my supervisors, Professor Simon Koblar and Associate Professor Monica Anne Hamilton-Bruce, to whom I will remain eternally grateful. Their unreserved support throughout my PhD has enabled me to persevere and challenge myself to produce research that hopefully has ongoing relevance and contributes to improving outcomes for patients with stroke. I can never forget the immense kindness, empathy and understanding I received from Simon and Anne through one of the most difficult personal situations in my life, the loss of my dear twin sister.

I am also greatly indebted to Professor Susan Hillier. Susan’s support, friendship and guidance throughout my research work was tremendous and something for which I will always remain indebted to her.

It was my great fortune, due to the multidisciplinary focus of research work within this thesis to work with brilliant researchers in many disciplines. My thanks to Professor Julie Ratcliffe and Doctor Rachel Milte for their guidance and to Doctor Stuart Howell and Doctor Susan W Kim for their support with statistical planning and analysis, and to Ms Debra Ray for her help with key considerations for the PERSPECTIVES study. I would also like to thank my fellow researchers at the Stroke Research Programme, University of Adelaide, particularly Austin Milton, Maria Gancheva and Karlea Kremer, for their support throughout these years. John Liddle edited the thesis and I am grateful for his assistance.

Last but most importantly, I would like to thank my husband Rajeev, who has always understood my aspirations and believed in me, even when I did not, and my son Vighnesh, whose total belief in my capabilities makes me proud and humble at the same time. This thesis would not have been possible without these two exceptional men in my life.
AUTHORSHIP DECLARATIONS

List of Publications


### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCD² Score</td>
<td>(Age, Blood pressure, Clinical features, Duration, Diabetes) Score</td>
</tr>
<tr>
<td>ADL</td>
<td>Activities of daily living</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>ARD</td>
<td>Absolute relative difference</td>
</tr>
<tr>
<td>ASA</td>
<td>American Stroke Association</td>
</tr>
<tr>
<td>ASPECTS</td>
<td>Alberta Stroke Program Early CT Score</td>
</tr>
<tr>
<td>BI</td>
<td>Barthel Index</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CIMT</td>
<td>Constraint-induced movement therapy</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CT</td>
<td>Computerised tomography</td>
</tr>
<tr>
<td>CTs</td>
<td>Cell therapies</td>
</tr>
<tr>
<td>DALYs</td>
<td>Disability-adjusted life years</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiography</td>
</tr>
<tr>
<td>ED</td>
<td>Emergency department</td>
</tr>
<tr>
<td>EMS</td>
<td>Emergency Medical Services</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GMP</td>
<td>Good Manufacturing Practice</td>
</tr>
<tr>
<td>GP</td>
<td>General practitioners</td>
</tr>
<tr>
<td>HALE</td>
<td>Healthy life expectancy</td>
</tr>
<tr>
<td>HS</td>
<td>Hemorrhagic stroke</td>
</tr>
<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>ICF</td>
<td>International Classification of Functioning, Disability and Health</td>
</tr>
<tr>
<td>ICH</td>
<td>Intracerebral haemorrhage</td>
</tr>
</tbody>
</table>
**ABBREVIATIONS (continued)**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>Ischaemic stroke</td>
</tr>
<tr>
<td>IST</td>
<td>International Stroke Trial</td>
</tr>
<tr>
<td>m TICI</td>
<td>Modified Thrombolysis in Cerebral Infarction flow score</td>
</tr>
<tr>
<td>MD</td>
<td>Mean difference</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>mRS</td>
<td>Modified Rankin Scale</td>
</tr>
<tr>
<td>NIHSS</td>
<td>National Institutes of Health Stroke Scale</td>
</tr>
<tr>
<td>NINDS</td>
<td>National Institutes of Neurological Disorders and Stroke</td>
</tr>
<tr>
<td>OR</td>
<td>Adjusted odds ratio</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised control trial</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>rt-PA</td>
<td>Recombinant tissue plasminogen activator</td>
</tr>
<tr>
<td>SAH</td>
<td>Subarachnoid haemorrhage</td>
</tr>
<tr>
<td>SLT</td>
<td>Speech and language therapy</td>
</tr>
<tr>
<td>SMD</td>
<td>Standardized mean difference</td>
</tr>
<tr>
<td>TCT</td>
<td>Trunk control test</td>
</tr>
<tr>
<td>TIA</td>
<td>Transient ischaemic attack</td>
</tr>
<tr>
<td>TIS</td>
<td>Trunk impairment scale</td>
</tr>
<tr>
<td>TUG</td>
<td>Timed Up and Go test</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>VER</td>
<td>Very early rehabilitation</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>YLL</td>
<td>Years of life lost</td>
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CHAPTER 1: INTRODUCTION

Stroke represents a neurological deficit resulting from an acute focal injury in the central nervous system (CNS) due to a vascular cause such as cerebral infarction, intracerebral haemorrhage (ICH), or subarachnoid haemorrhage (SAH) (1). Two main types of stroke are recognised based on the initial causative mechanism: ischaemic (IS) and haemorrhagic stroke (HS). Ischaemic stroke accounts for over 80% of the total number of strokes and specifically refers to central nervous system infarction accompanied by overt symptoms (2).

1.1 Definition of Stroke

While the terminology of ‘stroke’ was introduced in 1689, it was in 1976 that the World Health Organisation (WHO) defined stroke as “rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer leading to death, with no apparent cause other than of vascular origin”, for universal use (3). This definition included ischaemic stroke due to cerebral infarction or due to an arterial or venous blockage, and haemorrhagic stroke, resulting from a haemorrhage into CNS tissue. The cut-off time-period of 24 hours was an arbitrary decision at the time, based on very limited evidence (4).

Episodes of temporary brain dysfunction with vascular aetiology that typically resolved within 24 hours were termed ‘transient ischaemic attack’ (TIA) (5). In 2009, the American Heart Association/American Stroke Association (AHA/ASA) defined TIA as “transient ischaemic attack (TIA): a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia without acute infarction” (5). As brain imaging techniques have improved, it became apparent that the persistent brain infarction can occur much sooner and may not correlate accurately
with clinical presentation. An update in 2013 suggested the use of imaging data along with clinical presentation to differentiate between TIA and stroke (1). Australian clinical practice guidelines (2017) for stroke management define TIA as “focal neurological symptoms due to focal ischemia that have fully resolved”. These guidelines recommend that if an ischaemic lesion is present on brain imaging, then the same is classified as a stroke even if symptoms have fully resolved (6).

The International Classification of Diseases (ICD) system aims to standardize diagnostic classification for most diseases. The current (10th) revision along with its clinical modification (ICD-10-CM) published in 2016, defined cerebrovascular disorders as comprising TIA, cerebral ischaemic stroke, ICH, or SAH (7).

The updated definitions of stroke and its different subtypes, supported by guidelines across the globe such as those from the American Heart Association/American Stroke Association (2013), Australian Stroke Foundation (2017) and European Stroke Organisation (2008), incorporate both clinical and tissue criteria that have emerged due to significant improvement in sophistication of brain imaging (6, 8, 9). It is now possible to localize regions of brain infarction and haemorrhage at high spatial resolution, estimate regions of hypoperfusion that are potentially salvageable and identify smaller lesions such as silent infarcts and microhaemorrhages. These advances would likely increase the number of stroke diagnoses from roughly 3% to 15% of the population but can potentially address relatively underestimated consequences of these events such as cognitive deficits and recurrent strokes (1).
1.2 Disease Burden of Stroke

Changes in lifestyle and demographic patterns over the last few decades have led to an increase in the overall numbers of people affected by stroke. The Global Burden of Disease Study (2013) reported that stroke is the second highest cause of years of life lost (YLL) globally (10). With expanding access to protocolised care in the acute stroke setting, the mortality associated with stroke has been declining across the globe, though more so in developed as compared to developing countries. The mortality rates from IS and HS combined in developed countries were almost halved from 1990 to 2013 (112.9/100,000 and 67.2/100,000), while in developing countries IS and HS mortality rates were reduced by only approximately 15% (from 160.9/100,000 in 1990 to 136.9/100,000 in 2013) (10). However, the absolute number of people affected in the world over the same time-period, has increased for both IS: 291.2/100,000 (95% confidence interval (CI): 278.7 to 303.8) in 1990 to 299.1/100,000 (95% CI: 290.2 to 309.2) in 2013, and HS: 105.6/100,000 (95% CI: 102.0 to 109.2) in 1990 to 116.6/100,000 (95% CI: 113.1 to 120.5) (10). Krishnamurthi et al. (2015) highlighted the fact that between 1990 and 2013, there were significant increases in absolute numbers and prevalence rates of both HS and IS for younger adults (20-64 years of age) (11). The death rates for all types of strokes among younger adults, declined in both developing and developed countries to a variable extent (11). However, there was a 24.4% (95% CI: 16.6 to 33.8) increase in total disability-adjusted life years (DALYs) for this age group, with a 20% (95% CI: 11.7 to 31.1) and 37.3% (95% CI: 23.4 to 52.2) increase in HS and IS numbers, respectively (11).

In 2013, there were almost 25.7 million stroke survivors globally (71% with IS); 6.5 million deaths from stroke (51% died from IS); 113 million DALYs due to stroke (58% due to IS) and 10.3 million new strokes (67% IS) (10). The proportional contribution of stroke-related DALYs and deaths due
to stroke at a global level, compared to all diseases, increased from 1990 (3.54% (95% CI: 3.11 to 4.00) and 9.66% (95% CI: 8.47 to 10.70), respectively) to 2013 (4.62% (95% CI: 4.01 to 5.30) and 11.75% (95% CI: 10.45 to 13.31), respectively) (10). Stroke is second only to ischaemic heart disease globally as a contributor to DALYs in developing countries, and it is the third largest contributor to DALYs in developed countries (10).

With increasing life expectancy across the globe due to enhanced food security and improved control of communicable diseases, stroke is likely to represent an increasing burden for patients, families, society and governments (10). The gap between life expectancy and healthy life expectancy (HALE) has been widening over the past few decades. This increase has been attributed largely to the rise in prevalence of chronic diseases such as stroke, dementia, diabetes, and ischaemic heart disease (12).

1.3 Current Management Landscape: Ischaemic Stroke

The management of stroke has been steadily evolving to address the whole continuum of stroke care. The management strategies that have a supportive evidence base to date are aspirin, protocolised management in a stroke unit, decompressive craniectomy (where indicated), early blood flow restoration through pharmacological thrombolysis or mechanical thrombectomy, or a combination of both strategies and rehabilitation to complement all these treatment strategies (9). In 2018, AHA/ASA updated the guidelines for management of acute ischaemic stroke to summarise the existing evidence for the various components of stroke management and proposed standards for optimal care (8). In September 2017, The National Health and Medical Research Council and the Stroke Foundation released an update to Australian Clinical Practice Guidelines for stroke management (6). Similarly, there have been ongoing updates to guidelines for the
management of stroke in different countries to keep abreast with new research happening in this field (9). In order to meet the standards, set by the guidelines, well-defined stroke systems of care are required, that enable patient care pathways to coordinate and optimize the entire stroke care continuum, from primary prevention to rehabilitation (13). Systems include the designation of comprehensive stroke centres, development of regional strategies to guarantee appropriate interventions like thrombolysis and stroke unit care, inter-provider collaboration with telemedicine and establishment of performance measures for all these components (14). Ganesh et al. (2016) reported that the crude 30-day mortality rate decreased from 15.8% in 2003-2004 to 12.7% in 2012-2013 in Canadian provinces with established stroke systems as compared to remaining at 14.5% in provinces without such systems (13). The study provides evidence for population-wide reduction in mortality associated with access to stroke systems of care (13). In Australia, the National Stroke Foundation developed the Acute Stroke Services Framework (2015) and the Rehabilitation Stroke Services Framework (2013) (15, 16). These frameworks guide the planning, monitoring, and optimisation of acute and rehabilitation stroke services in the country to support the delivery of care in line with the clinical practice guidelines. The following sections discuss the key components of these guidelines:

1.3.1 Pre-Hospital Care: Early Assessment, Diagnosis, Triage

1.3.1.1 Emergency Medical Services (EMS)

All guidelines recommend that potential stroke patients be managed with urgency, as treatment is time critical (6, 8, 9). Berglund et al. (2012) reported that a shorter time to reach the stroke unit and an increase in thrombolysis frequency was achieved for patients by targeted prioritisation of EMS dispatch in response to calls with presentations likely to be stroke (17). Ekundayo et al. (2013) reported that timely access to EMS was independently associated with (18):
• quicker arrival to hospital (onset-to-door time ≤3 hours; adjusted odds ratio [OR] 2.00, 95% CI: 1.93 to 2.08)

• quicker imaging (door-to-imaging time ≤25 minutes; OR 1.89, 95% CI: 1.78 to 2.00) and,

• faster access to thrombolysis (door-to-needle [DTN] time ≤60 minutes; OR 1.44, 95% CI: 1.28 to 1.63).

Studies [O'Brien et al. (2012), McKinney et al. (2013)] have reported that preferential transfer of patients directly to a hospital with capacity to provide reperfusion therapies and stroke unit care led to decreased time to imaging and specialist assessment (19, 20). Lahiry et al. (2018) reported that a pre-hospital acute stroke triage protocol to prioritise transfer to a primary stroke centre in regional New South Wales, Australia, significantly increased the likelihood of eligible patients receiving thrombolysis (OR 17, 95% CI: 9.42 to 31.2, p<0.05) which was potentially cost-effective as well (average cost of $10,921 per DALY avoided per patient) (21).

The National Stroke Audit report released by the National Stroke Foundation in 2017 reports that 76% of overall stroke patients arrived at hospital by ambulance. However, only 58% of acute stroke services reported established protocols with local ambulance services to enable pre-notification and only 69% had protocols in place to implement bypass and transfer to regional stroke centres with facilities for thrombolysis. As a result, only 36% of patients with stroke in Australia reached hospital within the critical 4.5-hour time window for thrombolysis (22).

Some research groups have investigated whether providing thrombolysis in a specialized ambulance is safe and effective. Ebinger et al. (2014) reported ambulance-based thrombolysis resulted in decreased time to treatment without an increase in adverse events (23). Bowry et al. (2015) in a similar study corroborated the decrease in time to treatment in their study but indicated
that this strategy may result in a larger number of stroke mimic patients receiving thrombolysis in error (24). As adoption of mobile stroke units with capacity for performing onsite imaging and thrombolysis is increasing across the world, future data will indicate whether the overall impact on treatment metrics and long-term outcomes is sufficient to trigger change in management pathways (25, 26).

1.3.1.2 Transient Ischaemic Attack

Timely diagnosis and immediate referral to a stroke team of all patients with suspected TIA has been shown to result in meaningful long-term benefit in terms of significant reduction in recurrent stroke (27, 28).

Since the risk of stroke following TIA is high within the first two days (29), Australian practice guidelines strongly recommended that diagnostic work-up and implementation of optimal therapy should be completed within 24 hours of symptom onset (6). This includes assessment by a stroke specialist, extended monitoring with electrocardiography (ECG) as required and brain imaging [computerised tomography (CT) or magnetic resonance imaging (MRI)] (6). Both ASA guidelines and Australian guidelines highlight the importance of organised and effective communication between general practitioners (GP), EMS personnel, emergency department (ED) staff and the specialist stroke team to achieve this. Multiple studies such as Lavallee et al. (2007), Rothwell et al. (2007) and Dutta et al. (2015) have reported that establishment of dedicated TIA clinics have been associated with a significant fall in the number of 90-day recurrent stroke (27, 30, 31).

In pre-hospital settings, high-risk indicators such as crescendo TIA, current or suspected atrial fibrillation, current use of anticoagulants, carotid stenosis or high ABCD² score have been used, to identify patients for urgent specialist assessment (32). However, Leung et al. (2012) reported in a
cross-sectional study in Western Adelaide in South Australia that GPs lacked access to neurologists and that knowledge of relevant guidelines and deficient education was identified as a key barrier to timely diagnosis and referral to specialist care (33). Ranta et al. (2015) reported that use of electronic decision support in a primary care setting may accelerate diagnostic and triage decisions and improve outcomes (OR for risk of 90-day stroke, vascular event, and/or death = 0.27, 95% CI: 0.09 to 0.78, p = .016) (32). The ‘Stroke/TIA integrated decision support tool’ has been available to GP practices around New Zealand since 2015. The 2017 Australian guidelines acknowledge that access to such a tool in Australia would be of benefit in timely management (6). The Australian National Stroke Audit report (2017) observed that 83% of all acute services reported having a defined clinical pathway for assessing TIA patients but only 29% reported access to a rapid access TIA clinic for patients not admitted to hospital, where average waiting time was reported as 4 days (first quartile: 2 days, third quartile: 10 days) (22).

1.3.2 Hospital Management

1.3.2.1 Rapid Assessment in the Emergency Department

As per the National Stroke Audit (2017) report, 46% of patients with stroke in Australia present to ED) (22). Therefore, a well-defined protocol for prioritised evaluation of these patients is critical. In 2011, the National Institutes of Neurological Disorders and Stroke (NINDS) panel, proposed goals for time intervals for key milestones from first contact with a stroke patient in the ED to thrombolysis. These goals aimed to optimise the ‘stroke chain of survival’ by establishing time-sensitive targets for critical milestones in the ED such as identification, specialist evaluation, imaging and treatment of stroke to enable efficient and effective stroke care (34).
Table 1: NINDS: Stroke Chain of Survival

<table>
<thead>
<tr>
<th>Action</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Door to physician</td>
<td>≤10 minutes</td>
</tr>
<tr>
<td>Door to stroke team</td>
<td>≤15 minutes</td>
</tr>
<tr>
<td>Door to imaging initiation</td>
<td>≤25 minutes</td>
</tr>
<tr>
<td>Door to imaging interpretation</td>
<td>≤45 minutes</td>
</tr>
<tr>
<td>Door to needle</td>
<td>≤60 minutes</td>
</tr>
<tr>
<td>Door to stroke unit admission</td>
<td>≤3 hours</td>
</tr>
</tbody>
</table>

Reproduced with permission from Jauch et al. (2013) (34)

The 2017 Australian practice guidelines recommend the implementation of coordinated ED systems that can ensure prioritised assessment of every stroke patient, preferably by the stroke team or other experienced personnel. These systems require the use of multi-level interventions such as the use of validated screening tools and triage categories and targeted protocols for timely recombinant tissue plasminogen activator (rt-PA) administration, e.g., ‘Code Stroke’, and urgent access to imaging (35, 36). The National Stroke Audit reports that the availability of coordinated emergency department systems had risen from 86 to 92% in the period 2015-2017 across Australia (22).

A ‘Target: Stroke’ program was initiated in 2010 in the United States (US) with the initial aim to achieve DTN times within 60 minutes in at least 50% of ischaemic stroke patients treated with intravenous (IV) rt-PA through incorporation of multi-level coordinated care delivery (37). Xian et al. (2017) reported that the Phase II of the program resulted in an increase in the percentage of patients with DTN time of ≤60 min from 33.9% (1849/5460) in 2010 to 59.3% (10,020/16,901) in
2015. The study reported that the use of standardized stroke care procedures was critical to the achievement of DTN times within 60 minutes in ≥ 50% of acute ischaemic stroke patients treated with IV alteplase (38).

1.3.2.2 Stroke Units

In 2013, the Stroke Unit Trialists' Collaboration published a Cochrane review of 28 trials (5855 patients) evaluating benefit from organised stroke unit care (39). Substantial reductions were observed in: the odds of death recorded at final (median one year) follow-up (OR 0.81, 95% CI: 0.69 to 0.94, p = 0.005); the odds of death or institutionalised care (OR 0.78, 95% CI: 0.68 to 0.89, p = 0.0003), and the odds of death or dependency (OR 0.79, 95% CI: 0.68 to 0.90, p = 0.0007) (39). The provision of comprehensive specialized stroke care that incorporates rehabilitation and ensures uninterrupted continuum of care for patients with stroke, is perhaps the most important recommendation made in guidelines across the world (6, 8).

The 2017 Australian national guidelines strongly recommend that all people with stroke should be managed by a stroke unit with a multidisciplinary team and be admitted directly to a stroke unit (preferably within three hours of stroke onset) (6). It is also recommended that hospitals without a dedicated stroke unit, have established transfer protocols to guide urgent transfers to the nearest stroke unit hospital (6).

The Australian National Acute Stroke Services Framework issued in 2015 defined the minimum criteria for a stroke unit as follows (15):

1. co-located beds within a geographically defined unit
2. a dedicated, inter-professional team with members who have a special interest in stroke and/or rehabilitation (The minimum team would consist of medical, nursing and allied health.)

3. the inter-professional team meeting at least once per week to discuss patient care.

4. regular programs of staff education and training relating to stroke.

Different models of stroke unit care are reported depending on resource availability (39). These are: acute stroke unit (acute unit in a discrete ward for acute short-term care), comprehensive stroke unit (integrated acute and rehabilitation management in a discrete ward), stroke rehabilitation unit (discrete rehabilitation unit for transition from acute care), and mixed rehabilitation ward (rehabilitation provided on a general caseload ward).

The 2017 National Stroke Audit reported an increase in access to stroke unit care in Australia to 69% from 58% in 2013 with the number of stroke units increasing to 95 from 87 in 2015 (22). However, significant differences persisted in stroke unit access in metropolitan (77%) areas as compared to regional (47%) areas (22).

1.3.2.3 Early Reperfusion Strategies

Interventions to restore blood supply to the penumbral zone of the brain either by intravascular clot lysis with tissue plasminogen activators or endovascular removal of the clot are two significant milestones in the management of stroke (8).

1.3.2.3.1 Thrombolysis

Significant literature exists to support that timely intravenous thrombolysis in eligible patients is the cornerstone of optimal acute management of ischaemic stroke (40, 41). The 2018 AHA/ASA
guidelines recommend timely administration of rt-PA in eligible patients (8). Further to this, it suggests that patients eligible for IV alteplase should receive IV alteplase even if endovascular therapy is being considered (8). These recommendations are mirrored in Australian guidelines as well as other guidelines across the globe (6, 9).

Wardlaw et al. (2014) have followed the evolution of evidence in this field through the last two decades. Their first review on the subject was published in 1992 and their latest update in 2014 analysed evidence from 27 trials, involving 10,187 participants, testing different thrombolytics (42). A majority of these trials started treatment up to six hours after stroke and analysed IV thrombolytic administration (42). The data suggests that thrombolysis, delivered up to six hours after ischaemic stroke, was associated with significant reduction in the proportion of patients who were dead or dependent (mRS=3-6) at three to six months after stroke (OR 0.85, 95% CI: 0.78 to 0.93) (42). However, there was an associated increased risk of symptomatic intracranial haemorrhage (OR 3.75, 95% CI: 3.11 to 4.51), early death (OR 1.69, 95% CI: 1.44 to 1.98) and death (OR 1.18, 95% CI: 1.06 to 1.30) seen at three to six months after stroke. Intracranial haemorrhage was found to be the predominant driver of this excess early death (within 7-10 days) occurrence (42). The data indicates that delivery of thrombolysis in eligible patients within three hours of stroke was associated with an optimal benefit versus risk scenario, as this timing was more effective in reducing death or dependency (OR 0.66, 95% CI: 0.56 to 0.79) without any increase in death (OR 0.99, 95% CI: 0.82 to 1.21; 11 trials, 2187 participants) (42). An analysis of the impact of time to thrombolysis on outcomes reported that for every 1000 patients given rt-PA within three hours, 90 additional patients would be alive and independent (P < 0.0001) with no heterogeneity between the relevant trials, as compared with 10 additional patients, if treated between three and six hours after stroke (P = 0.58) (42). This provided further confirmation of
evidence that earlier treatment increased the proportion of patients with better outcomes than later treatment (42). However, results from the third International Stroke Trial (IST-3) trial reported a meaningful benefit, even when the treatment window was extended to 6 hours (43). At 6 months, a non-significant absolute increase of 14/1000 was reported in the proportion of people alive and independent but the shift in Oxford Handicap scores in the rt-PA group was significant compared to the control (OR 1·27, 95% CI: 1·10 to 1·47, p=0·001) (43). It is important to note that 53% (n=1617) of the study population in the IST-3 trial was greater than 80 years of age (43). Similar findings were reported from a subgroup analysis by Wardlaw et al. as part of the Cochrane review which indicated that rt-PA administration in the older age subgroup was associated with a meaningful benefit (OR 1·35, 99% CI: 0·97 to 1·88) (42). Overall, the Cochrane review by Wardlaw et al. concluded that benefit was still seen when the timing window was extended up to 6 hours (OR 0.84, 95% CI: 0.77 to 0.93, p = 0.0006; 8 trials, 6729 participants) but they highlighted significant heterogeneity between the relevant trials, weakening the strength of the available evidence (42). Additionally, the review concluded that similar reduction in death or dependency (mRS 3 to 6) by the end of follow-up was seen in participants treated up to 6 hours aged ≤ 80 years (OR 0.85, 95% CI: 0.76 to 0.95) versus > 80 years (OR 0.80, 95% CI: 0.64 to 0.99) (42).

Consequently, the 2017 Australian clinical guidelines and the 2018 AHA/ASA guidelines recommend that all patients with ischaemic stroke who meet specific eligibility criteria be commenced on intravenous alteplase (dose of 0.9 mg/kg, maximum of 90 mg) as early as possible (preferably within the first few hours but may be used up to 4.5 hours after onset) (6, 8). Coordinated and comprehensive systems of stroke management that incorporate appropriate infrastructure, facilities and network support (e.g. telemedicine), are essential to enable timely thrombolysis to be feasible for all eligible patients (6).
1.3.2.3.2 **Endovascular Thrombectomy**

Multiple studies [Goyal et al. (2015), Molina et al. (2015), Campbell et al. (2014), Saver et al. (2015), Berkhemer et al. (2015)] reported that mechanical thrombectomy with a stent retriever, initiated within 6 hours of symptom onset was associated with successful angiographic reperfusion (44-48). This was defined as a modified Thrombolysis in Cerebral Infarction flow score (m TICI) of 2b/3 and a favourable disability outcome at 90 days (defined as a favourable shift in mRS distribution) in an appropriate stroke population (44-48), who had:

- pre-stroke mRS score of 0 to 1
- causative occlusion of the internal carotid artery or middle cerebral artery segment 1 (M1)
- age (≥18 years)
- NIHSS score of ≥6
- Alberta Stroke Program Early CT Score (ASPECTS) of ≥6.

Saver et al. (2016) published a meta-analysis of individual patient level data from all the randomized phase III trials in which stent retrievers or other second-generation devices were used (49). A pre-defined subgroup analysis of 390 patients who achieved substantial reperfusion showed that each 1-hour delay to reperfusion was associated with a less favourable degree of disability (OR 0.84, 95% CI: 0.76 to 0.93; absolute relative difference (ARD): -6.7%) and less functional independence (OR 0.81, 95% CI: 0.71 to 0.92; ARD: -5.2%, 95% CI: -8.3% to -2.1%), but was not associated with significant difference in mortality (OR 1.12, 95% CI: 0.93 to 1.34; ARD 1.5%, 95% CI: -0.9% to 4.2%) (49). This study reinforced the importance of timely endovascular treatment but provided initial evidence to support an extension of the treatment window from 6 to 7.3 hours after symptom onset. This analysis also reported a treatment effect in the subgroup of 188 patients who were not treated with IV alteplase (OR 2.43, 95% CI: 1.30 to 4.55) indicating
that pre-treatment with IV alteplase was not an essential pre-requisite (49). While the guidelines still recommend that all patients should receive rt-PA, it is also recommended that thrombectomy should be organised for eligible patients without waiting to observe the clinical response with rt-PA (8).

The results of the DAWN trial, published in 2017, support a clinical benefit with mechanical thrombectomy performed between 6 and 24 hours from ‘last known well’ timepoint in a select patient group with large anterior circulation vessel occlusion who presented with a clinical imaging mismatch, i.e. a clinical deficit that was disproportionately severe relative to the infarct volume (50). The improvement in functional outcome at 90-days in the thrombectomy group versus the standard care group (mRS score 0–2, 49% versus 13%; adjusted difference=33%; 95% CI: 21% to 44%) was clinically meaningful (50).

In 2018, the ‘DEFUSE 3’ trial expanded the patient group likely to benefit with thrombectomy performed 6 to 16 hours from ‘last known well’ timepoint, to include patients with large anterior circulation occlusion likely to have salvageable ischaemic brain tissue, as identified by perfusion imaging (perfusion-core mismatch and ischaemic core less than 70 ml) (51). The study showed: a favourable shift in the distribution of the mRS at 90 days (OR 2.77; p<0.001); a higher percentage of functional independence (45% versus 17%, p<0.001); a lower 90-day mortality rate (14% versus 26%, p=0.05), but no significant between-group difference in the frequency of symptomatic intracranial haemorrhage (7% and 4%, respectively; p=0.75) or of serious adverse events (43% and 53%, respectively; p=0.18) in the treatment group (51). As a result, the updated AHA/ASA guidelines and the Australian clinical practice guidelines support an extension of the time window available for thrombectomy in the above patient group up to 24 hours (6, 8).
In 2017, the Australian National Stroke audit reported that despite the increase in availability of thrombolysis services (72%) and in the number of stroke units from 87 to 95, only 13% of patients presenting to hospital were eligible for thrombolysis (15). The mean rates of thrombolysis across the globe are reported to be between 5-20% (52). This underscores the fact that despite widespread knowledge of the benefit possible with timely thrombolysis, current systems of care all over the world lag in terms of real world implementation (52).

1.3.2.4 Early Assessment for Rehabilitation

Stroke practice guidelines universally recommend that all stroke patients undergo an early functional assessment (ideally within 24-48 hours) conducted by professionals with expertise in rehabilitation, who are part of the multidisciplinary team in the stroke unit (6). Determination of individual rehabilitation goals should be based on their residual level of ability to: communicate and to perform activities of daily living and be functionally mobile. This may be achieved using standardized tools such as the ‘Assessment for Rehabilitation Tool’ produced by the Australian Stroke Coalition Working Group (2012) (53). It is recommended that the findings of this formal assessment should be incorporated into the care transition and discharge planning (5).

1.3.3 Rehabilitation

Crichton et al. (2016) published findings from a population-based South London Stroke Register of 2625 patients with first-ever stroke, with > 10 years of follow-up data (54). At 15 years, 262 (21%) had survived, with mild disability in 33.8% of the survivors (95% CI: 26.2% to 42.4%), moderate disability in 14.3% (95% CI: 9.2% to 21.4%) and severe disability in 15.0% (95% CI: 9.9% to 22.3%) of the survivors. At least 1 in 10 of the 15-year survivors had lived with moderate-to-severe disability since their initial stroke and there was an accumulation of disability with time.
Similar findings have been observed in other stroke outcomes studies such as Anderson et al. (2004) and Hardie et al. (2004) (55, 56).

1.3.3.1 Definition of Rehabilitation

Rehabilitation is considered the current standard of care in the chronic phase of stroke and aims to augment the natural post-stroke recovery process and prevent further deterioration of function (6). The British Society of Rehabilitation Medicine defined rehabilitation as “a process of active change by which a person who has become disabled, acquires the knowledge and skills needed for optimum physical, psychological and social function” (57). The WHO defined stroke rehabilitation to encompass the coordinated delivery of intervention(s) provided by a multi-disciplinary team in conjunction with medical professionals, to improve patient symptoms and maximise functional independence and participation (social integration) using a holistic biopsychosocial model, as defined by the International Classification of Functioning, Disability and Health (ICF) (58).

1.3.3.2 Models of delivery

Australian clinical guidelines for stroke management (2017) recommend that stroke survivors are treated within a rehabilitation framework that ensures continued monitoring for improvement as well as identification and timely management of any deterioration, in consultation with stroke specialists (6). Rehabilitation is delivered across a variety of care settings such as acute or specialised rehabilitation wards within the hospital, in community outpatient settings and in the home. The National Stroke Rehabilitation Audit Report (2016) reported that 73% of hospital patients were managed in general rehabilitation wards and only 28% of overall stroke cases audited received targeted management in a dedicated stroke rehabilitation unit (6%) or neuro-rehabilitation unit (8%) or a combined acute/rehabilitation unit (14%) (59).
1.3.3.2.1 **Inpatient Rehabilitation: Early Mobilisation**

Stroke unit care has incorporated a commitment to early mobilisation as an essential element. Despite limited evidence in past years, it has been well accepted that prolonged immobilisation negatively affects multiple (musculoskeletal, cardiovascular, respiratory and immune) systems, that immobility-related complications start early after stroke and that preclinical research postulates that there may be an early ‘critical’ period of enhanced neural plasticity during which the injured brain is most responsive to targeted interventions (60).

The evidence regarding the type, dose and timing of these early interventions has been building steadily. A review and meta-analysis by Lynch et al. (2014) included five randomized controlled trials and 38 cohort studies (61). This review indicated a small improvement (non-significant) in the odds of improvement in BI with early mobilisation (OR 1·20, 95% CI: 0·77-3.18, p=0·23; OR 1·16, 95% CI: 0·61–2·18; p=0·66, with significant heterogeneity I²=66%). In 2015, the findings of the AVERT Phase III trial were published (62). This randomized controlled study, conducted in 2104 patients reported that patients who received a ‘very early mobilisation’ (VER) protocol (average of 6.5 out-of-bed sessions per day, starting 18.5 hours post-stroke) experienced a less favourable outcome at 3 months (62). Subsequent subgroup analysis indicated that this is more likely to be the case in individuals who had a more severe stroke or intracerebral haemorrhage (62).

While this changed practice to one that discourages very early, intensive mobilisation training, Bernhardt et al. later reported a pre-specified dose analysis that evaluated the impact of dose and timing of mobilisation on outcomes (63). The analysis indicated that more frequent but shorter duration mobilisation sessions might potentially result in better outcomes. Further to this, Reuter et al. (2016) reported insights from an analysis of the Baden-Wuerttemberg stroke registry regarding the application of VER in acute ischaemic stroke and intracerebral haemorrhage in real
world clinical practice (64). They indicated that in current clinical settings, the selection of patients receiving VER and its frequency was often driven by the need to optimize resources, and those at either extreme of the prognostic spectrum were least likely to receive therapy. This underscores the need for further research to identify the specific patient subgroups likely to most benefit from VER, to help in effective resource allocation.

1.3.3.2.2 EarlySupported Discharge

Early supported discharge (ESD) is an attractive model that links inpatient care with community services and provision of rehabilitation services within the home environment with the goal to establish skills that are appropriate to the patient’s home setting. The Cochrane review by Fearon et al. (2012) reported that ESD groups had a significantly shorter hospital stay equivalent to approximately seven days (65). Importantly, ESD was associated with decreased risk (65) of:

- death or institutionalisation (OR 0.78, 95% CI: 0.61 to 1.00, \( p = 0.05 \)) and
- death or dependency (OR 0.80, 95% CI: 0.67 to 0.97, \( p = 0.02 \)) respectively.

A recent update to this review published in 2017 included 17 clinical trials recruiting 2422 stroke patients (66). The OR for the outcome of death or dependency at a median follow up of 6 months (range 3-12 months) was 0.80 (95% CI: 0.67 to 0.95, \( p = 0.01 \)); for death was 1.04 (95% CI: 0.77 to 1.40, \( p = 0.81 \)) and death or requiring institutional care was 0.75 (95% CI: 0.59 to 0.96, \( p = 0.02 \)), respectively (66). Participants showed improvement in extended activities of daily living scores but the standardized mean difference (SMD) (0.14, 95% CI: 0.03 to 0.25, \( p = 0.01 \)) was small and the impact on participants’ activities of daily living scores was unclear. Furthermore, the evidence for benefits tended to be weaker at one- and five-year follow-up. Importantly, the greatest improvement in outcomes were seen in the trials evaluating a co-ordinated ESD team in
comparison to results in those services without a co-ordinated team (subgroup interaction at \( p = 0.06 \)) (66). In addition, patients with mild to moderate disability at baseline showed greater benefit than those with more severe stroke (subgroup interaction at \( p = 0.04 \)) (66). These studies support the recommendations by the Australian Clinical Guidelines for Stroke Management 2017, as well as the AHA/ASA Rehabilitation guidelines for stroke (2016). The guidelines reiterate that appropriately resourced ESD services with co-ordinated multidisciplinary team input can reduce long-term dependency, admission to institutional care and the length of hospital stay, especially in patients with mild to moderate disability (6, 8). Notwithstanding this, the Australian National Stroke Audit found that only 4% of overall patients discharged from acute care received EDS (22).

It is critical that goals for recovery are client-centred, clearly communicated and documented and are determined in collaboration with the stroke survivor and their family/carer. The beneficial effect of establishing clear goals and seeking input of the stroke survivor in articulating these goals has been reported in multiple studies (67-69).

1.3.3.2.3 Rehabilitation in community

Stroke rehabilitation for people living in the community, is delivered in either a centre, outpatient or day hospital setting. A meta-analysis published by Hillier et al. (2010) included 11 randomized controlled trials that compared home-based and centre-based rehabilitation and reported that home-based rehabilitation was associated with a significant increase in BI scores at 3-6 months versus centre-based rehabilitation (mean difference (MD) of 4.07 points, 95% CI: 0.81 to 7.44, \( p=0.99 \)) (70). However, the differences were no longer significant at 6 months (70). Rasmussen et al. (2016) reported on the findings of a randomized control trial (RCT) that assessed quality of life and disability outcomes among patients assigned to home-based or standard care (71). The home-based
rehabilitation group demonstrated significantly improved quality of life measured by EuroQol-5D: intervention median = 0.77 (Interquartile Range (IQR) 0.66 to 0.79) versus the control median = 0.66 (IQR 0.56 to 0.72, p=0.03) and lesser disability (reduced mRS): intervention median=2 (IQR 2 to 3); control median = 3 (IQR 2 to 4, p=0.04) (71). Coupar et al. (2012) published a Cochrane review that analysed the relative benefit from home-based rehabilitation for individuals with upper limb impairment following stroke (72). This review included four studies with a total of 166 participants. Three studies compared the effects of home-based upper limb therapy programs versus usual care and one study compared the effects of a home-based upper limb program with a similar hospital-based programme (72). No statistically significant difference in the activities of daily living (ADL) score, functional movement of the upper limb, extended ADL or upper limb motor impairment was found in either comparison (72). The 2017 Australian practice guidelines recommend that home-based rehabilitation may be considered as a preferred model for delivering rehabilitation in the community (6). However, centre-based care should be available to all patients with stroke in the community if home-based rehabilitation is unavailable (6).

1.3.3.3 Targets of Rehabilitation

1.3.3.3.1 Sensorimotor Impairment

Weakness is the most often reported impairment after stroke (73). Strength training and task-oriented training have been the ‘cornerstone’ strategies to manage weakness. Recently, electromechanical and robot-assisted training have been reported to assist passive and active movement training and augment motor learning (73). A Cochrane review by Mehrholz et al. (2018) analysed data from 45 trials (involving 1619 participants). Electromechanical and robot-assisted arm training improved: activities of daily living scores (SMD 0.31, 95% confidence interval (CI) 0.09 to 0.52, P = 0.0005; I² = 59%; 24 studies, 957 participants, high-quality evidence); arm
function (SMD 0.32, 95% CI 0.18 to 0.46, P < 0.0001, I² = 36%, 41 studies, 1452 participants, high-quality evidence); and arm muscle strength (SMD 0.46, 95% CI 0.16 to 0.77, P = 0.003, I² = 76%, 23 studies, 826 participants, high-quality evidence) (73). In 2014, Nascimento et al. published a review and meta-analyses that showed that the use of cyclical electrical stimulation had a moderate positive effect on muscle strength, along with a small-to-moderate improvement in activity (74). Importantly, the effect was seen across different levels of initial weakness and in both subacute and chronic phase stroke (74). Mehrholz et al. (2017) recently evaluated evidence regarding the effect of electromechanical-assisted gait training in combination with physiotherapy on walking (75). Data analysis from 36 RCTs (1472 participants) in this review provided moderate-quality evidence supporting the use of electromechanical-assisted gait training in combination with physiotherapy (75). The intervention increased the likelihood of regaining independence in walking (OR 1.94, 95% CI: 1.39 to 2.71, p < 0.001; I² = 8%) but did not indicate any significant benefit in terms of walking velocity or walking capacity (75). It was also noted that the benefit with this strategy was only seen in the acute phase and not in the chronic phase. However, the authors expressed caution about the clinical interpretation of these findings due to significant variability in study design, study population and types of devices used. Post-hoc analysis suggests that differences between the types of devices may have a significant impact on walking velocity but not on the overall ability to walk (75). Therefore, the current Australian practice guidelines support the use of electromechanical training in patients with upper/lower limb weakness who have less than antigravity strength (6).

Somatosensory impairments, especially in touch sensation and proprioception are detected in approximately 40% of stroke patients (76) and these can negatively impact on motor recovery and compromise a patient’s ability to lead a safe independent life and regain their levels of participation
prior to stroke (76, 77). The National Stroke Rehabilitation Audit indicated that 78% of Australian hospitals had local protocols for assessment of sensory deficits (59). Most research in the context of sensory impairment has focussed on the upper limb using either a sensory retraining approach or sensory stimulation approach. Though multiple interventions have been described in the literature such as tactile stimulation, mental imagery, mirror therapy, thermal therapy and pneumatic compression therapy, the overall impact of these interventions on recovery of sensation and ADLs has been small and inconclusive (76, 77, 78). Lynch et al. (2007) investigated sensory retraining for the foot and lower limb in a small RCT and reported no benefits (79).

1.3.3.3.2 Visual Impairment

Visual impairment most commonly presents as visual field loss and is seen in approximately 30–60% of stroke survivors (59, 80). Patients may have diplopia, difficulties with ocular convergence, impaired saccadic movement, over-sensitivity to light, nystagmus or dry eyes. These often interfere with activities such as reading, writing, moving around and driving (80). Interventions for visual impairments have mainly targeted deficits in eye movements, visual fields and visual-spatial or perceptual deficits by employing either restitutive or compensatory strategies (such as Fresnel prism glasses, computer-based visual retraining programs or visual scanning, multimodal audio-visual exploration training and virtual reality training) (80). Even though seven systematic reviews have been published in this field, conclusive evidence is still lacking due to the poor methodology of studies so far (80, 81).
1.3.3.3.3 Physical Activity

1.3.3.3.3.1 Amount of Rehabilitation

Systematic reviews (82, 83) and meta-regression analysis (84) have established that structured rehabilitation to maximise therapy time within individual tolerance limits, is associated with consistent, moderate benefit in improving walking ability, arm function and quality of life. Schneider et al. (2016) pooled data from 14 studies (954 participants) that focussed on upper limb activity, walking ability or a combination. Increased therapy time was associated with improvement in activity though there was significant heterogeneity between studies (upper limb and lower limb combined, SMD=0.39, 95% CI: 0.07 to 0.71, I²=66%) (82). English et al. (2016) published a meta-analysis and reported that the provision of therapy for seven days/week instead of the usual five days/week in an inpatient setting reduced the average length of stay in the rehabilitation unit but resulted in no significant difference in walking speed or functional independence and health-related quality of life measures at discharge (83). Winstein et al. (2016) reported the results from a large RCT (the I CARE trial) with no additional benefit found from doubling the amount of arm motor therapy (~ 28 hours/week) (84). Thus, the literature is still inconclusive regarding what may be the optimal quantum of therapy as well as the best strategy to deliver that consistently over the long-term. The 2017 Australian guidelines suggest that a minimum of three hours a day of scheduled therapy that ensures at least two hours of active task practice may be reasonable, but the feasibility of attaining this consistently is dependent on multiple factors such as level of disability, patient engagement and modality of delivery (6).

1.3.3.3.3.2 Cardiorespiratory Fitness

Compromised cardiorespiratory fitness is very common amongst stroke survivors as reported by multiple studies that documented peak oxygen consumption (VO₂ peak) values of stroke patients
to be 26-87% of values seen in age- and gender-matched normative values (85). Activities essential for independent living require peak VO$_2$ of 15-18 mL O$_2$/kg/min and limited cardiorespiratory reserve represents a significant functional and quality-of-life challenge (86). Saunders et al. (2016) published a Cochrane review including 58 trials (797 participants) that evaluated cardiorespiratory interventions, resistance interventions and mixed training interventions (86). Cardiorespiratory and mixed training interventions resulted in moderate improvement in: global indices of disability (combined disability scales) and physical fitness (peak VO$_2$), as well as a small improvement in mobility (maximum walking speed, preferred gait speed and walking capacity) and balance measures (86). Recent research indicates that generic exercise after stroke can result in clinically meaningful health benefits across other disability domains in addition to activity level (87). At the impairment level, exercise potentially improves bone health, fatigue, and executive functioning, memory and post-stroke depression. At the participation level, exercise training has been associated with increased likelihood of social participation, return to work and improved quality of life (87). All practice guidelines recommend early assessment of cardiorespiratory fitness and inclusion of individually tailored exercise interventions in the rehabilitation plan for all stroke survivors (6, 8).

1.3.3.3.4 Balance, Posture and Mobility

Balance dysfunction following stroke is common and sitting balance has been used as a prognostic indicator for motor recovery (Feigin et al. 1996) (88). Sitting balance is an important predictor of recovery. Sitting equilibrium after a stroke has been found to have moderate to strong correlation with: BI score at 1week (Loewen & Anderson, 1990) (89); walking ability at 6 months (Feigin et al. 1996) (88); and 6-week gait ability (Sandin & Smith, 1990) (90). Sitting training interventions such as lateral weight transfer training, trunk exercises, body vibration and the practice of reaching beyond arm's length while sitting, were studied in various studies with different design elements.
Veerbeek et al. (2014) examined evidence from six RCTs (150 participants) using these interventions (91). Only studies with repetitive training interventions for reaching beyond arm-length (3 trials, 50 participants) were associated with improvement in reach distance and ground reaction force. Bank et al. (2016) provided more recent data from 11 low to moderate quality RCTs published up to 2013, with cohort size between 9-65 participants (92). Meta-analysis of pooled data reported a non-significant improvement in Trunk control test (TCT) and significant improvement in Trunk impairment scale (TIS) (92). This is important in the context of the recommendations from the American Physical Therapy Association that suggest that TIS may be the preferred outcome measure for sitting balance due to its responsiveness and reliability across different patient settings (93). Additional randomised trials assessing novel interventions such as training on a tilted platform, weight-shift training, and combined transcutaneous electrical nerve stimulation (TENS) and task-related trunk training were published recently and provide initial data on their effectiveness (94, 95, 96). The ability to transfer from sitting to standing position is critical to independent functioning after a stroke. Rehabilitation approaches have focused on repetitive task practice, strength training and more commonly, a combination of both (91). These are delivered as part of general interventions such as task-specific walking training and circuit class therapy, ideally initiated early after stroke. Multiple reviews have reported on the evidence for improved sit-to-stand ability following repetitive task practice and general interventions respectively (91, 97, 98). Use of consistent biofeedback, e.g., the number of repetitions/session/day, and time to complete a specific number of sit-to-stands, may augment training effectiveness as reported by Stanton et al. (2011) (99). French et al. (2007) published a review of seven RCTs and reported moderate benefits on the ability to stand from sitting (SMD 0.35, 95% CI: 0.13 to 0.56) following repetitive task-specific training (97). Veerbeek et al. (2014) reported non-significant improvement in body weight
distribution, sit-to-stand, and balance (91). Pollock et al. (2014) pooled data from 13 trials (603 participants) that used different interventions such as repetitive sit-to-stand training and exercise programs that included sit-to-stand training, sitting training and augmented feedback (98). The authors reported that only four studies were of high quality. Only one study (48 participants) reported on the ability to sit-to-stand independently as an outcome, reporting significant increase in the odds of independent standing following training (OR 4.86, 95% CI: 1.43 to 16.50) (98). More commonly, studies reported on change in measures such as time taken to stand (7 trials) or lateral symmetry (5 trials), wherein significant improvements were seen (98).

Standing balance plays a key role in the ability to walk and perform various activities of daily living and in decreasing the risk of falls after stroke (100). Stroke survivors present with limitations in postural sway, weight transference, and maintaining their balance in standing posture (100). Evidence from systematic reviews supports improvement of standing balance following exercise training that specifically includes either functional task practice in standing and weight-shifting or walking training that includes a balance challenge, e.g., overground walking or walking on obstacle courses (91, 100, 101). Miklitsch et al. (2013) demonstrated that balance training on a dynamic surface as compared to a stable surface did not result in a significant difference as measured by Timed Up and Go (TUG) performance (between-group difference of 2.89 sec; p = 0.100) (102). Corbetta et al. (2015) published a meta-analysis of studies evaluating virtual reality training interventions (103) and reported that small improvements in standing balance (Berg Balance Scale increase of 2.1 points, 95% CI: 1.8 to 2.5) and mobility (TUG improvement of 2.3 seconds, 95% CI: 1.2 to 3.4) (103). Cheok et al. (2015) examined the use of virtual reality provided through use of a Wii balance board in addition to standard care, and reported significant improvement in mobility (TUG) (SMD 0.81 (95% CI: 0.29 to 1.33, p=0.002, I²=0%) but not in balance (104).
Impaired walking impacts up to 75% of the overall stroke population in Australia and this can interfere with independent mobility (22). Systematic reviews by different groups such as Mehrholz et al. (2017), van de Port et al. (2012), Veerbeek et al. (2014), English et al. (2017) and Laver et al. (2017), analysed and reported data on various interventions that targeted walking impairment (75, 91, 101, 105, 106). These included: task-specific overground training (19 RCTs; 1008 participants); rhythmic gait cueing (6 RCTs; 231 participants); joint position feedback (11 RCTs; 254 participants); electrostimulation by (a) neuromuscular stimulation (NMS) (18 RCTs with 551 participants), (b) electromyography-triggered neuromuscular stimulation (EMG-NMS) (2 RCTs, 68 participants) and (c) transcutaneous electrical nerve stimulation (TENS) (5 RCTs, 349 participants); virtual reality training (6 RCTs, 150 participants); mental imagery (6 RCTs, 231 participants) and use of an orthosis (4 RCTs, 137 participants) (75, 91, 101, 105, 106). These interventions are delivered using different modalities such as circuit class training, treadmill training (31 RCTs, 1768 participants), electromechanically assisted training with/without functional electrostimulation (19 RCTs, 915 participants), and community-based ambulation training (3 RCTs, 94 participants) (75, 91, 101, 105, 106). Repeated motor practice has been postulated to be the physiological basis of motor learning (107). Repetitive task training (RTT) underpins the majority of modalities listed above to deliver interventions aimed to improve walking deficits (107). French et al. (2016) published a comprehensive review of the impact of RTT on different walking measures: walking distance (change from baseline: MD 34.80, 95% CI: 18.19 to 51.41; nine studies, 610 participants); walking speed (SMD 0.39, 95% CI: 0.02 to 0.79; 12 studies, 685 participants); functional ambulation (SMD 0.29, 95% CI: 0.10 to 0.48; 5 studies, 419 participants) (108). Australian practice guidelines and ASA guidelines strongly recommend
tailored repetitive practice of walking delivered via circuit class therapy or treadmill training (6, 8).

1.3.3.3.5 Upper limb activity

In acute stroke patients in Australia, 69% present with upper limb impairment (22). The upper limb (UL) function is subdivided into proximal or ‘arm’ (i.e. shoulder/elbow) and distal or ‘hand’ function (i.e. wrist, hand and fingers) (6).

French et al. (2016) reported that there is low quality evidence for benefit in arm function (SMD 0.25, 95% CI: 0.01 to 0.49; 11 studies, 749 participants) and non-significant benefit in hand function (SMD 0.25, 95% CI: 0.00 to 0.51; 8 studies, 619 participants) for RTT delivered via different interventions (108).

Pollock et al. published a Cochrane review in 2014 that analysed 40 reviews investigating different interventions for improving upper limb function (503 studies with 18,078 participants) (109). This review reported that moderate-quality evidence exists for effectiveness of constraint-induced movement therapy (CIMT), mental practice, mirror therapy, interventions for sensory impairment, virtual reality and a relatively high dose of repetitive task practice. They also reported that unilateral arm training may be more effective than bilateral arm training (109).

Corbetta et al. (2015) published a Cochrane review of 42 trials (1453 participants) and reported that CIMT improved arm function, dexterity (hand function) and arm motor impairment (110). However, this was not associated with a significant effect on the overall ability to perform activities of daily living in the short- or long-term. Kwakkel et al. (2015) compared the impact of CIMT (1 hour/day initiated within 2 weeks of stroke) with usual care, in patients with some residual finger extension, and reported improvement in the CIMT group as compared to the control (111).
Mehrholz et al. (2015) reported that mechanically assisted arm training modestly improves arm function and activities of daily living, though the strength of the evidence is moderate to low due to variability in the types of devices used and the number of repetitions (112).

Laver et al. (2017) reported a small but significant benefit with virtual reality and interactive video gaming for arm function and activities of daily living when used as an adjunct to usual care in participants with mild to moderate arm impairment. However, this benefit was limited to the early (first 6 months) post-stroke period (106).

Australian and ASA guidelines recommend that all individuals with stroke should receive task-specific training and ADL training, tailored to individual capabilities and that this training should be increased in difficulty on a periodic basis depending on gain in function (6, 8).

1.3.3.3.6 Communication

1.3.3.3.6.1 Assessment

The 2017 National Stroke Audit of acute stroke services reported that 57% of stroke patients presented with communication and speech problems (22). Previous studies such as Bowen et al. (2012) and Hoffmann et al. (2013) have reported that about one-third of people have persisting communication deficit after stroke (113, 114). Practice guidelines recommend that stroke patients be assessed by a speech pathologist within 48 hours of admission to determine the type and severity of the communication impairment (6, 8).

1.3.3.3.6.2 Disorders of speech – Aphasia, Dysarthria and Apraxia

Aphasia is defined as an acquired impairment of the language system following brain damage and is seen commonly after a stroke involving the left hemisphere (114). The National Stroke Audit
Brady et al. (2016) published a Cochrane review that analysed data on speech and language therapy (SLT) from 57 small RCTs (3002 participants) (115). SLT significantly improved functional communication reading, writing, and expressive language (SMD 0.28, 95% CI: 0.06 to 0.49, p = 0.01), as compared to no SLT (115). This review also studied the emerging data from comparative studies of different therapy regimens (intensity, dosage and duration), delivery models (group, one-to-one, volunteer and computer facilitated) and theoretical approaches (constraint-induced therapy and semantic therapy) (115). There is currently no indication that delivery models per se have significant impact in terms of difference in efficacy (115). There is emerging data to suggest that higher intensity interventions were associated with bigger improvements in functional language skills but this came at the cost of higher drop-out rates and were likely not maintained long-term (115). The current practice guidelines support the provision of SLT as early as tolerated (6, 8).

Dysarthria is a collective term for a group of speech output disorder problems that result from impaired movements of the speech musculature including lips, tongue, palate, larynx and the respiratory muscles (113). Approximately 20% of stroke patients present with dysarthria and it often leads to restricted efficiency of communication and social participation (116). Mackenzie et al. (2014) reported that non-speech oro-motor exercises did not provide additional benefit to behavioural speech practice (117). Mitchell et al. published a Cochrane review in 2017 to analyse interventions for management of dysarthria (118). These interventions targeted:

- Impairment: non-speech and oro-motor exercises or external stimulation (via transcranial magnetic stimulation or acupuncture) of the muscles
- Activity: use of augmentative communication devices (non-technical materials such as an alphabet chart or text-to-talk computer devices)
- Participation: conversational training and behavioural therapy.

The review analysed data from four small studies in stroke, published up to 2016 and provided preliminary evidence for potential benefit from individually tailored interventions including behavioural techniques and use of augmentative and alternative communication devices (118).

Apraxia of speech represents a disorder of motor planning or programming resulting in difficulty in volitionally producing the correct sounds of speech (119). A systematic review by Ballard et al. (2015) indicated that the current level of evidence is low, as it is predominantly composed of case series or uncontrolled case studies (119). Both articulatory-kinematic and rate/rhythm-based interventions may facilitate clearer production of speech sounds, but this was not associated with improvement in overall communication (119).

Right hemisphere stroke can be associated with dysfunctional exchange of communicative intent through nonverbal and verbal means (120), often at a conversational level. It is characterised by prosody (flat melody of speech or difficulties interpreting emotion/intent contained in another person’s speech), expressive and receptive discourse (difficulties understanding intent in language) and pragmatics (disrupted functional use of language in context) (120). Research literature is currently limited (120). Practice guidelines based on consensus, suggest the use of cognitive-linguistic treatments to improve use of emotional tone (121) and semantic-based treatment to improve metaphorical comprehension (122).

1.3.3.3.7 Cognition and Perception

The National Stroke Rehabilitation Services Audit Report in 2016 stated that approximately 60% of patients present with some degree/type of cognitive and perceptual impairments (59).
1.3.3.7.1 Assessment

Cognitive impairments are comprised of deficits in different domains: attention, memory, orientation, language, executive functions, neglect, apraxia and agnosia. Cognitive deficits have been associated with doubling the risk of dependent living at 3 months (123) and tripling the risk of death (123). Practice guidelines across the globe recognise the significance of this risk and recommend screening for cognitive and perceptual deficits by trained personnel, for all stroke survivors prior to discharge from hospital (6, 8).

1.3.3.7.2 Disorders: Executive Function, Attention, Concentration, Memory and Perception

Approximately 75% of stroke survivors suffer from some degree of executive dysfunction (124, 125). The presence of dysfunction negatively affects the ability to regain independence in ADL, especially in patients who have concomitant limb weakness, and it can decrease the likelihood of success of many targeted rehabilitation interventions dependant on goal-oriented behaviour (124).

Cognitive rehabilitation strategies (125, 126) to address executive dysfunction can be:

- Restorative interventions: self-awareness training, intensive neurorehabilitation, standard neurorehabilitation combined with cognitive remediation, problem-solving/goal management/strategy training, autobiographical memory cueing, working memory training and verbal feedback
- Compensative interventions: intensive neurorehabilitation, standard neurorehabilitation, video-feedback, verbalisation, chunking and pacing, and directive feedback
- Adaptive interventions: interventions targeted to improve independence with ADL.

At present, there is limited evidence to support the effectiveness of cognitive rehabilitation strategies.
Attention is defined as selectively concentrating on a discrete aspect of information. Attention impairments may be specific (e.g. selective, sustained, divided) or generalised. Attention deficits during the acute phase have been reported to range between 46% and 92% (127). Approximately 20-50% of stroke survivors continue to suffer from attention deficit up to 5 years post-stroke (128). A Cochrane review by Loetscher et al. (2013) analysed data from 6 RCTs (223 participants) and found that cognitive rehabilitation improved measures of divided attention (SMD 0.67, 95% CI: 0.35 to 0.98, p < 0.0001) in the short-term but didn’t improve global measures of attention or functional outcome; nor did it provide persisting benefits (129).

Memory impairment after stroke has been estimated to affect between 23% and 55% of stroke patients at three months and between 11% and 31% at one year after stroke (130) and may affect different aspects such as language-based memory, visual-spatial memory, differentiating learning, recall, recognition and forced-choice memory. Nair et al. (2016) published a Cochrane review that analysed data from 13 trials (514 participants) (131). The review analysed various types of memory retraining techniques such as computer-assisted programs and training with memory aids (diaries or calendars), delivered in community (7 studies), inpatient (4 studies), and mixed community and inpatient settings (2 studies) (131).

The review reported improvement in immediate subjective memory measures (SMD 0.36, 95% CI: 0.08 to 0.64) (131). However, these benefits did not persist in the long-term. The review found no evidence of significant impact on level of independence in ADLs, mood, or in quality of life (131).

Perceptual impairment after stroke can present as: visual or object agnosia (the inability to process sensory information or recognize objects, faces, voices, or places); prosopagnosia (the inability to recognize faces); and perceptual disorders involving visuospatial or tactile sensation, location,
motion, colour or auditory processing (6). Though literature regarding their prevalence is currently scant, according to the National Stroke Rehabilitation Services Audit Report in 2016, some type of perceptual deficit is seen in about 25% of stroke patients (59). Rehabilitation strategies usually include functional training, sensory stimulation, strategy training and task repetition to target the specific perceptual deficit in the individual. However, there is very little evidence, at present, to support the use of these interventions (6).

1.3.3.7.3  **Limb Apraxia**

Limb apraxia is defined as impairment in planning and sequencing of movement, not caused by weakness, incoordination, or sensory loss. Lindsten-McQueen et al. (2014) estimated that the prevalence of limb apraxia in people with left hemisphere stroke ranges from 28% to 51% (132). This systematic review analysed data from eight studies: four RCTs and four pre-post designs. These studies employed different treatment approaches: errorless learning with training of details (one trial), gesture training (two trials), and strategy training (five trials) (132). The review provides preliminary indication of potential benefit with these treatment strategies but highlights an urgent need for validation through larger, adequately-powered studies (132).

1.3.3.7.4  **Neglect**

Spatial neglect is described as the failure to attend to sensory or visual stimuli on the affected side (133). The National Stroke Audit Report in 2016 stated that approximately 30% of stroke survivors in Australia demonstrated spatial neglect (59).

Rehabilitation interventions such as visual scanning with sensory stimulation, eye patching, simple cues, mental imagery and combinations of these trainings, have been used to manage neglect. Bowen et al. (2013) reviewed data from 23 RCTs with 628 participants (133). These studies
examined strategies such as computerised scanning training, pen and paper tasks, visual scanning training, eye patching and mental practice. The review concluded that there was limited evidence to support benefit with these interventions. Recent studies that examined use of a combination of these therapies (134, 135) and use of mirror therapy (136), reported meaningful benefit. Thus, current evidence for use of these interventions is inconclusive.

1.3.3.3.8 Activities of Daily Living (ADL)

ADLs are defined as routine self-care tasks that are part of everyday life and are usually divided into two types: basic and extended. Basic ADLs are tasks related to self-care and essential mobility and extended ADLs or instrumental activities of daily living (IADLs) are tasks that may not be necessary for fundamental functioning but are important for an individual to live independently (137). Around 87% of stroke survivors in Australia were considered to have difficulties with ADL (59). The majority of stroke survivors receive some intervention and ADL training in hospitals, including task-specific practice (91%) and training in the use of appropriate aids and equipment (62%) (59).

A Cochrane review that examined evidence for the use of occupational therapy for adults with problems in activities of daily living after stroke was published in 2017 to update data from recent studies (138). In this update, the authors (Legg et al.) reported on data from nine studies (994 participants). Targeted occupational therapy interventions improved overall ADL performance scores (SMD 0.17, 95% CI: 0.03 to 0.31, p = 0.02) and reduced the risk of poor outcome (death, deterioration or dependency in personal activities of daily living) (OR 0.71, 95% CI: 0.52 to 0.96, p = 0.03) (138). The review indicated a higher likelihood for individuals on occupational therapy to be more independent in IADLs (OR 0.22, 95% CI: 0.07 to 0.37, p = 0.005) (138). However, this
did not translate into any meaningful impact on mortality (OR 1.02, 95% CI: 0.65 to 1.61, p = 0.93), combined odds of death and institutionalisation (OR 0.89, 95% CI: 0.60 to 1.32, p = 0.55), or death and dependency (OR 0.89, 95% CI: 0.64 to 1.23, p = 0.47) (138). Furthermore, there was no benefit of these interventions on mood or distress scores (OR 0.08, 95% CI: -0.09 to 0.26, p = 0.35) (138). Overall, the quality of evidence was low due to the risk of patient selection bias, performance and detection bias, and limited availability of data for key outcomes of interest (138).

Australian stroke clinical practice guidelines strongly recommend that all stroke survivors undergo a comprehensive assessment of their ADLs/IADLs prior to discharge into the community. A continuum of care should be ensured while in the community through relevant task-specific practice and training in the use of appropriate aids and ongoing assessment and modification of intervention by trained clinicians (6).

Research in the field of rehabilitation has predominantly consisted of small trials with less rigorous design, due perhaps to the challenges that persist with the implementation of large scale, rigorous trials. Clinical practice has evolved in the field of rehabilitation, based predominantly on systematic reviews, which in turn are inherently limited in terms of strength of evidence by the lack of rigour in the studies included. The overall volume of stroke rehabilitation publications in the last four decades has grown considerably with approximately 35% of all the RCTs published in just the last five years (139).

Some of the challenges that need to be addressed urgently are the understanding of the underpinning biology of post-stroke recovery, the complex interactions between different domains of disability and the lack of feasibility of having single outcome measures that can adequately reflect changes across impairment, activity and participation attributes (139).
1.4 Current Gaps in Research: Ischaemic Stroke

The approaches to stroke research have predominantly been along the following pathological principles (139, 140, 141, 142):

- re-establish blood flow to the ischaemic region (reperfusion strategies)
- modulate varied molecular pathways involved in ongoing neuronal injury after the initial ischaemic insult (neuroprotection) and,
- augment endogenous neural recovery, either by acquisition of the new skills needed for optimum physical, psychological and social function with a given level of disability (rehabilitation) or by restitution of neural networks (CTs).

Of the above approaches, reperfusion is an established part of acute clinical management. However, research with the remainder of the approaches is still evolving and is discussed in the following sections.
1.4.1 Neuroprotection

The interruption of blood supply to the brain triggers a neuronal ischaemic cascade and leads to energy failure, and ultimately neuronal damage. Basic research has established that neurons are most susceptible to the acute onset of ischaemic damage (142). Further to this, ischaemic insult leads very quickly to damage of endothelial cells and pericytes, which compromise the integrity of the blood brain barrier (142). This leads to local release of Ca$^{2+}$, enzymes and cytokines (i.e., IL-1 and TNF-α), activation of resident immune cells (i.e., microglia) and recruitment of peripheral inflammatory cells (143). Large multiprotein complexes called inflammasomes, e.g. NOD-like receptor proteins, are early central players that efficiently amplify the initial immune response to cell injury and lead to ongoing recruitment of peripheral macrophages as well as an increase in reactive microglia and astrocytes in the peri-infarct zone (144). These different cell types in cerebral tissue are intricately co-located and directly or indirectly influence each other (144). The inflammatory cascade represents an interplay of different molecular pathways involved in: neuroinflammation, excitotoxicity, calcium channels’ over-activation and reactive oxygen species (ROS) production (145, 146). This cascade continues to be relevant in the sub-acute and chronic phase of stroke and plays an important role in both neural injury as well as repair (147).

Numerous chemical and biological candidates targeting different molecular and cellular aspects of this cascade have been investigated to date (148). Unfortunately, the potential seen with these therapies in the preclinical stage of research was not realised, as clinical trials of both pharmacological and non-pharmacological neuroprotective treatments reported negative results (148). Examples are the NEST-3 and FAST-MAG phase III trials. The NEST-3 trial (Neurothera Effectiveness and Safety Trial 3) examining the use of transcranial lasers in acute stroke reported futility in its interim analysis in 2014 (149). The FAST-MAG trial (Field Administration of Stroke...
Therapy–Magnesium) of pre-hospital magnesium infusion reported no difference in post-stroke outcomes between the intervention and placebo groups (no significant shift in the distribution of 90-day mRS, \( p=0.28 \) and no difference in the mean 90-day mRS scores, 2.7 in each group, \( p=1.00 \)) (150). Further to this, the investigators recently reported that there was no association of clinical outcomes with achieved magnesium levels, which provides further evidence that magnesium may not be neuroprotective in acute stroke (150). A Cochrane review by Zhang et al. (2012) analysed data from 34 RCTs (7731 patients) that investigated the use of calcium antagonists (nimodipine in 26 trials, flunarizine in three trials) (151). There was no benefit observed on the primary outcome of death or dependency at the end of follow-up (relative risk (RR) 1.05, 95% CI: 0.98 to 1.13), or on death at the end of follow-up (RR 1.07, 95% CI: 0.98 to 1.17) (151). In addition, subgroup analysis of different doses of nimodipine indicated that the higher doses were associated with poorer outcome (151).

There has been a recent resurgence of interest in neuroprotection with clinical studies ongoing with agents that target immunomodulation (Natalizumab), free radical scavenging (Edaravone) and excitotoxicity (PSD-95 inhibitor Tat-NR2B9c or NA-1). A phase II study of safety and efficacy of Natalizumab in patients with acute ischaemic stroke (ACTION) reported no difference in infarct volume growth from baseline up to day 5 with natalizumab compared with placebo (relative growth ratio 1·09, 90% CI: 0·91 to 1·30, \( p=0·78 \)) (152). However, the Natalizumab group demonstrated an improvement versus the placebo group. This improvement was defined as mRS scores of 0 or 1 at day 30 and at day 90 (OR 2·88, 90% CI: 1·20 to 6·93, \( p=0·024 \) and OR 1·48, 90% CI: 0·74 to 2·98, \( p=0·18 \) respectively) and BI score \( \geq 95 \) at day 90 (OR 1·91, 90% CI: 1·07 to 3·41, \( p=0·033 \)) (152). Isahaya et al. (2012) reported that Edaravone suppressed serum matrix metalloproteinase-9 (MMP-9) level in a prospective cohort of patients with acute ischaemic stroke (153). The ENACT
(Evaluating Neuroprotection in Aneurysm Coiling Therapy) phase II clinical trial with Tat-NR2B9c reported in 2012 that the Tat-NR2B9c (treatment) group sustained fewer ischaemic infarcts than the placebo group, as gauged by diffusion-weighted MRI (adjusted incidence rate ratio 0.53, 95% CI: 0.38 to 0.74) (154). Tat-NR2B9c is being investigated in an ongoing multi-country Phase III ‘A Field Randomization of NA-1 Therapy in Early Responders’ (FRONTIER) clinical trial in ischaemic stroke administered within three hours by paramedics in the field (155).

Since the key premise underpinning neuroprotection in acute ischaemic stroke is to enable administration of the agent as rapidly as possible following stroke onset in order to minimize infarct volume whilst awaiting reperfusion therapy, the limited time window of opportunity is likely to limit the overall impact of these agents, even if they are successful (156).

1.4.2 Rehabilitation

Research in preclinical stroke models and human stroke survivors confirms that a period of spontaneous recovery occurs early post-stroke with little or no active treatment (156). However, the duration and trajectory of this recovery varies across neural systems and across individuals (157, 158). While there is evidence to suggest that the degree and rate of recovery can be predicted post-stroke in several domains using proportional recovery algorithms, the biological mechanisms determining these are still to be understood clearly (159). The first Stroke Recovery and Rehabilitation Roundtable (SRRR) convened in 2016 and published their consensus on key concepts in recovery research in 2017 (138). ‘Recovery’ is a biological construct that comprises two components: (1) the improvement in each functional outcome across time, and/or (2) the mechanisms responsible for this improvement (160). The mechanistic processes underlying recovery are believed to be compensation or behavioural restitution (139).
The process of ‘compensation’ is defined as the ability to accomplish a task through using a new approach rather than using their normal pre-stroke behavioural repertoire (160). This does not require neural repair but may require learning new ways of using intact neural or muscular pathways or the use of assistive devices to compensate for the function lost due to stroke. Rehabilitation strategies predominantly activate compensation mechanisms to effect recovery of function after stroke (139).

**Figure 1. Critical time points post-stroke that link to the currently known biology of recovery**

![Phases of recovery](image)

1 Haemorrhagic stroke specific. 2 Treatments extend to 24 hours to accommodate options for anterior and posterior circulation, as well as basilar occlusion.

*Reproduced with permission from Bernhardt et al. (2017) (139)*

Behavioural restitution (true recovery) on the other hand, describes the full or partial restoration of the repertoire of behaviours that were available to the individual before stroke-induced injury (160). This process would require neural repair and restitution of injured neuronal networks. Cell therapy is likely to modulate recovery of function through this mechanism (162).
1.4.3 **Cell Therapies (CTs)**

1.4.3.1 **Definition of Stem Cells**

Stem cells are distinct populations of cells in an organism that have the capacity to self-renew by dividing and to develop into more mature, specialized cells (163). Stem cells can be unipotent, multipotent, pluripotent or totipotent, depending on their potential to mature into different numbers of cell types (163).

1.4.3.2 **Stem Cell Research in Stroke**

Research into stem cells has come a long way since the first *in vitro* cultures of stem cells in 1907, as reported by Maienschein in 2011 (164). Research in regenerative medicine over the past decades has demonstrated that endogenous stem cells and exogenous cell therapies can mediate different molecular pathways at different time points in the ischaemic cascade following stroke (165).

The acute phase of the ischaemic cascade begins directly after the occluding event (146). The lack of blood flow to the ischaemic area triggers local oxidative stress and excitotoxicity, which damages neural tissue and results in vasogenic oedema and the disruption of ionic and Na\(^{+1}\) and Ca\(^{+2}\) accumulation in cells at the ischaemic core, which leads to cell death (146, 166, 167). The massive release of glutamate and the Na\(^{+1}/Ca^{+2}/K^{+1}\) imbalance result in cortical spreading depolarization characterized by widespread depolarization of neurons and astrocytes that has been demonstrated in electrophysiological and imaging studies (168). The cells in the ischaemic penumbra begin expressing signals associated with neuronal injury that upregulate local neuroinflammation (169). Neuroinflammation is the pathologic hallmark of the subacute phase, which typically lasts for the first few days following the stroke onset, with the release of cytokines, chemokines, cellular adhesion molecules (CAMs) and matrix metalloproteases (MMPs) from
injured neuronal and non-neuronal cells, such as microglia and astrocytes (170, 171). Secretion of cytokines, chemokines, and CAMs, causes recruitment of peripheral neutrophils and macrophages through the injured blood brain barrier, resulting in ongoing damage to the neurovascular unit (171).

The ischaemic cascade continues into the chronic phase, wherein activated microglia, astrocytes and endothelial cells become dominant players (165). The activated microglia exert a dualistic role in neurological recovery through their relative polarization into different phenotypes at different stages of injury (171). The reactive microglia and astrocytes release trophic factors such as VEGF/Flk1 and Ang-1/Tie2, BDNF, nerve growth factor, VEGF, IGF-1, hepatocyte growth factor and GDNF (171). These factors promote angiogenesis, stabilize vasculature, enhance cell survival, proliferation and differentiation, trigger neuroblast proliferation, promote neurogenesis and trigger migration of endogenous neural stem cells from the subventricular zone (171).

There is extensive preclinical evidence from in vitro and in vivo models that different cell types being developed as (stem) cell therapies have the potential to induce endogenous brain repair processes, including neurogenesis, angiogenesis and synaptogenesis and provide trophic support to endogenous neural stem cells (165, 171, 172, 173). The administration of cell therapies in subacute stroke models has demonstrated suppression of pathways that modulate oxidative stress, reduction of mitochondrial activation and inhibition of apoptosis (165, 172).

Some degree of brain recovery is nearly always achieved as part of ‘spontaneous recovery’ seen in all stroke survivors (173). Cell therapies augment this endogenous recovery by potentially regenerating and repairing neural pathways (173, 174). Cell therapies potentially present a treatment paradigm that may uniquely combat both subacute and chronic inflammatory processes.
and drive brain regenerative mechanisms such as vasculogenesis, neurogenesis, angiogenesis and synaptogenesis to restore cerebral infrastructure and achieve neural repair (172-174).

Stem cells may potentially address the immense gap that currently exists in the management of stroke outside of the acute phase of early brain cell damage (174). Thus, stem cells represent a credible opportunity to promote active recovery across functional domains that hitherto remains an aspirational goal in the management of stroke (175).

1.5 Conclusion

Stroke has long represented a difficult therapeutic challenge. This challenge has translated into a significant public health burden in recent times as the number of people suffering from stroke and living with disabilities that limit their independence and gainful participation in society has been on a constant rise in both the developing and developed world (10). These unmet needs require that stroke research explores strategies to ‘recover’ function to levels that are as near as possible to pre-stroke levels of function. The need to better define recovery after stroke is an underpinning physiological concept that is crucial for any future progress in exploring strategies to recover neurological function lost consequent to stroke in an individual (156). As translational stroke research continues to evolve from secondary prevention and neuroprotection, and now attempts neural regeneration and repair, it becomes extremely critical that we examine elements of early phase clinical research in this area that are likely to become critical determinants of successful translation to viable clinical options (176).
CHAPTER 2: LITERATURE REVIEW

2.1 Burden of Ischaemic Stroke

The burden of neurological disorders has increased significantly over the last two decades and this group is now the worldwide leading cause of disability (10.2% of global DALYs) and the second-leading cause of mortality (16.8% of global deaths) (177). Stroke contributed to the largest proportion of total DALYs (47.3%) and deaths (67.3%) among all neurological disorders (177). This is likely to continue in the future due to ongoing demographic changes, including ageing of the population in developed countries and health transitions observed in developing countries, most notably the rise of ‘lifestyle diseases’ associated with increased risk for occurrence of stroke (177). Approximately 10 million new cases of stroke occur every year around the world (177). Although age-standardised rates of stroke mortality have decreased worldwide in the past two decades, the absolute numbers of people who have a stroke every year and who live with the consequences of stroke, and die from their stroke are increasing (10). In Australia, approximately 56,000 new cases of stroke are likely to occur every year (178).

Worldwide, stroke results in 6.5 million deaths annually of which 51% die from ischaemic stroke (IS) and 113 million DALYs (58% due to IS) are lost (10). In the context of Australia, this translates into 12,507 deaths annually (178). Moreover, the proportional contribution of stroke-related DALYs and deaths due to stroke compared to all diseases increased from 1990 (3.54% and 9.66% respectively) to 2013 (4.62% and 11.75% respectively) (10).

According to the Global Burden of Disease Study 2013, ischaemic stroke is the second highest cause of years of life lost (YLL) worldwide (11). In Australia, ischaemic stroke is responsible for
the third highest number of YLL, after ischaemic heart disease and cancer (179), and the quality adjusted life years lost per case is reported to be approximately 5.09 for IS and 6.17 for ICH (180).

The long-term disability experienced by stroke survivors worldwide represents an enormous global healthcare challenge. The cost of providing ongoing healthcare to people with stroke accounted for approximately 3–5% of all healthcare expenditure in the United Kingdom in 2017, with the total mean costs of healthcare attributable to stroke being £46,039 GBP per patient at five years after stroke (181). Gloede et al. (2014) reported lifetime costs per patient of approximately $103,566 AUD ($68,769 USD) and total lifetime costs of all first-ever cases of ischaemic stroke in Australia of $2 billion AUD, based on resource utilisation in patients followed up at 10 years following their incident stroke (182).

The approach to the management of stroke has been centred primarily on restoration of blood supply to the infarcted region of the brain and preventing re-occlusion (8). While IV thrombolysis and, more recently, endovascular thrombectomy have been transformative in the management of stroke and consequently for patient outcomes, the fact remains that progress in achieving a better health future after stroke during the last and present century has remained confined to only one (acute) aspect of the disease (183). It is well-recognised that while stroke may be acute in onset, it is very much a chronic disease with multiple molecular mechanisms that maintain ongoing injury and activate repair, simultaneously (184). Recent advances in basic research technologies has expanded our understanding of these mechanisms in the infarct brain microenvironment (145, 185). Translation of this knowledge into viable clinical options is arguably the next frontier and one that needs to be crossed with a sense of urgency to enable us to manage the significant stroke burden we are certain to face in the very near future (186).
2.2 Current Research in Ischaemic Stroke

Ischaemic stroke results in an acute, typically non-progressive blockade of blood supply to a region of the brain (1). However, the long-term implications of stroke are a consequence of dysfunction of neuronal networks that impair functional ability in stroke survivors (1, 187).

2.2.1 Mechanisms of Injury in Stroke

2.2.1.1 Ischemia-Reperfusion Injury

Primary damage to the neurovascular brain tissue caused by ischaemic stroke is characterised by neuronal death due to oxygen and glucose deprivation (188, 189). Cellular depletion of adenosine triphosphate (ATP) results in lactic acidosis, ion transport dysregulation, and extracellular glutamate accumulation (188). These metabolic changes lead to endothelial swelling and early disruption of the blood brain barrier (BBB) and neuronal death (190). These changes result in the loss of function mediated by the affected regions. Restoration of blood flow is critical to the survival of the area around the infarct core characterised by hypo-perfusion (penumbra) (189). However, this is associated with reperfusion injury in damaged cells, which causes free radical production, the activation of astrocytes and white blood cells which release cytokines and free radicals, and the production of synchronized neuronal activity (191). The initial, reversible damage to the BBB that occurs within hours after reperfusion is associated with the disruption of tight junction proteins from oxidative stress induced after reperfusion and the local accumulation of MMPs (192, 193). Oxidative damage to cellular molecules, upregulation of inflammatory mediators and MMPs, and modulation of tight junction proteins maintains ongoing damage to the BBB (193). Subsequent irreversible damage occurs 24–72 hours after reperfusion, mediated by upregulation of pro-inflammatory cytokines and infiltration of peripheral immune cells into the CNS (194).
2.2.1.2 Neuroinflammation

The initial event is followed by secondary damage mediated by ischemia and reperfusion resulting in a neuroinflammatory cascade that persists for days to weeks after stroke (143). Numerous mechanisms have been reported to be involved in this cascade, on a molecular and cellular level (143). These include: glutamatergic excitotoxicity, loss of intracellular calcium homeostasis, energy failure, oxidative stress, neuroinflammation, and axonal degeneration of synaptic inputs (anterograde degeneration) and projection neurons (retrograde degeneration) (156).

In the subacute phase, upregulated inflammation triggers release of cytokines, chemokines, CAMs, and MMPs and reactive oxygen species from injured neurons, microglia and astrocytes at the site of ischemia (156). This results in recruitment of resident immune cells such as microglia and peripheral immune cells such as neutrophils, macrophages and T cells (78, 171). This, in turn, sustains neuroinflammation and contributes to the breakdown of normal cerebral microstructure (BBB and synaptic networks). Activated microglia, astrocytes and endothelial cells may induce the release of molecules that block axonal sprouting, such as chondroitin sulphate proteoglycans (195, 196) or Ephrin-A5 (197). The resident immune cells clear the dead cells and are involved in the formation of a glial scar with aberrant extracellular matrix (ECM) and blood supply, as well as persistence of reactive astrocytes and resident activated microglia/macrophages (198, 199). Substantial remodelling of neurovascular architecture continues, which may result in compromised integrity of key functional tracts (200).

Temporal resolution of neuroinflammation in the brain is understood to be an active process, driven by active suppression of inflammatory mediators. There is evidence that an increase in signalling molecules like IL-10, TGF-β and arginase suppresses inflammation and exerts neuroprotective
effects on the surviving cells (199). Activated microglia release cytokines that facilitate axonal sprouting and dendritic morphogenesis, such as bone morphogenetic protein 7 (201); and several molecules belonging to the transforming growth factor (TGFβ) family such as GDF10 (200); and the Activin system (202, 203). GDF10 signals through the Smad 2/3 transcription factor to activate PI3 Kinase gene systems that mediate axonal sprouting (204).

Therefore, neuroinflammation plays an active role in both secondary brain injury and neurorepair after stroke and animal models of stroke indicate an active cross-talk between inflammation and neurogenesis (203, 205).

2.2.2 Mechanisms of Recovery in Stroke

2.2.2.1 Neuroplasticity

Neuroplasticity is the capacity of neuronal networks in the brain to respond to biological and/or environmental signals by changing their function and structure (206). This capacity varies during the lifespan from being prolific during embryonic development to limited in adulthood (206). Neuroplasticity comprises modulation of neurogenesis and ongoing remodelling of synapses and neural cells at a structural and functional level (206).

2.2.2.1.1 Neurogenesis

There is growing evidence supporting the presence of a distinct population of neural stem cells that contribute to ongoing neurogenesis in humans (207). These cells are located in distinct regions of the brain: the subventricular zone (SVZ), located adjacent to the lateral ventricle, and the subgranular layer of the dentate gyrus in the hippocampus, and possibly other regions of the hypothalamus such as the lining of the third ventricle and suprachiasmatic nucleus (208). Ongoing research into the functional relevance of these cell populations has clearly established the fact that
the brain is a plastic organ capable of regeneration and repair (209). Spalding et al. (2013) measured the concentration of nuclear bomb test-derived $^{14}$C in genomic DNA, to retrospectively birth date cells in the hippocampal region in human brain (210). The study demonstrated that substantial neurogenesis in the human hippocampus continued throughout life and postulated that this was crucial to learning and cognitive plasticity (210). Post-mortem brain studies demonstrate SVZ cell proliferation and neuroblast formation after stroke even in aged humans (211, 212).

Stroke triggers an increase in neurogenesis (213). These neurogenic niches are highly sensitive to oxygen deprivation; studies in vertebrate models have indicated hypoxia and inflammation are associated with both potentiation and suppression of neurogenic activity within these areas (214, 215). The complex interplay between neuroinflammation, neurogenesis and their impact on functional recovery after stroke is still being explored (216). Studies have reported that T cells, astrocytes and microglia interact to drive the proliferation of SGL and SVZ progenitor cells and direct the migration and differentiation of these cells \textit{in vitro} and \textit{in vivo} (216). On the other hand, TNF-α secretion by activated microglia leads to progenitor cell suppression (217). Resident microglia may potentially have a key modulatory role in determining the balance between these contradictory processes (218). Studies have shown that whereas acute activation of microglia results in suppression of neurogenic signals, chronically activated microglia are predominantly pro-neurogenic (218). Microglia release Insulin-like Growth Factor (IGF) in the hippocampal region and increase the proliferation, migration and maturation of NSCs (213). In addition to the increase in neurogenesis in the immediate period following stroke, there is evidence of a transient increase in angiogenesis that potentially enables neuroblasts to migrate along these newly formed blood vessels and home in to the infarcted area, assisted by chemical and cellular neuroinflammatory signals (213).
2.2.2.1.2 Reorganisation of Neural Networks and Synaptoplasticity

A key biological process in neuroplasticity is axonal sprouting and the formation of new connections in regions of brain damaged or partially deafferented from the stroke (219). The extent of initial stroke injury influences the topography of axonal sprouting (219). In small to medium sized experimental strokes, axonal sprouting occurs in peri-infarct cortical areas whereas in large infarcts, axonal sprouting from the contralateral cortex to the deafferented side of the cervical spinal cord is the predominant pattern (219, 220). Peri-infarct sprouting results in new, significantly different connections between motor cortex, premotor and somatosensory cortex (219). Contralateral corticospinal axonal sprouting observed in the rat, mouse and non-human-primate stroke models results in remapping of motor representations of the ipsilateral limb (221). Morecraft et al. (2015) indicated a role for somatosensory input in contralateral axonal sprouting (222). Infarction of the parietal somatosensory cortex, in addition to the motor cortex, was associated with suppression of contralateral sprouting (222). These anatomical mapping studies suggest that axonal sprouting starts early after stroke (within weeks) and remains active at several months after stroke (219). Post-stroke axonal sprouting demonstrates three distinct patterns determined by their temporal relationship to stroke onset and mediated by the interaction between glial growth inhibitor signalling and behavioural cues (219). Early after stroke, reactive sprouting occurs in the perilesional cortex as part of scar formation and tissue reorganization. The resultant endogenous brain remodelling after stroke may be associated with spontaneous recovery (219). Reparative axonal sprouting involves longer distance axonal sprouting activated in a specific environment characterised by blockade of NgR1 or EphrinA5, or upregulation of brain growth factor GDF10 (197, 204). This sprouting is limited to sensorimotor areas of the ipsilateral hemisphere or the contralateral spinal cord and remains directionally confined within the same functional domain.
The third pattern of unbounded axonal sprouting was observed in models where behavioural activity is increased at the same time as a blockade of the glial growth inhibitors. These patterns suggest a critical temporal relationship between: time since stroke onset; the dominant molecular/cellular signal in the microenvironment (blockade of glial growth inhibitors and stimulation of neurogenic pathways); and the behavioural cues to the injured brain functional pathway such as through rehabilitation (219). There is also evidence of synchronized low frequency neural activity seen in the hyper-acute stage of stroke (223). This activity leads to activation of downstream molecular pathways that trigger neuronal growth and stimulate co-activation of synapses in a specific region of neurons, resulting in the establishment and stabilisation of new neuronal connections (223).

Prabhakaran et al. proposed that it was possible to predict recovery in most patients with upper limb motor impairment (224). They reported that clinical predictors (acute upper extremity Fugl-Meyer Motor (UE-FM) score, subcortical lesion volume, age, time to reassessment, and y-intercept) could explain 89% of the variance seen in recovery within the first six months after exclusion of outliers with severe initial impairment. Thus, with the exception of the outlier subpopulation (characterised by low initial UE-FM score), recovery in patients was well approximated by a proportional relationship with the initial impairment (24–48 hours after stroke onset (recovery \( \approx 0.70 \times \text{initial impairment} \)) (224). Studies looking at motor function of the upper and lower extremities, speech and visuospatial neglect replicated this ‘70% proportional recovery rule’ (225-227). Thus, mechanisms driving spontaneous recovery after stroke generalize across neurological impairments and perhaps represent the extent of existing neural tract integrity and capacity for reorganisation of neural networks (158). The premise that existing neural tract integrity may be an important predictor for recovery was supported by studies that measured intactness of
the relevant neuronal networks using transcranial magnetic stimulation and diffusion tensor imaging, e.g. corticomotor pathways for upper limb motor function and frontoparietal attention networks for visuospatial neglect (228, 229). Clinical and imaging biomarkers that can establish this link between structural integrity and functional recovery may enable stratification concerning timing, quantum and nature of rehabilitation interventions and efficient selection of a study population for rehabilitation trials (158).

2.2.3  **Functional Recovery: Clinical Concepts and Terminology**

Recovery of functional ability after stroke is an aggregate of spontaneous recovery in injured areas and compensatory learning in uninjured parts of the brain (139). Spontaneous recovery encapsulates regeneration of new neurons to replace the injured ones and structural recovery, i.e. re-establishment of the complex arborisation of neurons and synaptic connections along with interlinkages between different neural networks as well as the restoration of neuro-vascular synergy (197, 230). Behavioural restitution describes the full or partial restoration of the repertoire of behaviours that were available to the individual before stroke-induced injury (160). This process requires restitution of disrupted neuronal networks, influenced by the local microenvironment and factors such as genetic reserve and pre-existing comorbidities such as hypertension, metabolic syndrome, hyperlipidaemia and diabetes (231). Cell therapies have demonstrated their potential to facilitate a microenvironment that increases endogenous NSC replication and promote neurogenic molecular and genetic pathways, thereby contributing to the restitution of disrupted neural networks after stroke (165, 232). Initial clinical evidence from Phase I trials have indicated potential functional benefits, although the underpinning biology is still incompletely understood (175, 233). ‘Compensation’ has been defined as the ability to accomplish a task through using a new approach rather than using their normal pre-stroke behavioural repertoire (160). This does not
require neural repair but may require learning new ways of using intact neural or muscular pathways or the use of assistive devices to compensate for the function lost due to stroke. Rehabilitation strategies predominantly activate compensation mechanisms to effect recovery of function after stroke (139). However, they may also augment spontaneous recovery, especially when applied early in the disease course (234).

2.3 Current Evidence for Potential Use of Cell therapies in Ischaemic Stroke

The CTs currently under research are using cells derived from both neural and non-neural tissue sources that have been used in various ways (172):

- undifferentiated stem or progenitor cells, such as adult progenitor cells (mesenchymal stromal cells, multipotent adult progenitor cells and haematopoietic stem/progenitor cells)
- very early neural precursor cells differentiated from embryonic stem cells (ESCs) following in vitro expansion and post-mitotic differentiation or production of homogenous immortalised cell lines by reversible oncogene transfection; and
- induced pluripotent stem cells (iPSCs) following retrograde manipulation by transfection of relevant transcription factors (Yamanaka factors) (235).

Depending on the level of differentiation, stem cells may demonstrate unlimited or limited capacity to differentiate into different cell types (236). Pluripotent stem cells, namely ESCs or iPSCs, have a wide proliferation potential, which may in turn increase their tumourigenic potential. In comparison, multipotent adult stem cells, such as neural stem cells (NSCs), haematopoietic stem cells (HSCs), extraembryonic stem cells and mesenchymal stem cells (MSCs) derived from bone marrow, dental pulp, adipose tissue, skin, menstrual blood and foetal membrane (amnion and chorion layers), have a limited differentiation potential with no reported incidence of tumour
formation to date. All these cell types have demonstrated capability for terminal neural differentiation, using varied differentiation techniques (172).

There is evidence to suggest that the location of origin and the nature of processing may play an important role in determining the relative propensity of stem cells to differentiate into different functional cell types (237, 238). In the context of stroke, different stem cell types have been reported to be clinically relevant as described in Table 2.
### Table 2: Summary of stem cell types

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Prospective Use</th>
</tr>
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<tbody>
<tr>
<td>Embryonic Stem Cells</td>
<td>Pluripotent&lt;br&gt;High rate of proliferation&lt;br&gt;Angiogenic and neuroprotective effects (238, 239).</td>
<td>Ethical concerns (240).&lt;br&gt;Tumourigenic risk (241).</td>
<td>Subacute/&lt;br&gt;Intracerebral&lt;br&gt;Allogeneic</td>
</tr>
<tr>
<td>Haematopoietic Stem Cells (HSCs)</td>
<td>Evidence of endogenous mobilisation associated with functional recovery (242).</td>
<td>Limited cellular potency (243).&lt;br&gt;Heterogeneous cell population (243).&lt;br&gt;Difficult to isolate and proliferate in clinically relevant amounts (244).&lt;br&gt;Some pro-inflammatory/adverse effects reported (245).&lt;br&gt;Limited in vitro and in vivo studies.</td>
<td>Acute/subacute/&lt;br&gt;chronic&lt;br&gt;Intravenous/&lt;br&gt;Intra-arterial&lt;br&gt;Autologous/&lt;br&gt;Allogeneic</td>
</tr>
<tr>
<td>Mesenchymal Stem Cells (MSCs)</td>
<td>Potentially can be harvested from nearly any tissue (246).&lt;br&gt;Multipotent (247).&lt;br&gt;Can be conveniently induced toward neural phenotypes (248).&lt;br&gt;Functional benefit in <em>in vitro</em> models (250).</td>
<td>Further research needed to determine tumourigenic risk (255).&lt;br&gt;Tissue source can influence function (237).</td>
<td>Acute/subacute/&lt;br&gt;chronic&lt;br&gt;Intravenous/&lt;br&gt;Intra-arterial&lt;br&gt;Autologous/&lt;br&gt;Allogeneic</td>
</tr>
<tr>
<td>Cell Type</td>
<td>Advantages</td>
<td>Disadvantages</td>
<td>Prospective Use</td>
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<tr>
<td></td>
<td></td>
<td>Cell identities have not been adequately characterized (258). Stimulation of atherosclerosis in mice (259). Anti-neuroinflammation mechanisms not clearly defined (256). May promote neuroinflammation in certain circumstances (256).</td>
<td></td>
</tr>
<tr>
<td><strong>Endothelial Progenitor Cells (EPCs)</strong></td>
<td>Multipotent (256). Potential to exert acute neuroprotection and provide chronic infrastructural repair (256). Angiogenic potential and support post-stroke BBB repair.(257).</td>
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<tr>
<td>Cell Type</td>
<td>Advantages</td>
<td>Disadvantages</td>
<td>Prospective Use</td>
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<tr>
<td>Neural Stem Cells (NSCs)</td>
<td>Multipotent (264). Anti-inflammatory effect in animal models (265). Increase endogenous NSC proliferation; and promote angiogenesis and neurogenesis (266, 267).</td>
<td>Differentiation potential may be restricted in severely hypoxic environment (268).</td>
<td>Subacute/Chronic Intracerebral</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Allogeneic</td>
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**Table 2: Summary of stem cell types**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Prospective Use</th>
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<tbody>
<tr>
<td>Induced Pluripotent Stem Cells (iPSCs) (continued)</td>
<td>Improve functional recovery and reduce infarct volume in stroke models (275). Promote neurogenesis and angiogenesis (276). Anti-inflammatory properties in vivo (275).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT2N (hNT)</td>
<td>Multipotent (279). Improve function in animal stroke models (280).</td>
<td>Tumourigenic risk (281).</td>
<td>Chronic Intracerebral Allogeneic</td>
</tr>
<tr>
<td>CTX0E03</td>
<td>Potential to promote angiogenesis, endogenous neurogenesis, and functional recovery in models of ischaemic stroke (286, 287). Safety and positive results recorded in clinical trial (288).</td>
<td>Few studies <em>in vitro</em> or <em>in vivo</em> to render significant conclusions. No evidence as to anti-inflammatory or immunomodulatory effects. Efficacy may be mediated by region of transplantation in brain.</td>
<td>Chronic Intracerebral Allogeneic</td>
</tr>
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</table>

*Reproduced with permission from Stonesifer et al. (2017) (172)*
MSCs are the most extensively researched extraembryonic cell type to date. These cells can be harvested from any tissue, including bone marrow, placenta, teeth, amnion and adipose tissue and can be easily induced to produce neural phenotypes (248). The International Society for Cellular Therapy (ICT) defined the minimum criteria for MSCs in 2006 (289):

- adherence to tissue culture plastic
- multipotency as demonstrated by in vitro differentiation into osteoblasts, adipocytes and chondroblasts
- expression of surface markers CD73, CD90 and CD105; and negative for CD34, CD45, CD14 or CD11b, C79a or CD19 and HLA-DR.

This was an important milestone towards standardising the characterisation of different types of stem cells, but there is growing recognition of a need to update and expand these to keep pace with developments in the field.

Neural stem cells (NSCs) are the other major group of CTs with promising potential for clinical application (290). NSCs can be extracted directly from neural tissue, such as the neuroectoderm in developing foetuses and the subventricular or subgerminal zone in adults (291). NSCs can also be produced by neuroinduction of ESCs, by blocking transforming growth factor-beta/bone morphogenetic protein (TGF-β/BMP) signalling pathways (292). Recent protocols, about the production of NSCs from iPSCs via a neural differentiation protocol involving blockade of dual-inhibiting SMAD signalling pathways (292) and from adult somatic cells by using the reprograming factors such as Oct4, Sox2, Klf4, and c-Myc, are increasingly becoming the preferred techniques to produce stable and homogenous NSC populations (293).
A need to develop criteria for standardization of characterization of different cell types is widely discussed, as they are progressing to clinical translation (294). Samsonraj et al. (2017) in their review have reported on key parameters that are important for comprehensive and standardized characterization of MSCs but are equally relevant to characterization of other adult stem cells such as NSCs, from different sources (295). These parameters relate to: assessment of cell growth potential; survival; quiescence and/or senescence (i.e., viability and growth; ‘colony-forming units-fibroblastic’ (CFU-F) efficiency; telomere length); cell identity (i.e., multilineage differentiation and surface marker expression); and the ability of stem cells to communicate with their microenvironment (i.e., immunomodulation and trophic factors quantification) (295). Knowledge of immunomodulatory action is important for anticipating graft rejection and the need for concomitant immunosuppression (295). The development of release criteria that validate the quality of stem cells for use in clinical trials against GMP (Good Manufacturing Practice) standards, would need to take these parameters into consideration (296).

2.4 Potential Opportunity with Cell therapies in Stroke

The discovery of endogenous niches of neural stem cells in the adult mammalian brain tissue in the 1990s revolutionised existing knowledge about the regenerative capacity of the brain and heralded an era of research into neuroplasticity and neural repair (297). Since then, regenerative medicine research has yielded growing evidence that therapeutic application of exogenous CTs can promote recovery from loss of neural function resulting from a diverse range of neurological disorders (298).

CTs may provide a therapeutic option with a potentially longer window of clinical application than is currently available with acute stage therapeutic options (298). They represent a novel treatment
paradigm that can potentially address both subacute and chronic neuroinflammatory and neurodegenerative processes while simultaneously promoting neurogenesis, angiogenesis and synaptogenesis in neuronal tissues (298, 299). Age is a well-established biomarker that influences the incidence, functional recovery and mortality with stroke in humans (300). Therefore, validated models for stroke in aged rats are very clinically relevant (301). Studies with human NSCs injected into aged rat brains showed them to successfully integrate into brain parenchyma and this was associated with improved performance on hippocampus-based memory tasks (302).

Studies that followed the fate of human/primate/mouse derived ESC and iPSC-derived neuronal subtypes and glial cells in rat stroke models, reported that these cell types differentiated into mature, integrated neurons with axonal extensions to distant sites and organised synaptic activity as well as into specific glial subpopulations such as astrocytes, and oligodendrocytes (303-306). Animal studies have demonstrated that the transplanted stem cells stimulated neurogenesis in the ipsilateral SVZ and subgranular zone of the dentate gyrus (307, 308).

From a pathophysiological standpoint, it is established that stroke results in a pan-necrosis of all cellular elements of neuro-vascular architecture (309, 310). While specific neural cell replacement is unlikely to play a major role in the difference in outcomes seen with CT use in stroke, recent evidence points to a potential role for CT to reconstruct neuronal circuitry (303, 311). Secondary cell loss continues to occur in the areas functionally related to the lesion site, for weeks after stroke (172, 312). Implantation of human induced pluripotent stem cells (iPSCs) into acute stroke models demonstrated preservation of subcortical structures like substantia nigra, perhaps via release of pro-neurogenic growth factors (305, 313). The stem cells also release paracrine factors that support sprouting angiogenesis surrounding the infarct core, as evidenced by the increased density of newly formed blood vessels in the brains of animals treated with bone marrow derived MSC and NSCs.
Intracerebral injection of human MSCs were associated with modification of the cerebral microvasculature and improved cerebral blood flow (314).

Research on the relative importance of these mechanistic pathways in ischaemic stroke induced injury in humans is still inconclusive but preclinical studies have reported both functional and structural benefit of the use of CTs in ischaemic stroke. Leong et al. in 2013 reported an overall improvement of 40.6% (95% CI: 37.1 to 44.0, p<0.001) in neurobehavioral outcome in rodent stroke studies (315). Furthermore, the functional outcomes did not demonstrate time dependency. A meta-analysis by Vahidy et al. (2016) concluded that the use of bone marrow mononuclear cells in animal stroke models resulted in a reduction in lesion volume (SMD −3.3, 95% CI: −4.3 to −2.3), improved function measured by the cylinder test (SMD −2.4, 95% CI: −3.1 to −1.6) and a trend for benefit in adhesive removal test and neurological deficit score (232).

iPSCs and iPSCs-derived mature cell types are likely to be the predominant focus of clinical translation as they bypass the ethical issues associated with blastocyst-derived CTs, may be easier to generate as a homogenous cell population and may have fewer immune issues if extracted and reprogrammed from a patient's own tissue (316).

Stroke, unlike other neurodegenerative diseases, results in non-selective loss of all cellular elements in the infarct lesion. Hence, replacement by a specifically matched stem cell derived population is neither feasible nor appropriate (309, 317). Preclinical stroke research has indicated that potential biological effects of CT, that are not reliant on cell replacement, are likely to be the main mechanism underlying the benefit with CT (290). Systemic cell delivery, most commonly via the intravenous route and investigated predominantly in acute or early subacute phases, is likely to modulate the initial neural injury and neuroinflammation and reinforce early reparative tissue
responses (290). On the other hand, the intracerebral delivery, predominantly into undamaged tissue close to the site of injury, in late subacute or chronic stages, is likely to rely on the pleotropic effects of these implanted cells as the dominant mechanism of action (174). Many studies have indicated that the functional improvement in stroke models was observed within the first 1–2 weeks following transplantation, i.e., before any functional neurons could have developed from the grafted cells (290). Interestingly, numerous studies have reported that the behavioural improvement early after transplantation of iPSC-derived NESCs was observed irrespective of the degree of graft survival and generation of neurons at later time points (313, 318).

2.5 Challenges in Execution of Early Phase Clinical Studies with CTs in Ischaemic Stroke

CTs are currently at the stage of clinical translation across the world. However, numerous challenges exist that can significantly impede progress in this field (319). The interpretation of current knowledge in stem cell research is difficult due to challenges in efficiently designing clinical studies (319). The International Society for Stem Cell Research (ISSCR) task force released ‘Guidelines for Stem Cell Research and Clinical Translation’ in 2016, as an update to the ‘ISSCR Guidelines for the Conduct of Human Embryonic Stem Cell Research’ (ISSCR, 2006) and the ‘Guidelines on the Clinical Translation of Stem Cells’ (ISSCR, 2008), that had covered different aspects of the clinical translation of CTs (294).

These challenges include: the present state of knowledge regarding the heterogeneity inherent in disease presentation; the unique propositions posed by use of CTs; practical considerations with the administration of stem cells as a therapeutic product; and ethical questions related to the sourcing and use of CTs. Furthermore, ensuring equitable but scientifically justifiable access for patients if this development process proves successful is an important challenge. Increasing
numbers of phase I clinical studies are reporting reasonable safety and potential for efficacy for CTs.

Therefore, it is timely to examine the above challenges. This research can potentially lead to a framework that enlists critical considerations in further clinical research with CTs and help enable efficient, consistent and relevant research output that is needed to address the ‘valley of death’ or failed clinical translation efforts for a number of promising preclinical candidates (320).

### 2.5.1 Study Design Considerations in CTs Research

Clinical translation of therapeutic candidates is a long process (321). It usually starts from first-use testing, called first in human or phase I studies, in appropriate human subjects, conventionally healthy volunteers. However, in the context of CTs, this is more likely to be individuals suffering from the target disease (322). The focus of these studies is to confirm the safety profile seen in the preclinical stage and establish useful and safe doses in humans. If successful, the development moves to phase II, where the main objective is to generate proof of concept (postulated benefit) and confirm safety in a larger disease population. Phase III is a key milestone that aims to prove whether the candidate provides a meaningful incremental benefit versus existing therapies, maintains a favourable risk-benefit profile and helps to identify an appropriate patient population likely to benefit. If the phase II/III data are supportive, the therapeutic candidates enter clinical practice via regulatory approval. Thereafter, phase IV studies continue to expand the understanding of the therapy in real world clinical practice and identify any safety signals that may arise with such a use (321).

Kondziolka et al. (2000) reported on the use of stem cells in patients with stroke in the first phase I study (323). In the last two decades, numerous phase I studies in stroke have been reported from
different parts of the world (324). These studies have been varied in terms of the stroke population, CT type, timing and route of delivery and clinical outcomes evaluated (325). Despite two decades since the Kondziolka paper, it is only in the last two years that transition to phase II with a focus on generating data on effectiveness has reported preliminary success (285, 288). This is in part due to the unique biological and therapeutic perspective that stem cells present and the inherent heterogeneity of a disease like stroke. The challenge for researchers in this field has been to define research methodology that best addresses these issues and enables a scientifically robust but efficient development strategy for CTs in stroke (319).

Researchers from academia and industry convened to discuss the challenges CTs present to stroke research. The discussions resulted in the ‘Stroke Treatment Academic Industry Roundtable’ (STAIR) preclinical recommendations and ‘CT as an Emerging Paradigm in Ischaemic stroke’ (STEPS) recommendations on consensus-based standards for pre-clinical and clinical development of CTs in ischaemic stroke (326-328). These recommendations have been undergoing revisions as newer data has emerged regarding mechanistic pathways underlying stroke injury and neuroplasticity and in mechanisms of action with different cell types. These include the importance of defining mechanisms of action in appropriate animal models prior to proceeding to human studies, investigating the impact of co-morbidities in pre-clinical models and selecting the optimum route and timing of delivery. In addition, STEPS identified rehabilitation and neuroimaging as areas for further research to determine their impact on future clinical trial design (328). RIGOR guidelines in 2013, built upon STAIRS and STEPS, recommended considering available preclinical evidence along with data for different routes and timing of administration studied in animal models whilst defining the various aspects of a clinical trial design (326).
Bioluminescence imaging (BLI) and immunohistochemistry techniques in numerous animal studies with different CTs have established that the transplanted cells rarely survive beyond 7–30 days post-implantation although the percentage of viable cells reported has varied depending on cell types and assessment techniques used (329, 330). Recent studies have reported success with using different techniques for enhancing survival of transplanted cells such as genetic modification with pro-survival genes, e.g., Bcl-2, hypoxic preconditioning and pharmacological preconditioning (Diazoxide/Minocycline/SDF-1/interferons) (165, 331). However, despite the short life of engrafted cells, they initiate molecular pathways that facilitate endogenous neurogenesis by upregulation of growth factor genes, promotion of angiogenesis, mobilisation of endogenous neural stem cells and modulation of neuroinflammation (165). There is robust evidence to support the fact that the potential biological effect of CTs may not depend on the efficiency of cell replacement (290, 332). It may be more critically determined by the cell type (lineage, proliferation potential and spatial conformity) and by the route and timing of cell delivery (290).

Therefore, while structural, immunological and biological characterisation of different cell types remains critical to optimize cell delivery and targeted biological effect, selective transplantation of specific cell phenotypes may not be as important to research success. In contrast, aspects of study design such as the choice of study outcomes, timing and routes of CT administration, and temporal relationship to rehabilitation, may have a more significant impact on the likelihood of these studies demonstrating effective ways to use CTs (309).

2.5.1.1 Selection of Outcomes Measures: Impact on Study Design Efficiency

Individuals suffering from stroke experience a unique combination of long-term disability due to the heterogeneous nature of stroke as a disease (54). Disability following stroke may present as
neurological dysfunctions (e.g. motor, sensory and visual) and limitations in performance of activities of daily living (ADL) and neuropsychological deficits (e.g. attention, memory or language). Furthermore, an under-represented aspect of stroke is the co-morbid occurrence of neuropsychiatric disturbances such as depression (54).

2.5.1.1.1 Framework for Disability Outcomes

The World Health Organization’s International Classification of Functioning, Disability and Health (WHO-ICF) released in 2001 provides a biopsychosocial holistic model to standardize the assessment of functioning of individuals and populations, and provide consistency across clinical disciplines, in the understanding and use of the term disability (58). Disability was defined as “the negative aspects of the interaction between an individual (with a health condition) and that individual’s contextual factors (environmental and personal factors)” and comprised three principal domains: impairment (structural and functional), activity limitation and participation restriction (58). Quality of life is the personalised evaluation of this disability or lack of functioning in the context of emotional satisfaction (333).
Adapted from Stucki G. Am J Phys Med Rehabilitation 2005; 84:733–740 (333)

To facilitate the use of this framework in clinical settings, the WHO developed the ICF Core Sets to contextualise disability to specific health conditions (334). These sets comprise aspects of impairment/limitation/restriction that are encountered, most frequently in typical patients suffering from that disease and describe a measurable outcome in clinical research or practice settings. ICF Core Sets (comprehensive and brief sets) for stroke enlist the most impactful aspects of functioning affected in patients after stroke (334). The brief ICF Core set for stroke includes 18 categories across the ICF components of functioning and contextual factors, based on consensus amongst a
multidisciplinary expert panel to create a list that was relevant but feasible for use in clinical settings (Table 3).

**Table 3: Brief ICF Core Set for stroke**

<table>
<thead>
<tr>
<th>ICF component</th>
<th>ICF category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body functions</td>
<td>Consciousness functions</td>
</tr>
<tr>
<td></td>
<td>Orientation functions</td>
</tr>
<tr>
<td></td>
<td>Muscle power functions</td>
</tr>
<tr>
<td></td>
<td>Mental functions of language</td>
</tr>
<tr>
<td></td>
<td>Attention functions</td>
</tr>
<tr>
<td></td>
<td>Memory functions</td>
</tr>
<tr>
<td>Body structure</td>
<td>Structure of brain</td>
</tr>
<tr>
<td></td>
<td>Structure of upper extremity</td>
</tr>
<tr>
<td>Activities and participation</td>
<td>Walking</td>
</tr>
<tr>
<td></td>
<td>Speaking</td>
</tr>
<tr>
<td></td>
<td>Toileting</td>
</tr>
<tr>
<td></td>
<td>Eating</td>
</tr>
<tr>
<td></td>
<td>Washing oneself</td>
</tr>
<tr>
<td></td>
<td>Dressing</td>
</tr>
<tr>
<td></td>
<td>Communicating- speaking and receiving messages</td>
</tr>
<tr>
<td>Environmental factors</td>
<td>Immediate family</td>
</tr>
<tr>
<td></td>
<td>Health professionals</td>
</tr>
<tr>
<td></td>
<td>Health services, systems and policies</td>
</tr>
</tbody>
</table>

*Reproduced with permission from Geyh et al. (334)*

This core set has been validated in studies that followed up the health status of ischaemic stroke survivors to test the feasibility of its use in clinical settings (335, 336). These studies reported that the most significant determinants of overall health status related to memory functions, muscle
power functions and attention functions (337). The issues with walking, speaking and receiving spoken messages (understanding) were consistently reported to be the dominant factors responsible for limitations of activity and participation (337).

2.5.1.1.2 Outcome Measures in Stroke Research

Numerous outcome measures have been used in clinical research to determine the extent of disability after stroke. These scales have been validated for use during different timeframes post-stroke to monitor the clinical impact of pharmacological and rehabilitative interventions in ischaemic stroke (338). This wide range of measures presents a challenge in standardization of outcome measures to be used in clinical studies, as recommended by guidelines such as STAIR, RIGOR and STEPS (328).

The choice of assessments employed to measure the success of CTs is critical as more clinical studies are being planned (338). The appropriateness of the outcome measures to the study population is vital. This can ensure optimal evidence generation that can translate into effective clinical strategies.

‘Hard’ clinical endpoints such as stroke mortality or recurrence have historically been important determinants of success of interventional trials in stroke. However, with mortality rates steadily declining, increasing number of studies are choosing the magnitude of change in clinically relevant disability outcomes as the primary endpoint for measuring effectiveness of therapy (339). This growing trend is recognised by regulatory authorities, who now recommend a measure of functional recovery as primary or co-primary endpoints for ischaemic stroke intervention trials (340). Different assessment tools have been used in studies investigating the impact of various ischaemic stroke interventions (341). However, there is no single outcome measure that describes
or predicts all dimensions of disability. A review of 491 randomized controlled trials of ischaemic stroke rehabilitation reported significant heterogeneity in outcome assessment (342).

Table 4: ICF disability domain measured in Stroke Outcome Measure Scales

<table>
<thead>
<tr>
<th>Body structure/function (impairments)</th>
<th>Activity (limitation)</th>
<th>Participation (restriction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glasgow Coma Scale</td>
<td>Barthel Index</td>
<td>London Handicap scale</td>
</tr>
<tr>
<td>Mini-Mental State Examination</td>
<td>Nottingham ADL Scale</td>
<td>Medical Outcomes Study</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>Glasgow Outcome Scale</td>
<td>Nottingham Health Profile</td>
</tr>
<tr>
<td>Hospital Anxiety and Depression Scale</td>
<td>Katz ADL Scale</td>
<td></td>
</tr>
<tr>
<td>National Institute of Health Stroke Scale (NIHSS)</td>
<td>International Stroke Trial simple questions</td>
<td>Stroke adapted sickness impact profile</td>
</tr>
<tr>
<td>Fugl Meyer Assessment</td>
<td>Modified Rankin Scale</td>
<td>Stroke Impact Scale</td>
</tr>
<tr>
<td>Orgogozo Stroke Scale</td>
<td>Oxford Handicap Scale</td>
<td>Stroke specific quality of life</td>
</tr>
<tr>
<td>Canadian Neurological Scale</td>
<td>Hamrin Activities Index</td>
<td>Frenchay Activities Index</td>
</tr>
<tr>
<td>Scandinavian Stroke Scale</td>
<td>Adams Disability Method</td>
<td></td>
</tr>
<tr>
<td>Toronto Stroke Scale</td>
<td>10-meter walk test</td>
<td></td>
</tr>
<tr>
<td>(Modified) Mathew Scale</td>
<td>Timed get-up-and-go test</td>
<td></td>
</tr>
<tr>
<td>European Stroke Scale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frenchay Aphasia Screening Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke Specific Scale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified Ashworth Test</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reproduced with permission from McArthur et al. (2014) (338)
An understanding of the clinimetric properties of a scale is vital to appropriate selection of an assessment tool that would be most suited to the demands of the study population and stroke characteristics being studied (343). Use of an inappropriate tool can adversely affect the study quality and may invalidate results. Adequate validity and responsiveness to detect clinically meaningful change within the time-frame of the trial have been reported to be essential though often unacknowledged key pre-requisites to ensure quality and efficiency of a trial (343).

The most widely accepted outcome measures scales such as the modified Rankin Scale (mRS), Barthel Index (BI), National Institute of Health Stroke Scale (NIHSS), Stroke Impact Scale (SIS) and Functional Independence Measure (FIM) capture important dimensions of ischaemic stroke-related disability but these scales have important limitations that are relevant to ischaemic stroke (343, 344). The principal measures scales are characterised/examined below for their strengths and weaknesses.

2.5.1.1.2.1 Modified Rankin Scale

The mRS describes ‘global disability’ and measures aspects across WHO-ICF domains of activity and societal participation (345). As a stroke outcome measure, it has key strengths that make it the preferred and most commonly used endpoint in stroke research as well as for regulatory assessment (342). The mRS is an ordinal hierarchical scale with grades from 0 (no symptoms) to 5 (severe disability) with an extra score of 6 to signify death, added in research settings. In contemporary ischaemic stroke studies, the mRS is often used both as a measure of baseline ability for patient selection and as an outcome measure of interest (345). The mRS has many potential strengths in that it demonstrates strong correlation with measures of ischaemic stroke pathology (for example, infarct volumes) and it has the ability to describe an almost full range of ischaemic stroke outcomes even though it is not designed to assess an individual’s psychosocial condition (338). The mRS
data collected at 90 days has been reported to be a robust indicator of long-term functional prognosis. This long-term predictive validity of the mRS supports its preferred use in RCTs (338). However, it may be less responsive to change due to a short range of available scores, which makes it poorly suited for measuring change over short time-periods, often seen in early phase trials (343). A systematic review of published reliability studies looking at different methods of administering the mRS assessment, found wide estimates of inter-observer variability (range for \(k = 0.25\) to 0.72) despite overall good agreement on pooled analysis (\(k = 0.61\)) (345, 346,347). Using various real-world datasets, power and sample sizes estimates have been generated from simulated trials modeled with varying levels of mRS reliability (348). These simulations suggest that improvements in mRS reliability (e.g., from \(k = 0.25\) to 0.50 or 0.70) can result in modest reductions in sample size required and consequent cost savings (348). Training of research teams on use of the scale, use of a structured format for mRS assessments and establishing a process of centralized evaluation, have been different ways that have been shown to be useful in improving inter rater reliability for mRS (348). Another key limitation of the scale is that it is heavily weighted towards physical functioning with no focus on important outcomes areas such as cognition, communication, language disorders, fatigue, pain or mood disorders (338).

2.5.1.1.2.2 National Institute of Health Stroke Scale

The NIHSS is a widely used scale that comprises 15 items used to assess severity of impairment of different neural systems (338). Items are graded on a 3- or 4-point ordinal scale on which 0 represents no impairment and add up to provide a total score (range= 0 to 42) with higher scores reflecting greater severity. Being relatively easy to execute in the acute stroke environment makes it a preferred outcome measure in both clinical practice and in research settings in acute ischaemic stroke trials (343). The tool is very useful for early stroke severity assessment and baseline scores
have strong predictive validity for outcome at 7 days and 3 months and has excellent inter-rater reliability in research settings (349). While a very useful tool to guide clinical management decisions in the short term, NIHSS has accepted limitations. The literature is divided regarding the robustness of the scale’s validity beyond the acute stage of ischaemic stroke, especially in the context of chronic functional recovery and ‘real world’ functional impact (343, 350). A ceiling effect is widely acknowledged in its administration, with lots of elements being untestable in high severity cases (338). The scale items favour left hemisphere events and posterior cerebral circulation events are poorly represented in the overall score (351, 352). Overall, the value of NIHSS as a long-term functional recovery index may be sub-optimal (338, 350).

2.5.1.1.2.3 Barthel Index

The Barthel Index, initially developed to be an index of dependence to evaluate nursing requirements, is perhaps the most widely used outcome measure of activities of daily living (338). Though not specific to stroke, its appeal lies in the ease of application, robust reliability (low inter-observer variability) and widespread use (338). Hsieh et al. (2007) reported that a mean BI change score of 1.85 corresponded to patient ratings of minimal clinically important difference (MCID), an estimate key to determining the adequate power of a study (353). However, BI demonstrates important limitations such as its relative insensitivity and lack of comprehensiveness (absence of stroke-specific domains such as communication/cognition) (354). This is reflected in the large reported ceiling and floor effects observed with BI, making it sub-optimal for use in patients at either end of the disability spectrum (338, 345).

2.5.1.1.2.4 Quality of Life Scales

Euro-Qol assesses the impact on quality of life and has been validated in stroke populations (355). However, only 61% of ischaemic stroke survivors in a study are able to complete the scale without
external assistance (355). The ischaemic stroke-specific QOL scale, validated in ischaemic stroke populations, incorporates twelve domains. It defines values for ‘minimal detectable change’ and ‘clinically important difference’ but similarly suffers from non-completion bias (356).

2.5.1.1.2.5 Stroke Impact Scale

Duncan et al. (2001) developed the Stroke Impact Scale (SIS) as a patient-reported outcome measure to assess multiple domains of stroke recovery without administering multiple tests (357). Though relatively new, its application may lower workload for the patient and increase utility for researchers (358). It is a stroke-specific, comprehensive, health status measure that incorporates domains from across the full WHO-ICF continuum (359). The limitations currently arise from the fact that limited normative data is available to date. In addition, the scale reports sub-optimally on the memory, communication, emotion and social participation domains (359).

2.5.1.1.2.6 Functional Independence Measure

Functional Independence Measure (FIM) is a measure that defines the burden of caring for an individual with stroke by assessing physical and cognitive disability (354). It is a less lengthy patient-reported measure with the ability to yield more detailed individualised information, which makes it an attractive tool in the clinical setting (354). Studies reported that a change in FIM score of 22 (total FIM), 17 (motor FIM) and 3 (cognitive FIM) could represent the MCID in a stroke population (354). These estimations are extremely useful to enhance the interpretability of changes seen in therapeutic intervention studies. However, FIM has its limitations in that the reliability of the scale is significantly impacted by rater training, which may result in high inter-rater variability (354). It is an ordinal scale with step changes for the component items and sensitive to the statistical analysis methodology used. Though it does not have a ceiling effect, the responsiveness to change may not be significantly better than the BI (354).
2.5.1.2 Statistical Methodology

Inappropriate choice of statistical methodology may have been a possible factor contributing to the disappointing results seen in many past stroke trials (338). The choice of analysis depends on the outcome measure of interest, as a number of these are ordinal in nature (360). Different approaches used so far comprise dichotomized analysis, selection of a composite global statistic that simultaneously analyses multiple outcome measures, responder analysis, and shift analysis.

2.5.1.2.1 Dichotomized Analysis

Dichotomized analysis uses logistic regression to dichotomize an outcome as a success or failure defined by a chosen cut-off point, e.g., mRS 0-2 versus 3-6 (345). This analysis is easy to perform and interpret in terms of clinical meaningfulness, e.g. via calculation of numbers needed to treat for a certain outcome (345). However, it results in the loss of clinically relevant information, as a step change in scales such as mRS or NIHSS represents a significant change in terms of different aspects of disability. In addition, transition across different health states holds variable relevance to individual patients and an arbitrary choice of a single transition may not be useful in assessment of benefit. Furthermore, the selection of cut-off point for this dichotomization in past studies has been inconsistent, making cross-study comparisons infeasible (348).

2.5.1.2.2 Responder Analysis

Responder analysis is a variant of dichotomized analysis that allows definition of success to vary according to baseline prognostic variables that are chosen \textit{a priori} as compared to a fixed one in simple dichotomized analysis (361).
2.5.1.2.3 **Shift Analysis**

Most recent trials have used shift analysis: an ordinal approach utilizing proportional odds logistic regression and the Cochran Mantel Haenszel methods to analyze change in outcome distributions over the full range of selected outcomes (362). This analysis examines benefits and harm experienced across all disease state transitions. Unlike the responder analysis, it does not make any prior assumptions of relationship between likely change in health state and baseline characteristics of study participants (345). The main disadvantage with shift analysis lies in its computational complexity. Although modern statistical software may provide a solution, the appropriate application in context of a specific clinical trial design requires the research team to have access to enhanced statistical expertise (363). It may also be a challenge to perform this analysis across the full range of scales with a high number of health state levels, e.g., NIHSS, although it has been used successfully with scales that use fewer levels, e.g. mRS (348). It has been reported that trials using ordinal logistic regression required smaller sample sizes (typically 28% smaller) than the dichotomous approach (348). This aspect can contribute significantly to numerous aspects of research: the cost of the trial, recruitment success, operational efficiency and robustness of data.

2.5.1.2.4 **Composite Global Endpoints**

Another commonly used approach is the use of composite endpoints that combine different scales (345). The NINDS trial reported the clinical effect of rtPA as a change in a composite score comprising NIHSS, BI, mRS, and Glasgow Outcome Scale (364). Makin et al. (2017) reported that use of a composite outcome comprising vascular, dependency and cognitive endpoints was associated with improved power in a trial in acute minor stroke and acute ischaemic stroke (365). However, these composite endpoints may be difficult to interpret and incompletely reflective of
the specific changes in the component outcome measures and prone to exacerbation of error if the component measures are correlated (345).

2.5.1.3 Trial Design

The inherent heterogeneity in neurological functions impacted by stroke has represented a challenge to recruit a homogenous patient population in the large numbers needed to ensure sufficient power (366). This is compounded further by the fact the rate and degree of spontaneous recovery expected differs between individuals based on the initial severity, initial neuronal reserve (influenced by factors such as co-morbid medical conditions and genetic factors) and timing and appropriateness of other established stroke interventions (226, 366). It is therefore plausible to consider that the overall stroke population is a composite of smaller subgroups defined by the shared characteristics such as the dominant functional system impaired or predicted trajectory of recovery. STEPS III recommended that development of stem cell therapies would benefit from the use of domain-specific end points in research, i.e., analysis of change in relevant domains of functioning would likely result in more clinically relevant data (328). However, the use of domain specific endpoints can provide useful information only if the populations being compared are sufficiently homogenous with regards to the domain of interest (367).

One of the strategies suggested to achieve the above objectives is to adopt a cluster randomized study design in preference to a conventional individual randomized study design (348). However, the use of cluster randomization may result in loss of rigour if appropriate analytical methods for power calculation and sample size calculations are not used. This is due to the fact that individuals in the same cluster tend to be correlated in terms of their characteristics and this reduces the effective sample size (348). Therefore, cluster trials’ size is inflated by a factor called the ‘design
effect’, which depends on the average cluster size and the degree of correlation within clusters (intracluster correlation coefficient or ICC) (348). ICCs are calculated as the inter-cluster variance divided by the sum of the intracluster and inter-cluster variance. ICCs provide an estimate of the proportion of variance that can be attributed to the cluster level (348). Knowledge and use of accurate estimates of ICCs for specific outcome measures is key to scientifically appropriate sample size calculations in cluster-randomised trials. ICCs for common outcome measures selected in stroke studies such as NIHSS, mRS and BI have been reported in previous studies (368). These estimates of relevant ICCs could be helpful in the planning of stem cell studies. The need for calculation of ICCs for other domain specific endpoints is acknowledged as an important unmet need (348).

2.5.1.4 Concomitant Rehabilitation with CT Delivery

Research supports the assessment, mobilisation and initiation of targeted rehabilitation early after stroke (after the first 24 hours) and as a standard of care in the chronic phase (139). There is an acknowledged need to understand the impact of rehabilitation as a variable in CT studies (328).

As the understanding of mechanistic pathways involved in both these treatment strategies has evolved, there is now biological evidence to support their concurrent use (369). The premise is that stem cells enhance brain plasticity and create a facilitative brain environment that responds more effectively to rehabilitative techniques to rebuild the dysfunctional neuronal networks leading to the recovery of function (370). In turn, targeted activity maintains the neuro-reparative pathways initiated in a post-stroke environment that are augmented by the presence of implanted stem cells (370). Imura et al. (2013) reported that NSC/NPCs transplantation in mice followed by treadmill exercise training was associated with significant functional motor and electrophysiological
improvement, increased differentiation of transplanted cells into neurons and astrocytes at the brain injury site as compared to mice that underwent transplantation alone. Furthermore, the expression of brain-derived neurotrophic factor and growth-associated protein 43 mRNAs were significantly upregulated early in the mice that underwent transplantation and the treadmill exercise compared to those in other experimental groups (371).

However, the provision of rehabilitation along with CTs introduces two challenges to designing stem cell studies. Firstly, current delivery of rehabilitation is inconsistent and while research in this field has expanded, there is an insufficient evidence base to standardize therapy for specific patient groups. Secondly, the combined delivery of stem cells and rehabilitation introduces complexity in the study design in terms of defining patient numbers that ensure sufficient power of the study analysis (348).

2.5.1.5 Summary

The numbers of early phase clinical trials using stem cell therapy for ischaemic stroke are on the rise. It is, therefore, imperative that we investigate the impact of study design components such as trial methodology, choice of outcomes measures used, the timing and route of delivery of stem cells and the impact of rehabilitation as a confounder in these studies. This will enable more appropriate and more efficient study designs for future clinical studies using cell therapies.

2.5.2 Regulatory Policy Considerations in CTs Research

Existing regulatory and policy frameworks for the review of therapeutic products across the world were based on examination of evidence generated through conventional development pathways, i.e. for small molecule pharmaceutical drugs (339). However, there are key differences relevant to the context of the development of cell therapies, which are amongst the most sophisticated
biological therapies developed to date. Most regulatory agencies are in the process of developing regulatory guidance to keep pace with the clinical translation of CT (340).

2.5.2.1 **Harmonisation of Legislative Terminology**

In 2015, the Federal Drug Authority (FDA) in United States of America issued guidance on the ‘Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products’ (322). The guidance reflects the acknowledgement on the part of the FDA that the design of early phase clinical trials of CT may often differ from the trial designs applied to conventional pharmaceutical products, due to either the distinctive features of these products or their clinical application. These differences include structural, functional and biological characteristics of the cell product, and manufacturing considerations that may be unique to these products, all of which can influence key elements of clinical trial designs. In terms of cell characteristics, the dynamic nature of living cells and their ability to respond to the microenvironment over time by changing their molecular and functional expression makes it challenging to attribute any signals regarding effectiveness or safety to a specific cell morphology. In addition, these cells retain an ability to migrate, which requires that methods to track bio-distribution profiles be included in trial designs to increase the likelihood of detecting ectopic effects. Inherent complexities of manufacturing CTs would likely affect the feasibility of product quantity (volume and concentration) available for clinical dosing as well as the timing of administration.

The FDA also recognised that conventional pharmacokinetic study designs are often not feasible in the context of cell products and that factors such as species specificity and immunogenicity make extrapolation from animal studies using conventional allometric scaling less reliable (372).
The above advice was recommended to be examined in conjunction with other guidance documents, particularly the ‘Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue Based Products: Minimal Manipulation and Homologous Use which was released by the FDA in November 2017 after multiple iterative changes to keep pace with the growing research in this area (373). It enables developers to distinguish between therapies that would require a substantial oversight as they are considered drug products under section 351 of the PHS Act in the US or that may be exempt per section 361 of the PHS Act. It defines the terminologies such as ‘homologous use’ and ‘minimal manipulation’ more elaborately to enable widespread understanding amongst the research and development community. In 2017, the FDA released a comprehensive policy framework for the development and oversight of regenerative medicine products, including novel CTs. The framework comprises four guidance documents (374):

- Same Surgical Procedure Exception under 21 CFR 1271.15: clarifies when the CTs are exempted from established regulations (375);
- Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue Based Products: Minimal Manipulation and Homologous Use: clarifies the existing terminology and defines actions against unsafe products (350);
- Evaluation of Devices Used with Regenerative Medicine Advanced Therapies (RMAT) (352);
- Expedited Programs for Regenerative Medicine Therapies for Serious Conditions (376).

The FDA will consider a drug eligible for regenerative medicine advanced therapy (RMAT) designation (377) if:
1. It is a cell therapy, therapeutic tissue engineering product, human cell and tissue product, or any combination product using such therapies or products, except for those regulated solely under Section 361 of the Public Health Service Act and part 1271 of Title 21, Code of Federal Regulations;

2. It is intended to treat, modify, reverse or cure a serious or life-threatening disease or condition; and

3. Preliminary clinical evidence indicates that the drug has the potential to address unmet medical needs for such diseases or conditions.

The adoption of this framework is likely to foster harmonisation with other regulatory bodies across the world and provide impetus to the faster translation of CTs in diseases with present unmet needs.

The regulatory framework for the evaluation of CTs in the European Union (EU), defined in 2008, is the ‘Regulation on Advanced Therapies (Regulation No 1394/2007)’ (378). This framework was expanded to provide further clarity by the release of the ‘Reflection paper on classification of advanced therapy medicinal products’ in 2015 (379). Regulatory bodies across the world have followed suit by establishing initial frameworks for research, development and clinical application of these innovative therapies in their respective countries. In Australia, the Therapeutic Goods Administration (TGA) regulates cell products, including blood products, vaccines and haematopoietic stem cells used in allogeneic transplantation (380). Autologous use of CT, defined as a single course of treatment with self-donated cells under the supervision of a medical practitioner who has the management of care responsibility, are not deemed to be therapeutic goods and are therefore exempted from TGA regulation. The autologous use of CT is currently regulated under clinical practice norms by the Medical Board of Australia and the Australian Health Practitioner Regulation Agency (AHPRA) in accordance with the Health Practitioner Regulation
National Law. In 2015, the TGA released a discussion paper providing its perspective on possible options for regulation of CTs which ranged from a continuation of the current pathway to a complete oversight by TGA as implemented in the context of other biological drugs (381). Following continued consultation, the TGA announced key changes to the existing legislation: the Biologicals Regulatory Framework, which took effect from 1st July 2018 (380). The framework provides specific guidance to define situations regarding the use of autologous CTs that need more rigorous oversight mechanisms from those that may not. This change will also bring the Australian process into better alignment with similar processes in the United States and the European Union.
### Table 5. Key changes in Regulatory Pathways for CTs in Australia

<table>
<thead>
<tr>
<th>Components of the New Biologicals Regulatory Framework</th>
<th>Details</th>
</tr>
</thead>
</table>
| Key Change                                            | 1. Regulation of autologous human cell and tissue products could be regulated either under the Biologicals framework or as blood and blood components.  
2. Biologicals classification and terminology definitions updated. |
| Autologous human cell and tissue products definition   | Autologous human cell and tissue (HCT) products are those that are removed from, and applied to, the same person, i.e. the donor and the recipient are the same.  
- These include some products commonly referred to as 'stem cell treatments'.  
- Where an autologous HCT product meets the definition of a blood component it may be regulated as a blood and blood component rather than under the Biologicals framework. |
| Advertising to consumers is prohibited                 | Autologous HCT products cannot be advertised to consumers from 1 July 2018, though services (that do not mention specific products) will still be permitted to be advertised |
| Regulatory pathways for supply of autologous human cell and tissue products | Risk-based regulation implemented |
| Explanation of key terms                              | 1. Autologous HCT products used in hospitals - excluded from regulation by the TGA, that meet all the following criteria:  
- collected from a patient who is under the clinical care of a medical or dental practitioner registered under a law of a State or an internal Territory |
Table 5. Key changes in Regulatory Pathways for CTs in Australia  (continued)

<table>
<thead>
<tr>
<th>Components of the New Biologicals Regulatory Framework</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explanation of key terms (continued)</td>
<td>• manufactured by that medical or dental practitioner, or by a person or persons under the professional supervision of that medical or dental practitioner in a hospital, for that patient who must be a patient of that hospital</td>
</tr>
<tr>
<td></td>
<td>• for therapeutic use in that patient by the same medical or dental practitioner, or by a person or persons under the professional supervision of the same medical or dental practitioner</td>
</tr>
<tr>
<td></td>
<td>• not advertised or promoted directly to consumers.</td>
</tr>
<tr>
<td></td>
<td>2. Autologous HCT products for use outside hospitals regulated with some exemptions such as: using 'unapproved' product pathways; inclusion on the Australian Register for Therapeutic Goods (ARTG); holding evidence that the manufacturing facility satisfies good manufacturing practice (GMP) requirements, if they meet all the following criteria:</td>
</tr>
<tr>
<td></td>
<td>• collected from a patient who is under the clinical care of a medical or dental practitioner registered under a law of a State or an internal Territory.</td>
</tr>
<tr>
<td></td>
<td>• manufactured by that practitioner, or by a person or persons under the professional supervision of that practitioner, for a single indication and in a single procedure on that patient by the same practitioner, or by a person or persons under the professional supervision of the same practitioner.</td>
</tr>
<tr>
<td></td>
<td>• for therapeutic application in a homologous use.</td>
</tr>
<tr>
<td></td>
<td>• minimally manipulated.</td>
</tr>
<tr>
<td></td>
<td>3. Autologous HCT products will be fully regulated as biologicals or blood components if they do not satisfy any of the criteria for exclusion/exemption specified above.</td>
</tr>
</tbody>
</table>
Table 5. Key changes in Regulatory Pathways for CTs in Australia (continued)

<table>
<thead>
<tr>
<th>Components of the New Biologicals Regulatory Framework</th>
<th>Details</th>
</tr>
</thead>
</table>
| New definitions related to classification             | 1. Classification of biologicals - updated:  
  - Class 2 biological: has been subject to a process that is minimal manipulation and is for homologous use  
  - Class 3 biological: has been subject to a process that is more than minimal manipulation and/or is not for homologous use  
  - Class 4 biological: is mentioned in Schedule 16 as a Class 4 biological that includes viable products that contain viable tissue of animal and/or human origin that are considered to pose a high risk, due to the level of manipulation and/or the current lack of safety data to appropriately classify them.  
  2. Minimal manipulation – new definition to link processing and intended use: if the process does not result in the alteration of any of the biological characteristics, physiological functions or structural properties that are relevant to the intended use of the cells or tissues.  
  3. Homologous use – new definition and recommendation that distinguishing between homologous and non-homologous use also applies to autologous use: the repair, reconstruction, replacement or supplementation of a recipient's cells or tissues with cells or tissue that perform the same basic function or functions in the recipient as the donor. |

*Adapted from the revised TGA Biologicals regulatory framework effective 1st July 2018 (380)*

Regulatory bodies and researchers are aligned in the view that consideration of the level of product manipulation and the intended use (autologous or allogeneic) should determine the level of
regulatory oversight required. CTs research has been led by academic groups until the very recent past. As different candidate cell types are moving along the translational spectrum, the knowledge and fulfilment of quality standards for data and cell biology have become critical determinants of the successful execution of stem cell studies.

2.5.2.2 Application of Standards for Good Manufacturing Practice

As research moves from bench to clinical translation, it is critical to understand that standards for manufacturing clinical biological agents are stricter than the standards for research-grade cell lines (382). Since the predominant types of CTs in the clinic are likely to be ones that have undergone prior differentiation to more committed cell lineage and typically more than ‘minimal manipulation’, these are likely to be subject to comprehensive review and full regulation like other biological products. In 2016 the ISSCR recommended that in view of the huge variation in cell types and tissue sources, individualized processing and manufacturing approaches should be used (294). The comprehensiveness of review of these cell processing and manufacturing protocols should be proportionate to the risk induced by the level of manipulation of the cells and their intended use, as well as the nature and size of the clinical trial. In line with broad GMP principles, standard operating procedures to ensure the quality of the reagents and consistency of protocols need to be established early in research (294). This is particularly important because the techniques for cell characterisation and standardisation of release criteria that are acceptable to regulators are still being developed for several cell types (294). In addition, given the innate risk with pluripotent stem cells (hESCs as well as iPSCs) for potential tumourigenicity, the release criteria need to ensure minimisation of risk from cell-culture-acquired abnormalities, such as karyotypic instabilities, as well as relevant global genetic and epigenetic parameters (294). ISSCR guidelines as well as
regulatory guidelines across the world have recommended adherence to GMP requirements from early stages of research as well as in innovative clinical use situations (294, 374, 380, 383).

2.5.2.3 Expedited Pathways for Approval and Concern for Unregulated Use

The regulatory bodies across the world recognize the need to define clear risk-based pathways that ensure patient safety in instances where CTs may have preliminary evidence of benefit in disease management where currently there is no other option available. The FDA has released draft guidelines on expedited pathways available for CTs: Priority Review and Accelerated Approval (374). A ‘Priority Review’ designation enables a shorter review cycle by the FDA. ‘Accelerated Approval’ allows for expedited review and approval based on data related to scientifically valid surrogate endpoints instead of established clinical endpoints. Meanwhile, the passage of ‘right to try’ (RTT) in USA, which can potentially be pursued for individual ‘gravely ill’ patients for access to cell therapy products ahead of FDA approval has raised concerns that some stem cell clinics are already willing to provide inadequately tested cell therapies utilizing this current gap in legislation (384). Similarly, the European Medicines Agency (EMA) launched the ‘PRIority MEdicines’ (PRIME) scheme in 2016, to enable early proactive regulatory dialogue regarding development plans for therapies of ‘major public health interest’ and which represent significant innovation (385). The European Commission introduced the ‘hospital exemption clause’ by the Regulation (EC) No.1394/2007 for Advanced Therapy Medicinal Products (ATMPs). This clause is applicable to those ATMPs that are prepared on a non-routine basis, i.e. individually prescribed, according to specific quality standards, for an individual patient (386).

Autologous delivery of CTs has been considered ‘innovative medical practice’, and this has led to multiple ‘stem cell clinics’ offering untested/unapproved CTs in routine practice (387). While
agencies like the FDA have worked to strengthen oversight and regulation mechanisms, the ISSCR issued a statement highlighting the risk to patient safety that these clinics represent. Increasing collaboration between researchers and regulators is likely to lead to more streamlined and effective mechanisms to ensure appropriate access while safeguarding patient well-being (294).

2.5.2.4 Summary

Early phase CTs research has been led by academic groups. However, the awareness of these regulations and ability to address them through appropriate design and operationalisation of these studies is critical. It is acknowledged that this is an important skill gap within the research community and capacity building in this area is vital to future translational success. It is therefore important to review existing pathways to identify similarities and differences across different countries and identify key areas that are critical to increasing the efficiency and scientific quality of early phase clinical studies with CTs in ischaemic stroke.

2.5.3 Ethical Considerations in CTs Research

Stem cell research and its potential application in different disease indications has been a subject of ongoing social debate (388). Unique aspects have emerged due to the novelty of the science, the incomplete understanding of neural regenerative processes, challenges in the context of existing theological and philosophical constructs, and the interplay of all of these factors within a changing political climate in which this research has been happening for the past four decades (389). One of the major ethical debates was the use of embryonic tissue as a source of stem cells for research, with concerns that this would amount to ‘killing’ human beings (at the embryonic stage) (390). Researchers have since worked on discovering alternative strategies: defining adult stem cell populations and re-programming somatic cells to pluripotent stage by techniques such as somatic-
cell nuclear transfer and induction with transcription factors (391, 392). Recently, novel protocols have been generated that enable trans-differentiation of terminally differentiated cells such as fibroblasts into neurons or neural precursors while avoiding an intermediate pluripotent cell state (393).

While these developments have influenced the evolution of stem cell research, other key areas have practical relevance as research transitions into the clinical phase. In the specific context of ischaemic stroke, issues such as the perspectives of patients on recovery following stroke and the consequent impact on the appreciation of risk and benefit as well as issues with patient selection and consent in clinical studies are likely important determinants of the eventual success and quality of these studies (394).

2.5.3.1 Study Design: Relevance to Stroke Survivors

The concept that patients have more to offer than mere participation has been gaining ground in the last few decades. This underscores the belief that research is more effective if it serves the real needs of the ‘consumers’ of research findings, i.e. patients (395). The value of involving patients in research planning and seeking their input regarding study design has been described previously (396). This has been reported to help in making the study activities more relatable and resonant with patient priorities (397). Patient input has been found to be very insightful in terms of what data should be collected and how these research findings need to be communicated back to the patient community (398). In the rapidly evolving field of stem cell research, it is paramount that patients be given adequate information and opportunities to participate in the conduct of research itself as empowered stakeholders (399).
2.5.3.2  Risk – Benefit Analysis and Communication

Early phase stem cell clinical research in ischaemic stroke is accompanied by uncertainties regarding associated risks. These risks include further deterioration in neurological function, tumourigenicity, aberrant differentiation and mistargeting of transplanted cells, and potential genetic risks associated with germ cell integration (400). The transplantation of stem cells is an irreversible phenomenon and subject to delayed effects due to cell proliferation following delivery (175). The use of preclinical animal models can provide relatively limited predictive information due to issues with species specificity, the limited period of follow-up and the fit of the model to human disease pathology (390). Delivery of stem cells in research settings, particularly in the context of autologous delivery, creates challenges with variability in the quality of individual donor cell batches. While standardised manufacturing protocols that address the requirements of good tissue practices (GTP) and good manufacturing practices (GMP) have not conventionally been required in early stage research, the unique characteristics of stem cells may require these to be established at an early phase in the clinical trials. This complicates the risk – benefit assessment conducted by oversight bodies and institutional review boards (IRB). These bodies may need to determine the adequacy of the type and duration of monitoring incorporated into study designs and the communication about this with prospective trial participants (389).

2.5.3.3  Selection of Trial Participants and Informed Consent

Since CTs carry a potential for extended or permanent deleterious effects, there can be no justification for recruiting healthy volunteers in early phase clinical trials (322). The researchers and IRBs have deliberated on the ethical and scientific rationale for selecting ‘seriously ill’ patients versus comparatively ‘healthier’ patients in early trials. While selective enrolment of patients with more severe disability may seem more justifiable, the risk of ‘therapeutic misconception’ is
particularly important in this cohort of participants (401). Lee et al. (2001) reported that participants often persisted with high expectations regarding outcomes despite receiving information indicating lower estimates of benefit during the informed consent process (402). One study reported that only 16% of highly educated individuals were able to interpret risk magnitude accurately (403). This, in conjunction with misestimation regarding potential benefit, is a challenge for the process to be truly ‘informed’ especially as it is difficult to articulate the risk–benefit profile comprehensively at an early stage of research (390). It is therefore critical to ensure that participants understand that CTs have the potential for life-long effects and informed consent should be viewed as an ongoing exercise to preserve participants’ autonomy in dealing with subsequent effects (404).

The neuro-behavioural impairments common after stroke may compromise a subject’s ability to make free and informed choices. This is a consideration for researchers in any stroke study, including those investigating CT and raises an issue about inequity of access to therapies with the potential to benefit and limit its eventual extrapolation to those patients with stroke who have behavioural limitations (404). The ISSCR (2016) recommendations suggest that the capacity to consent should be assessed formally along with the feasibility of proxy consent in stem cell research (294).

2.5.3.4 Summary

It may therefore be useful to understand the expectations of prospective trial participants about the risk-benefit information made available to them at the time of participation in early phase clinical studies as well the patient selection and consent process involved, in the context of stem cell research.
2.5.4 Health Economic Considerations in CTs Research

2.5.4.1 Cost of Stroke

Stroke represents a huge economic burden for patients, healthcare systems and society and this has been reported by multiple studies such as Gloede et al. (2014) who reported lifetime costs per patient in Australia of approximately $103,566 AUD ($68,769 USD) (182); Gustavsson et al. (2011) who reported that the annual costs per person for stroke care as 7,775 € PPP (power purchase adjusted Euro) which comprised of 5,141 € PPP (direct medical cost), 2,035 € PPP (direct non-medical cost) and 599 € PPP (indirect cost) (405). Di Carlo et al. (2009) reported that these costs for the United States were $65.5 billion (67% for direct and 33% for indirect costs) (406). Xu et al. (2018) analysed the total cost of health and social care for patients with acute stroke in England, Wales and Northern Ireland (407). The cost of stroke care in this study was £3.60 billion in the first five years after admission (mean per patient cost: £46,039) (407). Importantly, this study reported that social care costs accounted for a bigger proportion of total costs than direct health costs after the first year following stroke (407). Joo et al. (2014) reviewed data on indirect costs associated with stroke across 31 studies (408). The study revealed that indirect costs have been reported using diverse methods and definitions, which may account for the wide range (from 3% to 71%) in the proportion of the total cost of stroke that is represented by indirect costs (408).

2.5.4.2 Value Proposition of CTs and Implications for Research

Healthcare systems across the globe are struggling with the rising costs of providing care and cost is recognised as a fundamental dimension of healthcare quality. This follows from a need to define healthcare quality in terms of ‘value’: health outcomes achieved per unit of expenditure rather than simple cost reduction (407, 409). Innovative therapies like CTs are likely to have high initial costs
driven by the complexities in manufacture and delivery of these products, and so cost is a critical determinant of access to therapies in real world clinical practice (407). This increases the risk involved in research and development (R&D) investment in high technology products such as CTs. There is a recent argument for early health technology assessment in parallel with phase I/II clinical research (410). Generating economic evidence at an early stage can accelerate clinical translation by enabling strategic R&D decisions, preclinical market assessment, portfolio decisions, clinical trial design, and market access and pricing strategy arrangements (411). Hettle et al. (2017) reviewed existing health technology assessments in the context of the high level of uncertainty regarding data on the effectiveness of CTs at the current stage of research (386). They reported that the existing methodology was adequate despite the additional complexities in the context of the variability of current data. Explorative decision-analytic models used in different disease indications can simulate long-term effects of therapeutic strategies in terms of economic value (412). The application of one such health-economic model in Sweden by Svennson et al. (2012) showed that CTs offer the potential for cost offsets and cost savings in a long-term perspective by reducing the disability after stroke (413). However, the results of such modelling may vary between countries due to differences in treatment practices, distribution of disease burden, health access, pricing structures and probable impact of emerging data on safety and efficacy on future resource utilization.

2.5.4.3 Summary

CTs have resulted in improvement in functional recovery following stroke in preclinical and early clinical studies. This has the potential to positively influence the quality of life and reduce ongoing lifetime costs of care for stroke survivors. As these therapies enter the clinical phase of development, generation of economic evidence early in the development phase is likely to be
critical to ensure that development costs are in line with the potential benefits to consumers from these interventions (414, 415).

2.5.5 Conclusions

Stroke management has evolved from its initial focus on the acute event to the chronic, progressive condition that warrants ongoing monitoring and intervention after the event. The mortality of stroke has been declining over the last few decades, but the morbidity associated with stroke continues to represent a significant burden. Approximately 80% of stroke survivors return home with some level of residual disability and more than 30% of stroke survivors report persistent restrictions in participation at four years after stroke (e.g., difficulty with autonomy, engagement or fulfilling societal roles) (54).

Currently, significant unmet needs persist in many domains such as health-related quality of life, maintenance of daily activity and independence, and social reintegration. Therefore, there is a critical imperative to explore interventions at all stages of stroke. Research with CTs so far holds a unique potential for applicability throughout the stroke continuum. Evaluation of different aspects of research and the creation of a practical framework to enable efficient research output are critical. This evaluation and consequent findings can add to knowledge available to researchers engaged in early phase CT research in stroke and facilitate improved research outputs.
CHAPTER 3: STUDY DESIGN CONSIDERATIONS

3.1 Background

Clinical research with different types of CTs in ischaemic stroke has gathered momentum in the last decade. ClinicalTrials.gov, a database provided by the U.S. National Library of Medicine for clinical studies across the world, listed 150 clinical studies investigating different types of CTs in ischaemic stroke in July 2018. The ‘Stem Cell Therapeutics as an Emerging Paradigm in Stroke’ (STEPS) was initially convened in 2007 as a collaborative platform in the USA between academia, industry and key government bodies like the NIH and FDA and was updated last in 2014 (299). This resulted in a series of guidelines (I/II/III) which provide a high-level consensus approach to understanding barriers to: successful translation of preclinical research; increasing data regarding the mechanisms of action of CTs; and important elements of clinical trials in the context of the use of CTs in stroke (299).
Table 6: STEPS III Recommendations

<table>
<thead>
<tr>
<th>STEPS III: Suggestions for Phase II/III Efficacy Trials</th>
<th>Recommendations</th>
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<tbody>
<tr>
<td><strong>Entry criteria for Participants</strong></td>
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<tr>
<td>Understanding the properties of the cell therapy (CT) of interest</td>
<td>Exclusion of participants who are at high risk of known adverse events concerned with CT</td>
</tr>
<tr>
<td>Understanding the natural history of the stroke syndrome</td>
<td>More information needed regarding spontaneous stroke recovery and prognostication markers</td>
</tr>
<tr>
<td>Choice of study end points to assess hypotheses</td>
<td>Selection of endpoints aligned to study population</td>
</tr>
<tr>
<td><strong>Time window for patient selection</strong></td>
<td></td>
</tr>
<tr>
<td>Selecting an optimal time window for CT administration</td>
<td>Align to the intended pathophysiological targets known from preclinical studies</td>
</tr>
<tr>
<td><strong>Assessment of efficacy</strong></td>
<td></td>
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<tr>
<td>The use of domain-specific end points, which are sensitive to the differences in recovery</td>
<td>Use of domain specific endpoints along with established global endpoints</td>
</tr>
<tr>
<td><strong>Biomarkers of Activity</strong></td>
<td></td>
</tr>
<tr>
<td>Identifying markers that can define mechanisms of action and CT effects</td>
<td>Research is needed for their use</td>
</tr>
<tr>
<td><strong>Concomitant Rehabilitation Therapy</strong></td>
<td></td>
</tr>
<tr>
<td>Implications of including rehabilitation in study design</td>
<td>Rehabilitation could be a confounding factor in the assessment of efficacy</td>
</tr>
<tr>
<td></td>
<td>Concomitant rehabilitation can have labelling implications</td>
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Adapted with permission from Savitz et al. (2014) (328)
Numerous studies using different types of CTs, administered at different phases of stroke, were published in recent years. These studies have used diverse trial designs, patient populations and routes of administration for investigational CTs. There is robust preclinical evidence to support the premise that the predominant mechanism of action of all types of CTs is not cell engraftment, but the pleotropic effects they exert that augment endogenous neurovascular repair, support brain and synaptic reorganization, and reduce secondary tissue injury (232, 315). Early human studies of CTs in stroke have demonstrated adequate safety and provided preliminary evidence of a beneficial effect in acute, subacute and chronic phases of stroke. The publication in 2016 of two early phase clinical studies with SB623 and CTX0E03 (genetically modified stem cell types, isolated from adult bone marrow and foetal brain tissue, respectively) was an important milestone (285,288), as the first industry-driven clinical studies in the CT research space, hitherto driven by academic centre led trials. It is crucial to evaluate findings from these studies to understand the critical elements that need to be addressed pragmatically to ensure subsequent trial designs are optimized to generate good data on the relative effectiveness of these therapies.

A perspective on key developments in clinical CT research in stroke was published in *The Medical Journal of Australia* in 2017.
# Statement of Authorship

<table>
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<tr>
<th>Title of Paper</th>
<th>Regenerative neurology: meeting the need of patients with disability after stroke</th>
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<td>Overall percentage (%)</td>
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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.

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## Co-Author Contributions

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

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

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Regenerative neurology: meeting the need of patients with disability after stroke

If regenerative neurology restores function, it will meet a huge unmet need and change dogma

Treatment of stroke in the acute phase has come a long way with the development of paramedic, emergency department and stroke team pathways for hyperacute assessment and management with intravenous thrombolysis, endovascular clot retrieval and hemicraniectomy. Acute stroke units reduce mortality and morbidity by up to 20% or more. An estimated 80% of stroke patients survive for one year after stroke, with the large majority being left with chronic disability. In Australia and many other countries around the world, stroke is the leading cause of adult disability. It is estimated that up to 450 000 Australians have disability after stroke.

The only intervention currently available to stroke survivors is rehabilitation. Increasing evidence suggests that rehabilitation complements the natural functional recovery process that can often continue for months or years after stroke. However, there are persisting gaps in our understanding of the basic biological pathways that drive post-stroke recovery, and these pose challenges in applying evidence-based rehabilitation strategies in the real world. This becomes especially critical as patients often need a combination of rehabilitation strategies that cater for their specific disability and complement their potential for long-term recovery. These are often required beyond the period for which rehabilitation services are currently made available due to resource constraints. So where does that leave us in 2017?

Regenerative neurology or stem cell therapy may provide an answer to this unmet need by potentially restoring neurological function in an individualised manner. Many stem cell researchers and clinicians hold the view that the field of regenerative medicine may have as large an impact on humanity as antibiotics.

Basics of stem cells

Stem cells are unique in possessing two qualities — the capacity for self-renewal and the potential for multilineage differentiation. If a stem cell is pluripotent, it can give rise to cells derived from all three germ layers (ectoderm, mesoderm and endoderm) that differentiate into different tissues during embryonic development. On the other hand, a multipotent stem cell tends to generate limited cell types, often relevant to the organ from which the stem cell was derived — for example, haematopoietic stem cells (HSCs) tend to generate blood and immune cell types. Embryonic stem cells isolated from the very early embryo are pluripotent while adult somatic stem cells derived from adult organs, such as mesenchymal stem cells from bone marrow, are multipotent, similar to HSCs.

A significant clinical limitation to the use of embryonic stem cells therapeutically is the potential for them to form tumours, such as teratomas which have multicellular types from the different embryonic lineages (hair, bone, teeth, heart muscle, etc). In contrast, to date, multipotent cells such as mesenchymal stem cells are considered safer, with animal studies reporting no increase in tumorigenicity. In 2006, Yamanaka (2012 Physiology or Medicine Nobel Laureate) showed that somatic cells (skin fibroblasts) could be engineered genetically by four genes (known as the Yamanaka factors) to produce pluripotent cells similar to embryonic stem cells. This third type of stem cell is termed an induced pluripotent stem cell (iPSC). This discovery has radically transformed stem cell research and proffers the concept of personalised regenerative medicine. Early clinical trials have already started deriving iPSCs from an individual's fibroblasts for autologous (self-)treatment or personalised medicine. The findings of preclinical studies in stroke models have provided encouraging evidence for potential for neuroregeneration and useful insights into potential applicability in the future.

Chronic stroke and local injection

Last year was an exciting one for stem cell therapy in stroke patients. There were two high impact publications documenting early phase clinical studies with two different multipotent stem cells, SB623 and CTX1003. Both are genetically modified stem cell types, one isolated from fetal brain tissue and the other from adult bone marrow. Two independent research teams from reputable institutions in the United Kingdom and United States performed these studies with industry funding (ReNeuron and San Bio, respectively).

This research examined two key questions in relation to study design:

- Is it potentially useful to treat stroke survivors in the chronic phase when their disability has plateaued, sometimes as long as 3 to 4 years after stroke?
- Is intracerebral implantation of stem cells a feasible route of administration?

Published preclinical and preliminary clinical data indicate that the design of the studies was valid, although research opinion is often divided as to optimum timing and route of administration of cell transplantation.

Why was stem cell therapy not administered in the acute phase after stroke in these studies? There may be a number
of clinically pragmatic answers to this question — in the acute phase, patients may be too medically unstable to undergo neurosurgery. Moreover, patients are often still showing rapid improvement, so it would be problematic to measure any benefit above that of optimum acute stroke unit care, when disability has not yet plateaued.\textsuperscript{18}

Why was a neurosurgical implantation chosen? “Functional neurosurgery” is a fast-developing specialty and these neurosurgeons routinely implant electrodes for deep brain stimulation to treat Parkinson disease. Thus they have the expertise to inject, via a narrow bore cannula, deposits of stem cells into multiple sites within the human brain. One benefit to the patient of intracerebral implantation is that the cells remain within the brain and can be imaged non-invasively.\textsuperscript{19} An alternative route of administration used in earlier clinical studies was intravenous injection.\textsuperscript{20} Initially, this approach was considered safer than intracerebral implantation, but it is now appreciated that there is a theoretical risk of distant tumorigenicity, in that stem cells injected intravenously may deposit widely throughout a number of organs within the body (ie, lung, liver, etc.) and may interact with presymptomatic tumours.\textsuperscript{20}

**Is it safe?**

Early phase clinical trials characteristically involve small numbers of patients to minimise the number at risk if there is a serious treatment-related adverse event. In the two studies described above,\textsuperscript{16,17} 27 patients were followed for 12 months after treatment, which is a generally accepted timeframe. The studies stated that no adverse event directly attributable to the stem cell therapy was found. However, the neurosurgical procedure of creating a burr hole and entering the brain to administer the cells did result in appreciable anticipated adverse events (ie, haematoma, headache and other symptoms related to the consequent reduction of intracranial pressure). It is noteworthy that both studies will continue surveillance of all patients after 12 months to detect any longer term adverse events.

We propose an alternate perspective with respect to the claims that no stem cell-related adverse events occurred. Stem cells implanted into the brain are known from preclinical data to differentiate into neural cells and probably integrate within the brain.\textsuperscript{9} In theory, this cellular behaviour has the potential to form an epileptogenic focus. A small number of patients in each of the two high impact studies\textsuperscript{16,17} were reported to have seizures. With this limited clinical dataset it cannot be concluded whether their seizures arose from the neurosurgical procedure, as suggested in the publications,\textsuperscript{16,17} or was related to the stem cells. We propose that larger phase 2/3 studies should incorporate electroencephalography investigations to better understand the association of seizures with intracerebral implantation stem cell therapy.

The clinical data in these two early phase clinical studies supports the clinical feasibility and safety of intracerebral implantation of stem cells in patients with chronic disability after stroke. Both studies used an escalating dose of stem cell therapy. Cell doses of up to 10 million SB623 and 20 million CTX0E03 stem cells may be used for future larger phase 2 studies.

**So: does it work?**

This question will not be answered with any degree of certainty for a number of years as we await the results from large, multicentre, multinational, double-blind, randomised controlled clinical trials. While preclinical data from animal studies suggest an overall functional improvement of 40.6%, the extrapolation of these findings to human stroke pathophysiology is limited by: (i) species-specific differences; and (ii) the fact that controlled induction of cerebral ischaemic lesions in animals is not fully representative of the heterogeneous lesion load seen with human stroke.\textsuperscript{9}

Early clinical studies enrolled a heterogeneous mix of patient groups. Most of these studies were open label and single arm and thus not designed to answer the question of efficacy. Therefore, at present, it is difficult to postulate any differential benefit for specific patient or stroke subgroups.\textsuperscript{18} From a mechanistic perspective, there are a number of theories from preclinical data on how stem cell therapy may decrease post-stroke disability (Box), with neuroplasticity considered to be an important factor.\textsuperscript{21}

An aspect of immense practical relevance is that standardised rehabilitation was not provided to participants in these studies. There is an ongoing debate about the potential confounding effect of rehabilitation on functional and structural outcomes. However, rehabilitation is accepted as a standard of care to optimise natural recovery, and guidelines for stem cell research such as Stem Cell Therapy as an Emerging Paradigm for
Stroke (STEPS) recommend its inclusion in trial design. Stroke clinicians will know from everyday experience that significant improvement in neurological function many years after an ischaemic stroke is rarely observed. The two studies described above are very important in the field of regenerative neurology in that both found an associated improvement in function in the chronic phase of stroke among patients with different areas of stroke-induced injury. In light of the emerging evidence for long-term potential to relearn that can be harnessed by rehabilitation, stem cell implantation along with targeted and protracted rehabilitation could have a synergistic and biologically plausible impact on post-stroke recovery.

It is of fundamental interest that both studies described changes on magnetic resonance imaging (MRI) of the human brain after treatment. It was suggested that these MRI findings may not be explained by the neurosurgical procedure alone. These preliminary findings may present an opportunity for reverse translational research, from the clinic back into the research laboratory, to gain a better understanding of how changes in the human brain may occur after stem cell therapy.

At this juncture of stem cell research in stroke, there are three important points to be considered:

- The preclinical and early clinical data which suggest that stem cell therapy may be helpful are becoming encouragingly robust.

- The preponderance of failed translation efforts from preclinical to clinical therapeutics in stroke highlights that continued exercise of scientific rigor is critical.

- Ongoing stem cell tourism across the world and in Australia to reach centres that operate for financial gain without regard to research integrity or patient safety poses a significant danger to the credibility of this field.

The current regulatory framework in Australia for oversight of cellular therapies has significant gaps in scope as well as implementation. It is a matter of urgency that our politicians and regulatory authorities collaborate with their counterparts in the US, European Union, Japan and other regions where innovative approaches are being implemented to develop the field while creating adequate safeguards to protect patient interests.

Exciting scientific research is that in which the questions raised outweigh the answers. We suggest the quest to fulfil the unmet need for treating disability after stroke has taken a step forward.

Competing interests: No relevant disclosures.

Provenance: Not commissioned; externally peer reviewed.

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References are available online at www.mja.com.au.
3.2 Research Objectives

A systematic review of published clinical studies was undertaken to understand the choice of study design elements, endpoints, CT characteristics, dose and mode of delivery, and to analyse their impact on the quality of evidence generated in early phase clinical studies with CTs in ischaemic stroke.

3.3 Methods

A systematic review of all published clinical studies in stroke investigating cell therapies was conducted.

The protocol for the review, defined a priori was published in PROSPERO: International prospective register of systematic reviews in 2016.
# Statement of Authorship

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## Co-Author Contributions

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

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

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Safety and effectiveness of stem cell therapies in early phase clinical trials in stroke: a systematic review and meta-analysis

ANJALI NAGPAL, FONG CHAN CHOY, Susan Hillier, Stuart Howell, Anne Hamilton-Bruce, Simon Koblar

Citation

Review question
Is stem cell transplantation effective in patients with stroke?
Is stem cell transplantation safe in patients with stroke?

Searches
Databases such as PubMed, EMBASE, SCOPUS, Web of Science and the Cochrane Central Register of Controlled Trials (CENTRAL) trials registry of the Cochrane Collaboration will be searched. The specific search strategies will be created in consultation with a Health Sciences Librarian with expertise in systematic review searching. After the PubMed strategy is finalised, it will be adapted to the syntax and subject headings of the other databases. The International Clinical Trials Registry Platform Search Portal and ClinicalTrials.gov will be searched for ongoing or recently completed trials and PROSPERO will be searched for ongoing or recently completed systematic reviews. As relevant studies are identified, reviewers will check for additional relevant cited and citing articles.

Types of study to be included
We will include studies with all study designs, except case reports and segregate them into two groups for further analysis: controlled studies with comparator arm and studies without comparator arms.

Condition or domain being studied
Stroke (all types)

Participants/population
Inclusion Criteria:
Trials investigating use of stem cell therapy in adult patients who have experienced a stroke inclusive of all types of stroke, in any phase from acute to chronic phase, following stroke
Exclusion Criteria:
Trials investigating combination therapies including stem cells

Intervention(s), exposure(s)
All types of stem cell therapies inclusive of all study designs, all types of cell source (autograft, allograft or xenograft; embryonic, fetal or adult), route of administration (intracerebral/intravenous/intr-a- arterial/intrathecal) and dosage.

Comparator(s)/control
Non-exposed control group for analysis of studies with control comparator design

Context
Studies reported between 2005-2016 Studies reported in English

Primary outcome(s)
Effectiveness measures will be assessed using validated body structure/impairment (NIHSS/Fugl-Meyer assessment/modified Ashworth scale/European stroke scale); activity (Barthel index) and participation (Stroke Impact scale/ modified Rankin scale) measures.

**Timing and effect measures**
These outcomes will be assessed at 6 months.

**Secondary outcome(s)**
Post-procedure safety outcomes such as deaths, infections, stroke recurrence and neoplasms will be analysed.

**Timing and effect measures**
The minimum period of follow up considered will be 6 months.

**Data extraction (selection and coding)**

**Selection process:**
All studies identified using the search strategy described above will be screened independently by two review authors (AN and FCC) to identify studies that potentially meet the listed inclusion criteria outlined above. The full text of all potentially eligible studies will be independently assessed by the two review authors (AN and FCC). Any disagreement between them over the eligibility of particular studies will be resolved through discussion with senior reviewers (SK and SH).

**Data collection process:**
A standardised data extraction form will be used to extract data from the included studies for assessment of study quality and evidence synthesis. The data extraction form will be designed in consultation with statistician in the team (SH). Extracted information will include: study setting; study population and participant demographics and baseline characteristics; details of the intervention and control conditions; study methods; recruitment and study completion rates; outcomes and times of measurement; information for assessment of the risk of bias. Two review authors will extract data independently, discrepancies will be identified and resolved through discussion with senior reviewers (SK and SH). Missing data will be requested from study authors through email.

**Data items:**
Data will be collected from studies pertaining to year of publication, country where the trial was conducted, study design elements such randomization, blinding, treatment allocation and interventions in the control group. Characteristics of study participants such as demographics, phase and type of stroke, time between stroke onset and enrollment, time between stroke onset and administration of stem cell therapy and delivery of rehabilitation will be recorded. The outcomes assessed in different trials for determining safety, efficacy and feasibility will be recorded along with the period of follow up used in different studies.

**Risk of bias (quality) assessment**
Two review authors (AN and FCC) will independently assess the risk of bias at the study level for all the studies included in the review, considering the characteristics recommended by the International Cochrane Collaboration. These include allocation bias, assessment bias and reporting bias. A determination of risk of bias for each study and as a body of evidence will be illustrated and considered in final reporting.

**Strategy for data synthesis**
We will provide a narrative synthesis of the findings from the included studies, structured around the type of intervention, study design, target population characteristics, type of outcome and intervention content. The included studies will be segregated depending on study design into two groups for further analysis: controlled studies with comparator arm and studies without comparator arms.

We anticipate that there will be limited scope for meta-analysis because of the range of different outcomes measured across the small number of existing trials, within group. However, where studies have used the same type of intervention and comparator, with the same outcome measure, we will pool the results using a random-effects meta-analysis, with standardised mean differences for continuous outcomes and risk ratios for binary outcomes, and calculate 95% confidence intervals and two sided P values for each outcome.
Heterogeneity between the studies in effect measures will be assessed using both the Chi-squared test and the I-squared statistic. We will consider an I-squared value greater than 50% indicative of substantial heterogeneity.

Analysis of subgroups or subsets

The following subgroups will be considered, subject to feasibility:

1. Phase of Stroke at stem cell delivery:
   a. Acute and subacute (within three months of ischemic stroke) versus chronic (more than three months after ischemic stroke).

2. Treatment Characteristics:
   a. Source of stem cells: autologous or allogeneic
   b. Route of administration: intracerebral/intravenous/intra-arterial/intrathecal

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Dr Stuart Howell. The University of Adelaide
Dr Anne Hamilton-Bruce. The University of Adelaide
Dr Simon Koblar. The University of Adelaide

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Adelaide

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Stage of review
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Subject index terms status
Subject indexing assigned by CRD
**Subject index terms**
Genetic Therapy; Humans; Safety; Stem Cell Transplantation; Stroke

**Date of registration in PROSPERO**
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**Details of any existing review of the same topic by the same authors**

**Stage of review at time of this submission**

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**Versions**
30 June 2016

This information has been provided by the named contact for this review. CRD has accepted this information in good faith and registered the review in PROSPERO. CRD bears no responsibility or liability for the content of this registration record, any associated files or external websites.
3.4 Results

This review included 26 clinical studies that investigated CTs in stroke and were published in the 12-year time-period: 2005-2016. The number of studies published increased from nine studies in the period: 2005-2010 to 20 studies in the period: 2011-2016. It was interesting to note that a higher proportion of studies published since 2011 investigated autologous use of CTs (15 out of 20 studies) as compared to the earlier studies prior to 2011 [autologous (n=4); allogeneic (n=5)]. Mesenchymal stem cells were the predominant stem cell type studied in all these trials (n=18). A meta-analysis indicated a benefit in the CTs group in terms of functional impairment, activity and participation following stroke. Data synthesis across studies provided reassurance of the relative safety of the different CTs administered in these trials. Our review evaluated different aspects of the study designs used in the included studies.

The findings of the systematic review and meta-analysis were published in *Stem Cell Research & Therapy* in 2017.
# Statement of Authorship

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Signature | Date | 8/7/2017 |

## 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 to 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.

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<td>Fiona Chan</td>
<td>Initial Literature search and review of manuscript for publication</td>
<td>10/7/2017</td>
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<td>Stuart Howell</td>
<td>Data analysis including meta-analysis and review of manuscript</td>
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<td>Monica Hamilton-Bruce</td>
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Safety and effectiveness of stem cell therapies in early-phase clinical trials in stroke: a systematic review and meta-analysis

Anjali Nagpal1,*, Fong Chan Choy1, Stuart Howell2, Susan Hillier3, Fiona Chan4, Monica A. Hamilton-Bruce1,5 and Simon A. Koblar1,5

Abstract

Stem cells have demonstrated encouraging potential as reparative therapy for patients suffering from post-stroke disability. Reperfusion interventions in the acute phase of stroke have shown significant benefit but are limited by a narrow window of opportunity in which they are beneficial. Thereafter, rehabilitation is the only intervention available. The current review summarises the current evidence for use of stem cell therapies in stroke from early-phase clinical trials. The safety and feasibility of administering different types of stem cell therapies in stroke seem to be reasonably proven. However, the effectiveness needs still to be established through bigger clinical trials with more pragmatic clinical trial designs that address the challenges raised by the heterogeneous nature of stroke per se, as well those due to unique characteristics of stem cells as therapeutic agents. 

Keywords: Stem cells, Stroke, Clinical design, Outcomes, Regenerative medicine

Background

Stroke, classically characterised as a neurological deficit attributed to an acute focal injury of the central nervous system (CNS) by a vascular cause (infarction or haemorrhage), is a major cause of disability and death worldwide [1]. While stroke represents a single event of cell/tissue injury, it sets in motion a complex interplay of inflammation and repair involving neural, vascular and connective tissues, in and around the affected areas of the brain [2, 3]. Molecular and imaging research is generating new insights into mechanistic interactions at the cellular level [3]. The American Heart Association/ American Stroke Association (AHA/ASA) proposed an updated definition for stroke in 2013 that incorporates clinical and tissue criteria [4]. These criteria reflect the advances in imaging techniques and consequent understanding of disease pathophysiology in the past few decades. However, the translation of these advances into meaningful therapeutic options has until recently been met with limited success. While interventions for early re-perfusion such as thrombolysis and endovascular revascularisation have shown significant benefit, they are still subject to a limited window of opportunity [5, 6].

There is now a significant body of evidence from pre-clinical research which postulates that stem cells potentially modulate multiple pathways involved in endogenous neurogenesis, angiogenesis, immune modulation and neural plasticity, in addition to or instead of cell replacement [7–9]. These effects may potentially be harnessed for affecting structural and functional regeneration after stroke with a prolonged window of opportunity [10, 11]. An encouraging number of pilot and definitive early-phase clinical studies have been published in the last decade, signalling a critical milestone in clinical translation of stem cell therapies in stroke [12–39]. However, the interpretation of current knowledge in stem cell research seems challenging due to heterogeneity in the study design, publication bias and the possible confounding effects of concomitant interventions such as immunosuppressant use and rehabilitation. Early meta-analyses have attempted...
to investigate efficacy and safety data for particular cell types (e.g., mesenchymal cells) \[40\], stroke type (ischaemic) \[41\] or study design (single-arm studies) \[42\]. However, these analyses have been limited by the small number and size of studies considered. Other reviewers have taken more of an 'all-comers' approach to inclusion of all potential regenerative interventions including combinations of cell-based and biological therapies \[43\]. This approach, while attractive on a broader pathophysiological level, may present over-simplified assessment of the complexities involved in the use and investigation of living cells as therapeutic products.

**Objectives**
The present review and meta-analysis aims to assess the effectiveness and safety of cell therapies, studied as a monotherapy (inclusive of any type/source/route of administration) in adult patients with stroke (inclusive of all types and phases of stroke) and published in English.

**Methods**

**Protocol and registration**
The protocol for the review was prepared and registered on PROSPERO (international prospective register of systematic reviews) [Ref-2016:CRD42016039524], and is available online (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016039524).

**Eligibility criteria**
The review evaluated all studies investigating the use of stem cells in stroke, other than case reports, reported in the English language during the period 2005–2016. The included studies were segregated into two subgroups for further analysis: controlled studies with a comparator arm and studies without comparator arms.

**Inclusion criteria**
Trials investigating the use of stem cell therapy in adult patients who had experienced a stroke, inclusive of all types of stroke and in any phase from the acute to chronic phase, were included.

**Exclusion criteria**
Trials investigating combination therapies including stem cells with other therapies.

**Intervention(s) of interest**
Stem cell-based interventions with any type (autograft, allograft or xenograft; embryonic, fetal or adult) of cell source, route of administration (intracerebral/intravenous/intra-arterial/intrathecal) and dosage.

**Search strategy**
Databases including PubMed, EMBASE, SCOPUS, Web of Science and the Cochrane Central Register of Controlled Trials (CENTRAL) registry of the Cochrane Collaboration were searched until November 2016. The specific search strategies were created in consultation with a health sciences librarian with expertise in systematic re-view searches. After the PubMed strategy was finalised, it was adapted to the syntax and subject headings of the other databases. The International Clinical Trials Registry Platform Search Portal and ClinicalTrials.gov were also searched for trials completed recently. AN, FC and FCC checked for additional relevant articles. The authors of articles were contacted via email when pertinent information was missing in the published manuscripts and additional data thus obtained were included in the final analysis (Additional file 1).

**Study selection**
All studies identified using the search strategy described were screened independently by two review authors (AN and FCC). AN and FCC independently assessed full texts of all eligible studies. Any disagreement regarding the eligibility of a particular study was resolved through discussion with senior reviewers (SAK and SHi).

**Data collection process and data items**
A standardised data extraction form was used to extract data from the included studies for assessment of study quality and evidence synthesis. The data extraction form was designed in consultation with the methodologist on the team (SHo). Extracted information included: study setting (year of publication and country); study population demographics and baseline characteristics; details of the intervention and control conditions, if applicable; recruitment and study completion rates; information for assessment of the risk of bias; and study design elements such as randomisation, blinding, treatment allocation and interventions in the control group, outcomes and times of measurement.

Two review authors (AN, FCC) extracted data independently and differences identified were resolved through discussion with senior reviewers (SAK, SHi, AHB). Study authors were contacted via email for missing data.

Characteristics of study participants such as demographic characteristics, the phase and type of stroke, time between stroke onset and enrolment, time between stroke onset and administration of stem cell therapy and delivery of rehabilitation were recorded. The outcomes assessed in different trials for determining safety, efficacy and feasibility were recorded along with the period of follow-up used in different studies.
Risk of bias (quality) assessment
AN, FCC and SHi assessed the risk of bias in individual studies for the two subgroups included in the review, considering the characteristics recommended by the International Cochrane Collaboration [44].

Summary measures
Primary outcomes
Primary outcomes of interest were based on the WHO ICF framework [45] and included effectiveness measures assessed at the 6-month time point using validated scales for body structure/impairment measures (e.g., National Institutes of Health Stroke Scale (NIHSS)/Fugl-Meyer assessment/Modified Ashworth Scale/European Stroke Scale), activity measures (e.g., Barthel index (BI)) and participation measures (e.g., Stroke Impact Scale/Modified Rankin scale (mRS)).

Secondary outcomes
Post-procedure safety outcomes such as death, infections, stroke recurrence and neoplasms were considered. The minimum period of follow-up was established as 6 months.

Strategy for data synthesis
A narrative synthesis of the findings from the included studies was carried out structured around the type of intervention, the study design, the target population characteristics and the effectiveness and safety outcomes measured. The included studies were segregated depending on study design into two subgroups for further analysis: controlled studies with a comparator arm and studies without comparator arms.

Method for meta-analysis
The data were analysed by SHo using STATA/SE v14.1 (StataCorp LP, College Station, TX, USA).

For the single-arm studies, patient data were used to calculate a difference score, which represents the change from baseline to day 180. Meta-analyses were performed using only the mean and 95% confidence limits of the difference scores. Data from the controlled studies were explored using treatment effects (treatment vs control group) at 6 months. Baseline data for each study were inspected to ensure that the randomisation produced groups which did not differ in terms of mean scores for the three scales under investigation (NIHSS, BI and mRS), for which there were adequate data available. For both subgroups, a separate meta-analysis was performed for each instrument. The meta-analyses were performed using a DerSimonian–Laird random effects model to account for potential heterogeneity across studies [46]. Pooled estimates were presented as the standardised mean difference (SMD) with 95% confidence intervals.

Heterogeneity was summarised using the I-squared statistic.
A formal evaluation of heterogeneity and publication bias was planned in the event of data being available from an adequate number of studies.

Results
Study selection
The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [47].

Twenty-six studies, which fulfilled the defined inclusion and exclusion criteria, were selected for further data synthesis (Fig. 1).

Study characteristics
The type of stem cell intervention (cell type; source; route of administration; time between stroke onset and administration of stem cell therapy; delivery of rehabilitation), study design and target population characteristics (phase and type of stroke; time between stroke onset and enrolment) are presented in Table 1.

The majority of studies (n=18) utilised autologous adult human bone marrow-derived mesenchymal/mononuclear cells [13, 16-22, 24-26, 30-34, 36, 37, 39]. The remaining studies utilised varied allogeneic cell sources such as human neural stem cells derived from fetal tissue (n=3) [15, 29, 38], mesenchymal cells from umbilical cord blood (n=1) [23], neuronal cells derived from embryonic tissue (n = 2) [12, 14] and autologous peripheral blood haematopoietic stem cells (n = 1). One study investigated the use of a xenograft (porcine fetal cells; n = 1) [16].

The most common route of delivery of stem cells was intravenous (n = 11) followed by intracerebral (n = 9), intra-arterial (n = 6) and intrathecal (n = 2).

Studies without a comparator arm
Fifteen studies were evaluated in the single-arm study subgroup (Nexperimental = 131). Ninety-six participants received stem cell transplantation within 3 months of the incident stroke, of which only one patient had a haemorrhagic stroke. Seventy-nine patients received stem cell transplantation more than 3 months post stroke, of which 65 participants had ischaemic stroke and two patients had haemorrhagic stroke.

Studies with a comparator arm
Eleven studies were evaluated in the controlled study subgroup (Nexperimental = 330; Ncontrol = 329). Four of these studies evaluated the impact of stem cell transplantation within 3 months of the incident stroke. The patients in these studies were more likely those with
haemorrhagic stroke ($N_{\text{experimental}} = 170; N_{\text{control}} = 136$) than ischaemic stroke ($N_{\text{experimental}} = 70; N_{\text{control}} = 70$). On the other hand, seven studies that reported transplantation of stem cells more than 3 months post stroke had more patients with ischaemic stroke ($N_{\text{experimental}} = 79; N_{\text{control}} = 116$) than haemorrhagic stroke ($N_{\text{experimental}} = 11; N_{\text{control}} = 7$).

### Synthesis of results

#### Safety

Studies reported a varied period of safety follow-up to a maximum of 60 months following stem cell delivery. Safety events of particular interest are presented in Table 2. The most commonly reported adverse events included headache and fever, mostly self-limited and often related to the cell delivery procedures, particularly when administered via intracerebral/intrathecal routes.

Overall, 16 deaths were reported in participants receiving stem cell therapies. The cause of death was reported to be recurrent stroke ($n = 3$), infections ($n = 3$), cardiac causes ($n = 8$) and pulmonary embolism ($n = 2$). However, none of these events was ascertained as related to the therapy administered. The longest follow-up data published were from Lee et al. [19], who reported an adjusted hazard ratio (HR) of $0.344$ ($95\%$ CI: $0.115–1.031$, $p = 0.057$) for the mesenchymal stem cell group vs control for survival.

Twenty-one events of seizures were reported across 10 studies in patients receiving stem cells. The majority of these episodes were described as not related to the investigational therapy. These resolved with anti-epileptic treatment with no subsequent recurrence. Overall, five cases of tumours were reported (eccrine poroma ($n = 1$), lung cancer ($n = 2$), malignant melanoma ($n = 2$)). None of these were attributed to the stem cell therapy as
Table 1 Disposition of study design & intervention characteristics (Continued)

<table>
<thead>
<tr>
<th>Study variable</th>
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<th>Number of subjects</th>
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<tr>
<td>Not reported</td>
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MSC mesenchymal stem cells, MNC mononuclear cells, RCT randomised controlled trial

Table 2 Early-phase stem cell studies in stroke: safety events

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<tr>
<th>Event</th>
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<td>27</td>
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<tr>
<td>Tumours</td>
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<td>0</td>
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<tr>
<td>Seizures</td>
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<td>5</td>
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<tr>
<td>Recurrent stroke</td>
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<tr>
<td>Haematoma</td>
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<td>0</td>
</tr>
<tr>
<td>Pain</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Infections</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Fever</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Headache</td>
<td>14</td>
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Table 1 Disposition of study design & intervention characteristics

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<td>Brazil</td>
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<td>China</td>
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<td>Taiwan</td>
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<td>5</td>
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<td>Russia</td>
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<td><strong>Stroke phase</strong></td>
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<td>Chronic</td>
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<td>Haemorrhagic</td>
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<td>Human bone marrow-derived MSC/MNC</td>
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<tr>
<td>Human fetal neural stem/progenitor cells</td>
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<td>Umbilical mesenchymal stem cells</td>
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<td>Porcine mesenchymal stem cells</td>
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<tr>
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<tr>
<td>Intravenous</td>
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<tr>
<td>&gt; 3 months</td>
<td>14</td>
<td>283</td>
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aCo-transplantation of neural stem cells and umbilical cord MSC
bOne study had two unmatched sequential cohorts investigated under different routes of administration
A treatment cycle in one study used transplants via intracerebral route followed by 4 weeks of intravenous infusion
dOne study reported administration of stem cell therapy in two settings (1st setting before and 2nd after 3 months of stroke)

patients had well-recognised risk factors for tumour (lung cancer; melanoma) in their past history prior to receiving stem cells.

Effectiveness

**Studies without a comparator arm**

Meta-analysis evaluated available data from eight single-arm studies that reported the impact of stem cell therapies at 6 months post treatment, on recognised validated body structure/impairment (NIHSS), activity (BI) and participation (mRS) measures (Figs. 2 and 3). NIHSS scores showed a modest decrease (SMD= -4.13 (95% CI: -5.51 to -2.76; p = 0.000)), although I² = 86.20% indicated significant heterogeneity across the studies.
A similar, although numerically smaller, trend towards improvement was indicated by a decrease in mRS (SMD = -1.63 (95% CI -2.16 to -1.10; p = 0.017); $I^2 = 66.60\%$) and an increase in BI (SMD = 38.41 (95% CI 27.99–48.83; p = 0.163); $I^2 = 44.80\%$).

**Studies with a comparator arm**

Meta-analysis of data from six controlled studies that reported the impact of stem cell therapies on NIHSS, BI and mRS at 6 months post intervention revealed similar directional trends in the change of all three outcome measures.
parameters. However, the difference in effect size between experimental and control groups was very small (Figs. 4 and 5). NIHSS scores indicate a decrease (SMD = -0.75 (95% CI -1.29 to -0.22; \( p = 0.008 \); \( I^2 = 74.8\% \)). Similarly, mRS scores indicate a decline (SMD = -0.25 (95% CI -0.50 to -0.01; \( p = 0.726 \)). BI scores demonstrated an improvement (SMD = 0.39 (95% CI 0.13-0.66; \( p = 0.113 \); \( I^2 = 43.80\% \)).

Assessment of risk of bias
All studies had at least one or more source of bias (Fig. 6a, b).

Allocation
The sequence generation was adequate in 4/26 included studies. The method for sequence generation was not specified in three studies. The treatment allocation was concealed in only six out of 26 studies.

Blinding
None of the studies incorporated binding of participants and study personnel. Ten out of 26 studies had binding of outcome assessment.

Incomplete outcome data
Most studies reported having complete data for all included participants. In three studies, data on outcomes at 6 months were missing.

Selective reporting
All studies presented per-protocol data.

Other potential sources of bias
While selective publication of studies with significant results is regarded as a potential source of bias, there was no clear evidence of this in the current review. The impact of other sources of bias is equally hard to quantify.

Additional analysis
Further subgroup or sensitivity analysis was not deemed feasible due to the small number of studies and limited data availability.

Discussion
Summary of evidence
The current review indicates a trend towards improvement across varied domains of functional impairment in patients with stroke given stem cell therapies. The quantum of improvement is small from studies that had a control comparator. A high level of heterogeneity was
observed in both subgroups (with/without comparator). This may probably be due to the differences in cell types used and the route, dose and time of administration and other elements of the study design. An exploration of these factors as the source of the identified heterogeneity was not found to be feasible owing to the small number of patients involved. Therefore it is currently difficult to draw any meaningful conclusion about the most appropriate dosage and route of administration or the phase of stroke in which these therapies are likely to provide most meaningful benefit. Nonetheless, we believe that our study provides insights into the overall effect of stem cell therapy and provides a starting point for future research on this issue.

It is reassuring that the safety profile of these therapies has been reasonable, with no alarming signals to date, especially in relation to tumorogenicity. Most of the adverse events were self-limited and resolved spontaneously or with appropriate management. The events of most note were seizures, headache and events associated with procedures used for administering these therapies. In addition, all studies reported successful recruitment to target and successful administration of investigational therapy in study participants.

It is interesting to note that a greater proportion of more recent studies (since 2010) have investigated cells derived from an autologous cell source (15 out of 20 studies), whereas earlier studies had a similar number of studies with autologous ($n = 4$) and allo-geneic ($n = 5$) sources of cells.

**Implications for clinical practice**

There has been a steady increase in the number of studies published over the years (nine studies before 2010 and 20 studies published in the period 2010–2016). There is now an increasing body of evidence that administration of stem cell therapies in patients in different phases of stroke is feasible and encouragingly safe across different routes of administration. The key objective of early-phase clinical studies is to prove the concept and investigate preliminary safety of use in humans. To that end, our review and meta-analyses support the feasibility and safety of varied cell types delivered through different routes.

However, the strength of evidence to support effectiveness of these therapies is not robust. This is a challenge often seen in the early phase of development due to the small size of the studies typical at this stage. The direction of change indicates a potential benefit, which is consistent across both groups of studies (with or without a control comparison) and across outcome measures representing changes at the level of body function/impairment and those focused on daily activity and quality of participation in daily life. To gain stringent, clinically meaningful data as to potential benefit will require further research through well-designed phase 2/3 studies.

**Implications for research**

With early-phase clinical studies investigating stem cell therapies reporting encouraging results, the field seems set to move into a phase where definitive effectiveness
assessment becomes critical. The translational success with stem cell therapies in stroke, exciting as it may be, has posed questions that need addressing. The present review provides assurance for probable safety of cell therapies in patients with stroke and potential for further research. Our meta-analysis at this early phase of research is limited by significant heterogeneity in trial design and therapeutic strategies researched in these studies. However, the results of the analysis provide an early indication of potential benefit that should be
explored through further research. This is necessary to avoid costly failures as in the past with neuroprotective interventions in stroke. Currently there is persistent ambiguity regarding clinical meaningfulness of interventions, despite increasing volumes of research data. Most importantly, the review reiterates the need to conduct adequately powered studies using well-characterised cell therapy products and investigating impact on standardised clinical recovery outcomes.

Stem Cell Therapies as an Emerging Paradigm in Ischemic Stroke (STEPS I/II/III) formulated recommendations on quality standards for pre-clinical and clinical research involving cell therapies [48, 49]. While these represent a much-needed framework to standardise regenerative research in stroke, most of the published studies had started prior to formulation of these guidelines. In fact, the challenges in design, feasibility and ethical aspects of these studies provided the impetus for the formulation of these recommendations to a significant extent. The ability to characterise the cells under investigation has been enhanced significantly with increased capabilities in immune phenotyping and molecular transcriptional profiling of investigational cell types. Recent studies have investigated more selective cell types as compared to earlier ones, which used naïve cells predominantly. These studies have referred to prior evidence of safety and impact on structural, functional and imaging parameters in rodent models in most instances. However, it is pertinent to note that the extrapolation of these findings may not always be straightforward. Numerous factors such as the differences in pathophysiological mechanisms of stroke between rodents and humans, the interplay of co-morbid conditions in humans and the current dearth of evidence for the impact of stem cells in animal models simulating such baseline characteristics need further investigation.

Two studies [18, 27] investigated cell disposition using cell labels (99mTc and CD34-nano-iron complex) and reported variable homing and persistence of labelled cells in the brain. The study by da Fonseca et al. [18] also demonstrated distribution to other organs following IA administration. Numerous other studies have also reported extra-cerebral distribution of stem cells following IV/IA administration, which may have potential impact on eventual dosing and safety [18]. While these tracers might provide an indication for the initial distribution, they are limited in their ability to provide long-term information relevant to the lifetime of the implanted cells. Multimodal fate imaging using bioluminescence and fluorescence imaging with functional MRI has generated evidence for use for long-term viability and biodistribution of stem cells [50]. This can potentially inform the period of safety follow-up considered adequate in early-phase research. For instance, a safety follow-up of 6 months is considered adequate for mesenchymal cell types, while a period of at least 1 year is recommended for most cell types [41, 49].

**Study design—future considerations**

STEPS III proposed that the inclusion criteria for phase 2/3 studies should be structured based on properties of the cell therapy under investigation, particularly if there are any safety signals detected in pre-clinical and phase 1 studies. While this is evidently sound science, it may be important to note here that the predominant proportion of phase 1 studies have failed to detect any obvious cell-dependent adverse events, specifically linked to a particular cell type. Exclusion of patients with significant co-morbidities might still therefore be required in the interest of safety, although this approach would limit extrapolation of eventual results to the general stroke population.

However, an issue of greater clinical relevance is the selection of trial endpoints in phase 2/3 studies. While recommendations from expert groups involved in stroke research have been highlighting the need to validate and adopt domain-specific endpoints, its true utility can only emerge if domain-specific endpoints are used to power these studies. There is increasing evidence validating the usefulness of domain-specific measures in quantifying and predicting potential trajectory of recovery [51]. However, for these to be more consistently utilised the following issues may need to be addressed.

The objective of phase 3 studies has traditionally been to prove effectiveness in as broad a proportion of the target population as is feasible considering evidence from pre-clinical and early-phase studies. Considering the heterogeneous nature of stroke, it may be more meaningful to investigate stem cells in specific areas of impairment caused by stroke. This may necessarily restrict patient inclusion to specific disability, but may provide more specific domain-centric outcome measures.

However, such study designs may face challenges from regulatory authorities who prefer studies to be powered to established global endpoints, discouraging the developers from choosing such endpoints. This is borne out by the present review, where most studies have reported temporal changes in NIHSS/mRS/B1. There is therefore an urgent need for researchers, clinicians and regulators to collaborate to review evidence on domain-centric out-come measures and provide guidance on how these could be incorporated in future trial design.

In addition, it is important to consider the unique pharmacodynamics of stem cells in the post-ischaemic microenvironment in the brain. The engrafted cells and consequent activation of paracrine pathways are potentially unique to the individual area and severity of ischaemic injury in a given individual. Even though the...
broad mechanistic direction of repair and plasticity may be similar across individual patients, the interactions between cells and target brain tissue are determined uniquely by an individual’s genotypic and phenotypic particulars. Thus it is reasonable to postulate that the individual’s natural course of recovery can impact the quantum of change seen in functional/structural outcomes [52]. Emerging data from the field of rehabilitation research have put forth an interesting concept of ‘the Maximum Proportional Recovery Rule’, which proposes that 70% of maximum possible change (i.e. spontaneous recovery) occurs in the first few months post stroke. Recent data support the applicability of this rule across different domain-specific impairments [53]. Potentially useful prescriptive algorithms that can plot a prognostic trajectory for this recovery by combining clinical, neurophysiological and neuroimaging data are being developed [54]. These algorithms, if validated across domain-specific populations, may provide a practical tool for stratifying patients into more homogeneous subgroups.

Because the effectiveness of rehabilitation and cell therapies may be driven by unique patient characteristics differentially, it may be pragmatic to consider delivery of stem cells accompanied by targeted rehabilitation as an ‘intervention package’ using a service delivery premise. The measures of effectiveness with such restorative interventions are often continuous variables that require definition of the minimal clinically important difference (MCID) that is acceptable to prove benefit. The necessary next question is whether the conventional randomised controlled design is the ‘best fit’ for generating data to inform clinical practice in this fast evolving field.

Cluster randomisation with factorial design to incorporate multiple interventions (i.e. stem cell transplantation and rehabilitation) may be a pragmatic design to consider [55]. Study design can incorporate clusters of patients defined by domain-specific impairment receiving targeted, standardised rehabilitation in addition to stem cells. The effectiveness can then be assessed in terms of quantum of change on domain-specific endpoints.

An equally important area of research is defining the time points in stroke evolution more consistently in line with emerging tissue and imaging evidence. The chronic phase of stroke represents the area of greatest unmet medical need. However, it is interesting to note that while there are increasing data from rehabilitation and stem cell research in chronic stroke, the clinical determination of stroke as ‘chronic’ is heterogeneous, making any comparison/pooling of data difficult.

Limitations
The findings of the present review and meta-analysis should be examined in the light of a number of study limitations. First, high levels of heterogeneity were observed across studies, which differed in terms of therapeutic characteristics such as route of administration, timing after stroke and dose. We acknowledge this limitation and therefore have been conservative in our pooling and in our analysis techniques. Unfortunately, there were too few studies to explore these factors as potential sources of heterogeneity either through subgroup analysis or meta-regression. As a result, we are unable to draw any inferences about the optimum dose or route of administration. Such investigations may be more feasible as further studies appear in the literature.

Second, most of the studies included had small numbers of patients which may have resulted in small study effects, particularly in single-arm studies where the samples rarely reached double figures [56]. Small sample size is expected in early-phase research but this made any additional subgroup analysis unfeasible. Third, potentially relevant studies had to be excluded because of the lack of published information and non-availability of the additional information on request. Lastly, language bias remains an issue as we searched only English-language databases and journals.

Conclusions
This review and meta-analysis provides further evidence for the safety and feasibility of cell therapies for stroke. There is reasonable evidence to suggest feasibility, safety and potential effectiveness of these therapies. In view of the heterogeneity of disease per se and the nascent characterisation of therapies, the review poses important questions that are critical to translational success. Further progress in this field will require execution of phase 2/3 clinical trials with study designs that ensure homogeneity of stroke characteristics, potentially with domain-specific characterisation of disabilities and targeted provision of rehabilitation and with appropriate robust control to answer the fundamental question of effectiveness.

Additional file

| Additional file 1: Search Strategy. (DOCX 20 kb) |

Acknowledgements
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Availability of data and materials
All data generated or analysed during this study are included in this published article (and its supplementary information file).
Authors’ contributions

AN created the protocol and conducted the data search, selection, extraction, comparisons and meta-analyses and data synthesis. FC conducted the preliminary data search. FCC was involved in the protocol design, data search, selection and extraction. SHo, SHi, MAH-B and SAK provided inputs into the protocol design, meta-analyses and synthesis of results. AN and SHo performed data comparisons and meta-analyses. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Published online: 30 August 2017

References


3.5 Conclusion

This review and meta-analysis supported the overall safety and feasibility and potential effectiveness of CTs for stroke. In view of the heterogeneity of the disease per se and uncertainty about the optimal type, dose and delivery route of these therapies, the review highlighted the need to adopt pragmatic study design strategies. This would enable generation of clinically relevant data in homogenous subpopulations and matched controls, based on domain-specific characterization of disabilities, standardized co-delivery of rehabilitation and selection of aligned outcome endpoints appropriate to answer the fundamental question of effectiveness and progress forward in clinical development. Adoption of these aspects can accelerate legitimate research and facilitate participation of patients in research. These in turn may provide an effective deterrent to the existing risk that vulnerable patients face, of being misinformed about the unsubstantiated efficacy and safety of poorly characterised stem cell treatments offered at stem cell clinics across the world.
4.1 Background

The inherent complexity of CTs and the still-evolving understanding of their mechanistic pathways of action are challenging to understand especially when used in the context of heterogeneous diseases such as stroke (146). While conventional small molecule drugs and, to some extent, biologicals have extensive characterization and well-regulated pathways of oversight, CTs have an unpredictable developmental pathway because of their dynamic structural and functional profiles (417). The fact that there are very few candidate CT products that have progressed beyond the initial exploratory clinical studies in human subjects corroborates this lack of predictability (418).

In recent years, feedback sought from researchers in academia and industry concerning challenges in the development of regenerative medicine products has highlighted the lack of awareness and understanding of regulatory pathways as a significant deterrent to progress in this field (419). This seems to be more prominent amongst academic researchers, which potentially explains the hitherto unfulfilled opportunity afforded by numerous successful clinical studies published in this field (419). Timely and practical access to expertise in regulatory science is critical for clinician-scientists, who are still the predominant drivers of translational research with CTs (419).

4.2 Research Objective

The key elements critical to the execution of early phase studies in CTs in ischaemic stroke were analysed and described in a narrative review.
4.3 **Methods**

4.3.1 **Search Strategy**

The following databases were searched for identifying relevant studies: PubMed/MEDLINE, Google Scholar, Scopus, Web of Science, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Cochrane and websites of regulatory bodies in various countries and legal literature databases such as AustLII and Thomson Reuters Westlaw. The reference list of all identified reports and articles were hand-searched for additional studies. The search included papers published until April 2016. A range of keywords and index terms for the search included: ischaemic stroke; stem cells; cell therapies; regulatory policy; public policy; and regenerative medicine.

4.3.2 **Selection of Literature**

The first round of screening involved the scanning of titles and abstracts of identified studies and reports. Following this, full texts of the selected literature were assessed in the context of their relevance to CTs research.

4.3.3 **Data Extraction and Analysis**

The primary author completed the extraction process independently. It was reviewed for comprehensiveness and appropriateness by the review team. Synthesis of key themes emerging from the literature was undertaken and a descriptive analysis presented in the narrative review.

4.4 **Results**

The narrative review was published in *Advanced Drug Delivery Reviews* in 2016.
# Statement of Authorship

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| Submitted for Publication  
| Accepted for Publication  
| Unpublished and Unsubmitted work written in manuscript style |
| Publication Details | Advanced Drug Delivery Reviews |

## Principal Author

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## 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.

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Stem cell therapy clinical research: A regulatory conundrum for academia

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ABSTRACT

The encouraging pace of discovery and development in the field of regenerative medicine holds tremendous potential for bringing therapies to the clinic that may offer meaningful benefit to patients, particularly in diseases within or suboptimal therapeutic options. Academic researchers will continue to play a critical role in developing concepts and therapies, thus determining whether regenerative medicine will be able to live up to this potential that clearly excites clinicians, researchers and patients alike. This review summarises recent developments in regulatory frameworks across different countries that aim to ensure adequate oversight of the development of regenerative medicine products, which are unique in structural and functional complexity when compared to traditional chemical drugs and fully characterised biological drugs. It discusses the implications of these developments for researchers aiming to make the challenging transition from laboratory to clinical development of these therapies and considers possible pragmatic solutions that could accelerate this process that is essential to maintain research credibility and ensure patient safety.

Keywords:
Cell therapy
Regenerative medicine Academic research Regulatory policy Innovative medical practice

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1. Introduction

Regenerative medicine has come to be a widely accepted term for the varied research efforts made in the last few decades to understand the basic science underlying regeneration of human tissue, organs and cells and translate this growing knowledge into potential therapeutic modalities for diseases hitherto not amenable to management or possible cure [1]. Stem cell therapies hold the potential to provide effective disease modification and possible cure for these diseases that have posed a tremendous challenge to clinicians and a heavy burden in terms of impaired quality and quantity of life for patients [2]. These diseases are associated with a burgeoning cost of suboptimal care for the community and the healthcare system. Successful regenerative medicine has the potential to address all these issues [3].

Numerous stem cell therapies are currently in an exciting but critical clinical translational phase of development, as borne out by clinical trial registries across the world. As of June 2016, there are 4479 studies investigating use of different cellular therapies in various disease indications, of which 998 are industry-sponsored studies, as shown by listings on the National Institute of Health global clinical trial registry 1. In stroke, for instance, of the 48 ongoing studies, 18 are sponsored by industry 1. It is interesting to observe that most ongoing research to date has initially been conducted in academic institutions. Industry has until recently, adopted a very cautious attitude in terms of involvement in development of these therapies [4]. Basic research is yet to provide broadly acceptable answers to key questions concerning structural and functional characterisation of different cell therapies. The challenges posed in terms of regulatory uncertainty and potential commercialisation models have meant that the key drivers in this field have been academic institutions and small to medium enterprises. Lack of experience in addressing regulatory requirements and limited financial and human resources often challenge such entities. Ascell based therapeutics move into clinical translation phase, these issues assume critical significance as failure to address these efficiently can be a significant roadblock in procuring funding and approval for meaningful clinical studies critical to ensuring accelerated translation in this field [5].

The inherent complexity of stem cell products and the still evolving understanding of the basic science underlying their mechanistic pathways of action pose a difficult challenge, especially when applied to chronic diseases where there is still an incomplete understanding of disease pathophysiology. The characterisation of chemical drugs has been relatively well understood, leading to advanced standardisation and regulation. However, the structural characterisation and mechanism of action for cellular products is poorly understood presently. Additional work around the validation and global standardisation of preclinical efficacy assays is needed, which makes these therapies not amenable to standard pharmacokinetic characterisation. This unfortunately makes the regulatory pathway difficult and unpredictable. These aspects create multiple challenges for scientists involved in the development of such therapies as they navigate their way through the complexity of development [6].

In recent years, feedback sought from researchers in academia and industry concerning challenges in the development of regenerative medicine products has highlighted the lack of awareness and understanding of regulatory pathways as a significant deterrent to progress in this field [7-9]. This seems to be more prominent amongst academic researchers, which may potentially lead to the loss of many innovative developments in this field [7-9]. In light of the frantic pace of scientific advancement in molecular biology and its application in the area of regenerative medicine, it becomes even more critical that speedy, accurate and practical access to expertise in regulatory science is made available to academic researchers and clinicians, who are still the predominant drivers of translational research in the field of regenerative medicine. Recognition of this need for mechanisms for interdisciplinary collaboration is likely the first step towards accelerating the future pace of development of regenerative medicine.

In this review, we provide a concise description of key developments in regulatory pathways in regenerative medicine across the globe, aimed particularly at researchers in academic settings. This will enable an expanded understanding of the key challenges faced in the development of regenerative medicine products and provide a summary of approaches initiated to address them.

2. Regulatory pathways in different jurisdictions

2.1. United States of America

The Food and Drug Administration (FDA) in the United States has been issuing guidance periodically for development of human cells, tissues, and cellular and tissue-based products (HCT/Ps) for clinical use utilising a tiered, risk-based approach [10]. The extent of FDA oversight required in the development process is dependent on two key considerations: the level of cell manipulation (minimal more than minimal) and the intended use of cell therapy (homologous/non-homologous) [11]. An HCT/P is regulated under section 361 of the Public Health Service Act 1944, which entails an abbreviated review, if it is minimally manipulated, is intended for homologous use only and does not involve the combination of the cells or tissues with other materials, which may raise new clinical safety concerns. The products that undergo more than minimal manipulation and/or are used in a non-homologous manner are deemed ‘biological products’ (Fig. 1). These undergo an extensive development process with the approval of clinical trials in humans requiring compilation of pre-clinical evidence, the submission of an Investigational New Drug Application (IND) and the submission of a Biologics License Application (BLA) under section 351(f) of the Public Health Service Act 1944 and related regulations.

The FDA issued ‘Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products’ in 2015 [12] providing recommendations for clinical translation of HCT/Ps that fulfil the criteria for being a biological drug product, thereby requiring regulatory oversight under section 351 (Table 1). These recommendations pragmatically acknowledge the fact that the distinctive characteristics and feasibility challenges with these products influence the design considerations of early-phase clinical trials of HCT/Ps. The recommendations also acknowledge the limitations in the extrapolation of pre-clinical data to inform early phase study design especially in context of highly humanised or species-specific cell based products. In addition, the FDA developed recommendations for preclinical assessment of cell therapy products, which reflect the authority’s openness to move beyond the established pre-clinical guidance based on small molecule therapies, supported by reasonable and scientifically sound evidence [13].

These recommendations lay particular emphasis on the characteristics of cell therapy products such as their ability to express molecules and factors that affect and are in turn, affected by the local microenvironment, and their ability to migrate and differentiate in vivo into undesired cell types. In addition, the impact of potential viral vector contamination and any adventitious therapy/intervention (e.g. immunosuppression/invasive procedures/combination therapies) needs to be evaluated in detail to ensure the safety of potential research participants in clinical trials.

The FDA recognises that the challenges with manufacturing these products may determine feasible doses and emphasises potential issues with the variability within different lots of the products. The guidance underscores the importance of establishing and maintaining GMP standards early in development of the product.

Whilst the principal intent of early phase trials is the assessment of safety and feasibility, as most cell therapy products are likely to be investigated in disease populations to justify the risk inherent in these therapies, the recommendations encourage preliminary assessment of efficacy and obtaining ‘proof of concept’ data in humans in early phase trials to better inform further development. To that end, activity
assessments in the trial design are required to be detailed and justified in the context of the given disease indication and cell therapy product. The FDA guidance on Good Tissue Practice (GTP) requirements for cell therapies provides safeguards to minimise the risk of communicable disease transmission by HCT/Ps and prevent contamination during manufacturing [14]. Depending on whether the product is autologous or allogeneic, additional requirements such as donor screening may also be applicable.

2.2 European Union (EU)

In Europe, cell therapies are evaluated under Advanced Therapy Medicinal Products (ATMP), which include three major types of products specifically, gene therapy, somatic cell therapy and tissue engineered products, as set out in Regulation (EC) No. 1394/2007 and Commission Directive 2009/120/EC amending Directive 2001/83/EC [15,16]. These regulations lay down additional scientific and technical requirements regarding the testing of ATMPs for human use. The regulations deem that these therapies be considered as drugs if they are intended for non-homologous use and have undergone substantial manipulation. In this case, the relevant guidelines for development of biological therapies will be applicable (Fig. 2). The European Medicines Agency (EMA) recognises that evaluation of ATMPs requires specific expertise and has set up a Committee for Advanced Therapies (CAT) to evaluate the quality, safety and efficacy of each ATMP. The CAT acknowledged that studies for generating quality and nonclinical safety data for ATMPs are often conducted either by small and medium-sized enterprises or in academic institutions [17]. The EMA framework proposes a system of evaluation and certification wherein the CAT, independent of any marketing authorisation application, can evaluate the data from such studies. It also evaluates the implementation of Good Clinical Practice (GCP) and/or Good Laboratory Practice (GLP) in these studies, which may facilitate future application for clinical trials and marketing authorisation application based on the same data.

The EU regulatory pathway recognises the need to incorporate flexibility in evaluation of the manufacturing of these products in light of the specific technical characteristics of advanced therapy medicinal products [18]. Thus, whilst decreasing that the manufacture of advanced therapy medicinal products should be in compliance with the general principles of Good Manufacturing Practice (Commission Directive 2003/94/EC) [19], there are recommendations to develop guidelines specific to ATMPs to accurately reflect the specific challenges in their manufacturing process.

2.3 Japan

Japan’s new regenerative medicine legislations aimed at accelerating the development of regenerative medicine products came into force in November 2014 (Fig. 3). The Pharmaceutical, Medical Devices and Other Therapeutics (PMD) Act 2014 will regulate the commercial development of regenerative therapeutics [20]. The act lays down provisions for accelerated approval, contingent on provision of early safety and indicative evidence of therapeutic benefit of an investigational therapy studied in well-designed Phase 1/2 trials, after review by the Office of Cellular and Tissue based Products within Pharmaceutical and Medical Devices Agency (PMDA) [21]. The seven-year period following conditional approval entails mandatory capture and provision of in-clinic data on efficacy and safety through defined mechanisms. By the end of this period, the sponsor either applies for final marketing approval (the equivalent of a Biologic License Application [BLA] in the US) or withdraws the product.

In addition, the Act on the Safety of Regenerative Medicine 2014 (ASRM) defines the pathway to oversee the cell therapies administered in medical practice using processed cells and in the context of academic clinical research [22]. The law has enabled a pathway of oversight that

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Fig. 1. Oversight considerations for cell based therapies in United States of America.
involves a tier-based, risk-dependent analysis and enables accreditation of cell processing centres for safer and resource efficient manufacturing. The institution that provides the cell therapy has to report annually on safety evaluation and scientific acceptability of these products to the Committee for Regenerative Medicine and Ministry of Health, Labour and Welfare.

2.4. Canada

The Biologics and Genetic Therapies Directorate, Health Canada, provides regulatory oversight for the clinical development of regenerative medicine products (Fig. 4) as stipulated mainly by the Fox and Drug Act 1985 [23]. Safety of Human Cells, Tissues and Organs for Transplantation Regulations 2007 (CTO Regulations) [24] and the Guidance document - Safety of Human Cells, Tissues and Organs for Transplantation Regulations 2013 [25]. The CTO Regulations currently regulate minimally manipulated cell and tissue products that are intended for allogeneic and homologous use only.  

Health Canada reviews the conduct of clinical trials investigating the use of these products in human subjects. It also issued a ‘Guidance document for Preparation of Clinical Trial Applications for use of Cell Therapy Products in Humans’ in 2015 [26], that emphasised the need for appropriate characterisation and adherence to GMP requirements early in development and highlighted the need for proactive discussion to address ambiguity in this regard. Institutional human research ethics boards review clinical research in accordance with the ‘Tri-Council Policy Statement (TCPS): Ethical Conduct for Research Involving Humans’, as well as other local and international guidelines. In 2014, the TCPS was amended to incorporate the ‘Guidelines for Human Pluripotent Stem Cell Research’ formulated by the Canadian Institute of Health Research (CIHR), which requires approval by a Stem Cell Oversight Committee for research activity funded by one of the national research funding agencies [27].

2.5. Australia

In Australia, cellular therapy products are regulated under the ‘Australian Regulatory Guidelines for Biologicals’ released by the Therapeutic Goods Administration (TGA) in 2014 [28] (Fig. 5). ‘The National Statement on Ethical Conduct in Human Research 2007’ updated in 2015 provides guidance for clinical research undertaken in Australia [29]. The Prohibition of Human Cloning for Reproduction and the Regulation of Human Embryo Research Amendment Act 2006[30], place further stipulations specific to research with embryonic stem cells. Whilst materials used in initial clinical research may be exempted from requirements for GMP, the TGA requires that investigational products used for phase 2 and 3 clinical trials meet GMP requirements [31].

Clinical Trial Notification or Exemption pathways (CTIN/CTX) enable access to investigative products in the context of clinical trials [32]. Whilst CTIN entails only a notification to the TGA, CTX mandates a complete review by the TGA before a trial can commence. The sponsors of the trials (either academic or industry) determine, in consultation with institutional human ethics review committees (HERCs) and
Fig. 3. Oversight considerations for cell based therapies in Japan.

Fig. 4. Oversight considerations for cell based therapies in Canada.
relevant authorities, the most appropriate pathway dependant on the understanding of risk involved with a given investigational therapy. The primary accountability for oversight of research activities still vests with the relevant institutional authority and HERCs.

In the context of cell therapies, HERCs often may not have sufficient local expertise to review such research proposals. Bodies such as the Advisory Committee on Biologics (ACB) and the Gene and Related Therapies Research Advisory Panel (GTRAP) are available to provide such expertise.

The autologous use of stem cell therapies, in the context of provision of a single course of treatment with self-donated cells provided or manufactured under the supervision of a medical practitioner who has the overall ‘management of care’ responsibility for the patient, is not regulated by the TGA as specified in the Therapeutic Goods (Excluded Goods) Order [33]. The Medical Board of Australia and the Australian Health Practitioner Regulation Agency (AHPRA) in accordance with the Health Practitioner Regulation National Law, 2009, currently regulate provision of such therapeutic options under ‘innovative clinical practice’. In 2015, the TGA released a discussion paper with possible options for regulation of such cell therapies that range from continuation of the current pathway to a complete overhaul by the TGA, as implemented in context of other biological drugs [34]. The discussion paper sought to obtain public opinion on the need to create a framework for regulating autologous stem cell therapies, some of which are provided in private non-academic and for profit stem cell clinics in Australia at substantial costs to the patient, with limited scientific basis and usually without preclinical safety or efficacy studies.

The lack of a clearly defined pathway for use of either autologous or allogeneic stem cells in humans within federal legislation framework in Australia presents a confusing situation for investigators. Unfortunately, this uncertainty has the impact of slowing progress in regenerative medicine research in Australia. It also provides an opportunity for non-scientific use of stem cells in clinical practice in Australia, which can be detrimental for patients and this young field’s reputation.

3. Challenges for academic research – considerations for the future

3.1. Harmonisation across different regulatory bodies: challenges in standardisation of terminology

Whilst the individual regulatory bodies around the world are continuously evolving their oversight frameworks, certain common themes are emerging across these different regulatory environments, which may be the starting point for future harmonisation. Such efforts for convergence, if successful, would be key contributors to future acceleration in the translation of advanced cell/gene therapies to the clinic.

The definitions of key attributes in cellular therapies such as ‘minimal manipulation’ and ‘homologous/non-homologous use’ have hitherto varied in scope across different jurisdictions. Whilst different agencies, notably FDA and EMA, are clarifying these terms [18,35], there is need for convergence of these definitions across regulatory jurisdictions. This could prove a significant enabler for the success of multinational research collaborations that are critically required at this stage of translation with most cell therapies. This is borne out by the fact that sharing of expertise and new knowledge being generated is cited by most stakeholders as the need of the hour [36,37]. However, practical success in this direction can only be made if operational uncertainties due to divergent regulatory environments can be decreased, thereby creating opportunities for researchers to collaborate across the globe.

At present, a significant proportion of cell therapy products under investigation represent heterogeneous populations of cells rather than a high purity single population of cells. Given the unique attributes of these therapies that make them straddle the boundaries between biological drugs and surgical/transplantation products, developers will certainly benefit from working together and learning from experts through practical examples and innovative approaches towards characterisation and standardisation. Establishment of such research networks can also increase the commercial attractiveness of these therapies, potentially increasing the much-needed investment from industry. The majority...
of product development research involving cell therapies is occurring in academic centres involving scientists and clinicians. These academic researchers often lack the technical, financial and human resources to navigate the necessary but complex regulatory process required for clinical translation of these therapies.

In the last few years, studies from the US, Canada and UK have reported on the level of awareness and understanding of the existing regulatory framework as applicable to regenerative medicine amongst clinician scientists and academic researchers [7,8]. These studies have indicated consistently that whilst there is an elementary awareness of possible regulatory requirements, most academic research teams find the current regulatory pathways difficult to understand and implement in their development projects. This clearly can pose significant challenges to clinical translation and eventual commercialisation.

Researchers, whilst accepting the importance of regulations to ensure safety and quality of regenerative medicine products, have indicated a need for modifying the product testing and quality requirements applicable to cell therapies. The complexity in the application of often difficult to understand regulatory requirements, the ambiguity regarding the level and extent of evidence needed regarding effectiveness and safety and the classification of investigational products is often overwhelming for academic research teams. These teams usually lack the regulatory and quality control and assurance expertise available in industry. The enthusiasm to advance these therapies that potentially hold significant benefit for patients has been seen researchers move into clinical trials with less than complete understanding of the biology of the therapy with attendant implications for safety. However, an important factor with cell therapies is the fact that their duration of action is potentially life long and irreversible. In this context, it is imperative to create global networks for knowledge sharing that may enable research teams to complement each other towards a more comprehensive understanding of different cell types in terms of their molecular and functional characterisation. This would provide critical opportunities to avoid repetition of negative experimentation. Creation of research consortiums focused on particular stem cell types similar to the disease specific research networks that currently exist could potentially enable inter-and cross-disciplinary exchange to address components of the development process that are common within and across the varied disease indications in which clinical application is investigated. This is likely to increase the efficiency and success of researchers in this field tremendously. However, for this to be a practical reality, government, legal and regulatory structures have to evolve pragmatically to create these platforms for academic or industry-led research whilst ensuring protection of intellectual property and the ability to publish innovative findings in high impact journals.

Importantly, we propose that basic scientists and clinical research teams should be actively supported to seek access to their local regulatory agencies early in the development of potentially new regenerative medicine therapies. The documents cited in this review provide starting points for future dialogue. Most regulatory agencies are very willing to speak with academic research groups. Whilst it has been possible to administer investigational product that was manufactured without demonstration of GMP compliance in Phase 1 studies, it is critical for researchers to understand that they will be unable to move beyond Phase 1 without transition to GMP compliance, a step that can prove expensive in terms of time and money.

With respect to the preparation of stem cells for human use, it is also important for research teams to understand the importance of foetal calf serum in manufacturing processes, there are other important issues. For example, murine sourced monoclonal antibodies used in cell selection may be a significant problem.

Regulatory agencies can facilitate the transition to clinical trials for academic and clinical researchers by establishing single point contacts for advice and providing this information on their websites. The regulatory agencies have been participating in information sessions on basic regulatory and safety requirements at clinical and research meetings. However, given the complexities of regenerative medicine products, a system that enables early, product-specific dialogue that could start at the beginning of product development, could go a long way in building regulatory competence amongst developers, especially those from the academia.

### 3.2. Access to expertise in manufacturing

Stakeholders in this field agree on the need for established technical standards, in terms of quality, safety and efficacy, through demonstrated adherence to GLP, GMP and GCP whilst conducting research through pre-clinical and clinical phases [38]. Having said that, the challenges in establishing appropriate quality standards are underscored by the fact that most agencies have committed to developing guidance specific to advanced medical therapies. The

### Table 1

<table>
<thead>
<tr>
<th>Clinical trial design considerations</th>
<th>Early-phase trial objectives</th>
<th>Choosing a study population</th>
<th>Safety, feasibility assessment, dose exploration, activity assessment</th>
<th>Choice of healthy volunteers not appropriate in most studies</th>
<th>Assessment of the overall benefit-risk profile in diseased population depending on severity of disease</th>
<th>Impact on interpretability of study outcomes</th>
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<tr>
<td>Control group and blinding</td>
<td>Demonstration of lack of other treatment options</td>
<td>Concurrent control group and blinding are generally not as critical but are useful especially in diseases where the natural history is not clearly understood</td>
<td>Blinding may not be feasible in certain therapies and risk of placebo/sham interventions may be unacceptable.</td>
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<tr>
<td>Dose and regimen</td>
<td>Important to collect data on characteristics of the administered product and clinical outcomes to enable correlative analyses to help in dose definition.</td>
<td>Repeated dosing might not be an acceptable risk in most studies until availability of preliminary data on the product’s toxicity and duration of activity.</td>
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<td>Treatment plan</td>
<td>Staggered administration within a cohort or between cohorts - the staggering interval should be long enough to monitor for adverse events prior to treating additional subjects at the same/increasing dose.</td>
<td>Cohort size determined by safety considerations and manufacturing capacity.</td>
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<td>Monitoring and follow-up</td>
<td>Assessments targeting specific safety issues that could be anticipated with these products; pre-defined study stopping rules.</td>
<td>Duration of follow-up period in which the product might reasonably be thought to present safety concerns should be justified.</td>
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**FDA guidance for industry: Considerations for the design of early-phase clinical trials of cellular and gene therapy products - key facts.**
unique and incompletely characterised structural and functional attributes of these therapies are challenging for regulators and developers alike, especially when they are more familiar with fully characterised chemical and biological drugs, rather than living cells. This is clearly an area of regulatory science, that needs to expand exponentially and interactively to keep pace with scientific progress in product discovery [39]. Research to identify key functional characteristics and possible biomarkers of efficacy, potency and safety risks such as tumorigenicity and unwanted biological effects requires long-term support, probably best done through institutions with basic science and commercialization expertise. Regulatory agencies can potentially facilitate widespread access to the findings of such research by exploring pragmatic ways to share those aspects, which have generic significance to these therapies whilst protecting intellectual property. This may contribute to translational success and ensure safety of the patients participating in clinical trials [40]. Ensuring GMP compliant manufacturing of cell therapies for the clinical phase of development is perhaps the biggest challenge for academic research teams seeking to translate their research clinically. The complexity of the cellular products often results in product characterisation and associated assays and standards being developed at the same time as the product itself. On the other hand, the regulatory reviewers are themselves at a stage of learning about the application of existing GMP requirements, the body of evidence that is needed in the context of a given product and what is practically feasible in light of the current stage of technology. This understandably protracts the decision-making process and adds to the time and money required. The move from a small-scale laboratory to clinical grade production of investigational product is daunting, especially for investigators from academic institutions, predominantly due to lack of funding and access to expertise in regulatory science and manufacturing. The National Heart Lung and Blood Institute (NHLBI), National Institutes of Health (NIH) led the way to address this issue by establishing the Production Assistance for Cellular Therapies (PACT) program in 2003 which provides (i) clinical product manufacturing support for phase 1 and 2 trials and (ii) translational development support, to enable the translation of laboratory-based techniques into GMP compliant production methods and Standard Operating Procedures (SOPs) for investigator initiated projects [41]. The fact that PACT has been able to support numerous projects with encouraging success and the present remit of its manufacturing support being limited to treatment of heart, lung, and blood diseases, begs for similar programs to be developed for diseases involving other organs (e.g. the brain) with significant disease burden such as stroke and neurodegenerative disorders. The CelICAN network, another promising example, was started in 2009 in Canada and incorporated as a not-for-profit corporation in 2014. The CelICAN presents a potentially replicable model of a national enterprise comprising different stakeholders such as academic researchers with demonstrated interest in the field, industry, clinicians, funding bodies and regulatory bodies. The network aligns the requirements for manufacturing facilities with expertise in processing cell products for clinical trials to ensure capacity, scalability and quality management processes to ensure efficient trial execution.

In 2016, EMA launched PRIME scheme [43] to enable early proactive regulatory dialogue between the applicant and the EU regulatory network regarding the development plan of therapies of ‘major public health interest’ and which represent significant innovation, through ongoing advice from relevant stakeholders. Whilst sponsors can apply to be considered for PRIME based on preliminary clinical evidence (proof of concept), EMA has expressed openness to provide exceptional earlier access to academia and small to medium enterprises if there is compelling nonclinical data (proof of principle) in a relevant model and first in human studies indicate adequate period of exposure for preliminary pharmacological and tolerability data.

3.3. Mechanisms for accelerated access: implications for cell therapies

The regulatory bodies across the world have developed different mechanisms for providing accelerated access to therapies, particularly in disease indications with current unmet need.

The FDA has put into place a number of potential pathways to decrease the time to market availability of therapies that serve clear unmet needs such as Fast Track Approval [44], Breakthrough Therapy Designation [45], Priority Review and Accelerated Approval [46]. The Fast Track process is aimed to expedite the review and in-clinic availability of investigational biological drugs and cell products for medical conditions with present unmet needs. Accelerated Approval allows for expedited review and approval based on data related to scientifically valid surrogate endpoints instead of established clinical endpoints. Breakthrough Therapy designation is the newest mechanism that is used if existing preliminary data on a therapy indicates substantial improvement in outcomes in a disease indication with an unsatisfactory therapeutic status quo. A number of cell-based therapies are currently being reviewed under the Fast Track Approval process.

An interesting development is the passage of ‘right to try’ (RTT) laws in certain states in the US. The RTT can potentially be pursued for individual ‘gravely ill’ patients for access to cell therapy products ahead of FDA-approval. The practical utility of these laws, however, has already been questioned as they are not applicable to current federal legislations and may draw resources away from efforts to develop effective treatments and may further complicate the FDA pathway for compassionate use of medications [47]. This also raises concerns in that some stem cell clinics are already willing to provide inadequately tested stem cell therapies utilizing the current gap in the capability of the FDA to ensure adequate regulation.

The EMA issued a ‘Guideline On Compassionate Use of Medicinal Products’ in 2006 which allows for access to potentially beneficial therapy to a group of patients ahead of regulatory approval but left its implementation to individual EU member states [48]. The European Commission introduced the ‘hospital exemption clause’ by the Regulation (EC) No.1394/2007 [49] for ATMPs that is applicable to those ATMPs that are prepared on a non-routine basis, i.e. individually prescribed, according to specific quality standards, for an individual patient. These therapies are provided in a hospital under the exclusive professional responsibility of a medical practitioner within the EU. This provides a mechanism to provide individual patient-centred care in relevant clinical situations, whilst ensuring product quality and patient safety (through the requirement of a system for patient and product traceability).

3.4. Dilemma named ‘innovative medical practice’

Growth of stem cell therapies, in particular autologous therapies, has brought forth an important issue that has defied consistent definition for many years [50]. The boundaries between research and innovative medical practice are being re-examined in the wake of an explosion in ‘stem cell clinics’ offering various cellular therapies in routine practice. Most of this activity involves the use of autologous fat-derived stem cells with little or no supporting basic scientific preclinical data. Provision of cell therapies under the guise of ‘innovative medical practice’ may encourage widespread adoption of therapies that may be ineffective at best and harmful at worst. Academic researchers are understandably very concerned with these practices, which can serve as a significant disincentive and message to the lay public that fundamental research is not required. Thus, these practices can challenge future scientific translational success in this field. Even now, the provision of cell therapies in such clinics is largely self-regulated in most parts of the world. Medical practitioners’ regulatory authorities in different countries, variably regulate this practice and lack adequate legislative, financial or skilled human resources to provide effective oversight for practice involving this fast advancing field [51].
The stem cell industry has burgeoned not only in the developing regions such as Mexico, China, India and south-east Asia but also in developed countries such as the US, Germany and Australia [36,52–55]. A recent report by Turner et al. indicates approximately 351 distinct ventures providing commercial cell therapy interventions at 570 centres across the US for a range of diseases [56]. This is a significant reflection on the limited success of the initiatives of the FDA, in recent years, towards increased involvement and the clear struggle for effective oversight of a fast expanding field. Berger et al. present an interesting perspective that the stem cell industry is a significant presence across the globe and not restricted to countries with poorly defined regulatory pathways [56]. In addition, an important consideration may also be that developed countries such as the US and Australia have higher numbers of clinics per capita, highlighting the ease with which access to such therapies is possible in these countries [57]. There is a formidable risk for patient safety, due to rampant lack of clarity on source and quality of cell therapies being administered at these sites. The aggressive and often misleading marketing of these therapies results in patients undergoing interventions with questionable safety and effectiveness [56].

Whilst the negative impact on patient safety is evident and increasingly being highlighted, the implications for research success have perhaps not garnered as much attention [56,57]. On the one hand, patients undergoing such interventions become unavailable or ineligible for bona-fide clinical research, which increases recruitment challenges and cost of research. On the other hand, the discredit resulting from adverse safety incidents in recipients of these services, severely undermines the confidence in the future potential for this research field. This has both immediate and far reaching impact on attractiveness of this research area for potential funding and investment in clinical translation from government and industry alike as well as the interest amongst academics to pursue research in this clearly emotive and polarised field. Clearly, this presents a cause for concern for all stakeholders in regenerative medicine and poses significant threat to future translational success in regenerative medicine.

A pragmatic attempt to address this issue, which, if successful, may provide a way forward, would be the establishment of a dedicated pathway for the oversight of research as well as routine medical practice. Japan has been highly progressive in this respect enacting the Act on the Safety of Regenerative Medicine that came into effect in 2014 and aims to provide oversight of provision of cell therapies in medical practice [21]. This pathway enables medical institutions to outsource cell culturing and processing to industry/centres with the required expertise and accreditation under much more streamlined process and mandates collection of necessary safety and efficacy data.

4. Conclusions

Regulation and innovation in clinical translation are closely linked and determine the success of commercialization of scientific discoveries. Changes in regulatory frameworks, often driven by scientific advances, can potentially enable or hinder innovation. Advancing regulatory policy and creating modalities for widespread access to this expertise, especially for academic research communities, will ensure robust interdisciplinary collaboration and foster shared learning critical to successful translation. As observed in different countries throughout the world, an environment of facilitative legislation is crucial. The hypothesis is that regenerative medicine may result in a paradigm shift in clinical medicine, not seen since the introduction of antibiotics. These therapies, if successfully translated into clinical practice, may provide meaningful options to society, currently reeling under the burden of diseases that take a substantial toll on lives of so many across the globe.

Acknowledgements

Stroke Research Programme, School of Medicine, Faculty of Health Sciences at The University of Adelaide, Australia provided support for the conduct of the research and/or preparation of the manuscript for publication.

References


[52] M. Murcie, M. Pera, Regulatory loophole enables unproven autologous cell therapy to thrive in Australia, Stem Cells Dev. 23 (Suppl. 1) (2014) 34–38.


Online resources:
4.5 Conclusions

Regulatory science has evolved in the last few years to keep pace with the research using CTs. The regulatory bodies across the world have sought to harmonise terminologies used in CT research and development. In addition, guidance is evolving regarding quality and manufacturing standards expected in such research. Future clinical studies need to adequately justify the rationale for choice made with regards to study design, CT production and delivery methods, and overall study monitoring and management to enable successful clinical translation.
CHAPTER 5: ETHICAL CONSIDERATIONS

5.1 Background

As clinical studies with CTs are planned, researchers are faced with the dual challenge of maximizing trial recruitment while supporting individuals’ informed decision-making. This is further complicated by the limited data on long-term safety and efficacy with CTs available in early phases of research. Patient participation in the conduct of research as an active stakeholder beyond being a passive recipient has been encouraged world over, to sharpen the focus of research design and implementation to increase relevance to the patient community and to develop channels for communication between researchers and the patient community.

5.2 Research Objectives

A qualitative interview-based study was carried out with stroke survivors to understand key ethical considerations that impact on the execution of early phase studies with CTs.

A qualitative thematic analysis was carried out to identify the perspectives of ischaemic stroke survivors on:

- the relevance and importance of an early phase clinical study such as TOOTH (The Open study Of dental pulp stem cells (DPSC) Treatment in Humans) using adult human dental pulp stem cells in chronic ischaemic stroke
- consent issues with participation in such research
- the relevance of the planned outcome measures to individuals who have had personal experience of ischaemic stroke.
5.3 Methods

The protocol for a proposed phase I/II study investigating autologous use of dental pulp stem cells in patients with ischaemic stroke (TOOTH Study) was published in the *International Journal of Stroke* in 2016. The PERSPECTIVES Study was designed to collect insights from stroke survivors via face-to-face, semi-structured interviews on specific aspects of the design of the TOOTH Study. The methodology and relevant ethics approval for the PERSPECTIVES study are described in detail in the manuscript in submission.
# Statement of Authorship

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<th><strong>Title of Paper</strong></th>
<th>TOOTH (The Open study Of dental pulp stem cell (DPSC) Therapy in Humans): Study protocol for evaluating safety and feasibility of autologous human adult dental pulp stem cell therapy in patients with chronic disability after stroke</th>
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| **Publication Status** | ✔ Published
- Submitted for Publication
- Unpublished and Unsubmitted work written in manuscript style |
| **Publication Details** | International Journal of Stroke |

## Principal Author

| **Name of Principal Author (Candidate)** | Anjali Nagpal |
| **Contribution to the Paper** | Conceptualisation of protocol, preparation of manuscript and submission for publication |
| **Overall percentage (%)** | 65 |
| **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: 08/01/2016

## Co-Author Contributions

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

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

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| **Contribution to the Paper** | Conceptualisation of protocol, Review of manuscript |
| **Signature** | Date: 08/08/18 |

| **Name of Co-Author** | Monica Anne Hamilton-Bruce |
| **Contribution to the Paper** | Conceptualisation of protocol, Review of manuscript |
| **Signature** | Date: 17/8/2018 |

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| <strong>Contribution to the Paper</strong> | Conceptualisation of protocol, Review of manuscript |
| <strong>Signature</strong> | Date: 09/08/18 |</p>
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<td>Christopher Levi</td>
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<td>Songluo Shi</td>
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<td>Leanne Carey</td>
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TOOTH (The Open study Of dental pulp stem cell Therapy in Humans): Study protocol for evaluating safety and feasibility of autologous human adult dental pulp stem cell therapy in patients with chronic disability after stroke

Anjali Nagpal¹, Karlea L Kremer¹, Monica A Hamilton-Bruce²,³, Xenia Kaidonis¹, Austin G Milton², Christopher Levi⁴, Songtao Shi⁵, Leeanne Carey⁶,⁷, Susan Hillier⁸, Miranda Rose⁷, Andrew Zacest⁹, Parabjit Takhar¹⁰ and Simon A Koblar³,¹¹

Abstract
Rationale: Stroke represents a significant global disease burden. As of 2015, there is no chemical or biological therapy proven to actively enhance neurological recovery during the chronic phase post-stroke. Globally, cell-based therapy in stroke is at the stage of clinical translation and may improve neurological function through various mechanisms such as neural replacement, neuroprotection, angiogenesis, immuno-modulation, and neuroplasticity. Preclinical evidence in a rodent model of middle cerebral artery ischemic stroke as reported in four independent studies indicates improvement in neurobehavioral function with adult human dental pulp stem cell therapy. Human adult dental pulp stem cells present an exciting potential therapeutic option for improving post-stroke disability.

Aims: TOOTH (The Open study Of dental pulp stem cell Therapy in Humans) will investigate the use of autologous stem cell therapy for stroke survivors with chronic disability, with the following objectives: (a) determine the maximum tolerable dose of autologous dental pulp stem cell therapy; (b) define that dental pulp stem cell therapy at the maximum tolerable dose is safe and feasible in chronic stroke; and (c) estimate the parameters of efficacy required to design a future Phase 2/3 clinical trial.

Methods and design: TOOTH is a Phase 1, open-label, single-blinded clinical trial with a pragmatic design that comprises three stages: Stage 1 will involve the selection of 27 participants with middle cerebral artery ischemic stroke and the commencement of autologous dental pulp stem cell isolation, growth, and testing in sequential cohorts (n=3). Stage 2 will involve the transplantation of dental pulp stem cell in each cohort of participants with an ascending dose and subsequent observation for a 6-month period for any dental pulp stem cell-related adverse events. Stage 3 will investigate the neurosurgical intervention of the maximum tolerable dose of autologous dental pulp stem cell followed by 9 weeks of intensive task-specific rehabilitation. Advanced magnetic resonance and positron emission tomography neuro-imaging, and clinical assessment will be employed to probe any change afforded by stem cell therapy in combination with rehabilitation.

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Introduction and rationale

Stroke represents a significant global disease burden with 33 million stroke survivors estimated worldwide in 2010, amounting to 4% of the total disability-adjusted life-years (DALYs).¹

Up to 85% of all strokes are ischemic in origin with the middle cerebral artery (MCA) being the most common site of occlusion.² All evidence-based treatments for ischemic stroke currently available target the acute phase: aspirin, management in an acute stroke unit, thrombolysis, and decompressive surgery.³ In the chronic phase, rehabilitation still remains the predominant therapeutic intervention.⁴,⁵

As of 2015, there is no chemical or biological therapeutic agent that has been proven in large clinical trials to actively enhance neurological recovery during the chronic phase after stroke. While there is some preliminary evidence for the use of selective serotonin reuptake inhibitors (SSRI) to promote functional recovery independent of depression, this still needs to be substantiated in large, well-designed clinical trials.⁶

Cell-based therapy

Cell-based therapy is at the stage of clinical translation globally, from preclinical studies to early phase human trials being conducted in many countries to investigate safety and feasibility in a number of neurological diseases.⁷⁻⁹ There is strong experimental evidence that stem/progenitor/precursor cell therapy may improve neurological function (Figure 1) through five mechanisms of action: neural replacement, neuroprotection, angiogenesis, immune-modulation, and neuroplasticity.¹⁰⁻¹³

In stroke, the predominant mechanism/s of action may be influenced by the timing of treatment. In the acute phase, neuroprotection, immune-modulation, angiogenesis, and neural replacement may play more important roles in restoring function. In comparison in the chronic stroke phase, improvement in neurological function is likely to be driven by neural replacement, angiogenesis, and neuroplasticity.⁹ Stem cells are now recognized to secrete a range of paracrine growth factors, which may modulate neurogenesis, angiogenesis, and immunomodulation.¹²⁻¹⁴

There are primarily three human tissue sources for stem cell treatments: embryonic, reprogrammed somatic cells, or adult organs. Pluripotent stem cells, namely embryonic or induced pluripotent stem cells, with their wide differentiation potential may also present a risk of tumor formation.⁹ On the other hand, multipotent stem cells, such as neural stem cells, mesenchymal stem cells, and dental pulp stem cells (DPSC) have a limited differentiation potential, which makes them potentially safer for clinical use with no authenticated risk of tumor formation to date.⁹,¹⁵⁻¹⁸

There has been extensive preclinical research with respect to stem cell therapy in animal models of ischemic stroke. A systematic review of published animal rodent studies using stem cell-based therapy reported an overall improvement in neurobehavioral outcome of 40.6% (37.1–44.0; P < 0.001).¹⁶ The demonstration of functional improvement independent of time since stroke indicates that the stem cells may exert neuroprotective effect as well as modulate neuroregeneration, neuroplasticity, and/or angiogenesis.¹⁶

To date, there are numerous published reports of early phase clinical trials using different types of stem cell therapy for stroke.¹⁹⁻⁸ The majority used autologous precursor cells from the bone marrow with remainder using allogeneic neural and umbilical-derived stem cells.

Administration of stem cells during the acute stroke setting has inherent risk of morbidity and mortality as the patient may be medically unstable, making a case
for further research into feasibility of treatment institution in the chronic phase.

Different routes of administration of stem cells have been investigated, with intravascular delivery being the predominant route, followed by intracranial and intrathecal routes. Experiments in animal models suggest that in the acute stroke setting, the blood–brain barrier (BBB) is damaged and open to cellular transmigration and cytokine-mediated chemotraction of stem cells toward the site of infarction. Thus, in the setting of an acute stroke clinical trial, intravascular delivery of stem cells is likely to be efficacious.

However, in the chronic stroke setting, the pathophysiological environment changes with relative restoration of BBB integrity and dampening of cytokine-mediated chemotraction of cells toward the infarction. The intracranial delivery may potentially be an efficacious route of administration in the design of a human clinical trial in the medically stable period of chronic stroke.

**DPSC**

Human DPSC were discovered in 2000 from young adults with impacted molar teeth. It was found that the dental pulp tissue within the tooth harbored a unique adult stem cell population. Recently, it was shown that DPSC originate from glial stem cells, which has confirmed the unique ecto-mesenchymal ontogeny of DPSC and their propensity to differentiate down the neural lineage. It is postulated that neurological diseases may best be treated by neural stem cells. A clinically accessible source of neural-like stem cells such as dental pulp from the adult tooth is likely to be very attractive option.

There is strong experimental evidence from in vitro and in vivo studies, that DPSC generate bone fide neurons, which display neuronal morphology, express neuronal markers, and generate action potentials. The first direct evidence that DPSC induced neuroplasticity within a receptive host nervous system was reported using a chick embryo assay (Ikaros assay) in which DPSC were found to secrete a cytokine, SDF-1/CXCL12, which chemotacted trigeminal axons toward the implanted DPSC. It has been reported in the rodent stroke model that neural plasticity may underlie the improved neurobehavioral function afforded by DPSC treatment.

The first formal preclinical evidence for use of adult human DPSC (6 × 10⁵) in rodent brain, given 24 h following ischemic stroke, showed improved neurobehavioral recovery over a 4-week period in comparison with vehicle-control treated animals. Numerous preclinical studies in over 100 rodents have used intracranial transplantation of DPSC without tumorigenic consequences. To date, there are four preclinical studies that have demonstrated that DPSC therapy improves neurobehavioral function in a rodent model.
of MCA ischemic stroke.\textsuperscript{10,47–49} Inoue et al. reported that administration of human DPSC-derived conditioned medium resulted in improvement in motor function and infarct volume.\textsuperscript{49}

Recently, we found that DPSC from healthy teeth of aged donors retained their proliferative, multipotent, and neurogenic potential, which has significant potential clinical implications as the majority of prospective patients likely to benefit for this therapy are likely to belong to this age group (manuscript in preparation).

Currently, there are two clinical trials in which autologous DPSC have been used for tissue engineering. The first was in seven patients for oro-maxillo-facial bone repair, which was found safe and successful.\textsuperscript{50} The second is an ongoing Phase 1 clinical trial aimed to repair immature permanent teeth, which have been accidentally injured in childhood.\textsuperscript{51} Human adult DPSC, therefore, present an exciting potential therapeutic option for management of post stroke disability.

We, therefore, propose the “first-in-human” autologous DPSC therapy clinical trial, TOOTH—The Open study Of DPSC Therapy in Humans, investigating the use of autologous stem cell therapy for stroke survivors with chronic disability, with the following objectives:

1. Determine the maximum tolerable dose (MTD) of autologous DPSC therapy;
2. Define that DPSC therapy at the MTD is safe and feasible in chronic stroke; and
3. Estimate the parameters of efficacy required to design a future Phase 2/3 clinical trial.

The present manuscript provides an a priori description of the research protocol.

**Methods**

**Design**

TOOTH is a Phase 1, open-label, single-blinded clinical trial with a pragmatic design that comprises three stages, with each previous stage informing the next:

- **Stage 1** will involve the selection of 27 participants with MCA ischemic strokes and the commencement of autologous DPSC isolation, growth, and testing (Figure 2).
- **Stage 2** will involve the transplantation of DPSC in sequential cohorts of participants with an ascending dose and subsequent observation for a 6-month period for any DPSC-related adverse event to determine the MTD (Figure 3).
- **Stage 3** will investigate the neurosurgical intervention of the MTD of stem cell therapy in combination with rehabilitation. Nine weeks of intensive task-specific rehabilitation will be administered following

![Figure 2. A schematic representation of TOOTH design.](image1)

![Figure 3. Identification of the Maximum Tolerable Dose (MTD) of DPSC.](image2)
stem cell therapy. Advanced magnetic resonance (MR) and positron emission tomography (PET) neuro-imaging, rigorous clinical assessment and group experimental design analyses will be employed to probe any change afforded by stem cell therapy in combination with rehabilitation.

**Patient population**

A Participant Selection Committee will coordinate selection of 27 stroke survivors following an MCA ischemic stroke (18 non-dominant hemisphere and 9 dominant hemisphere stroke), who have reached a stable level of chronic motor, sensory, and/or language disability (Table 1). Participants will be selected from Adelaide, Australia for ease of regular follow-up for the duration of study. Aphasia-friendly modifications to participant information and consent forms, and training of consent personnel in communication will be made to enable participants with aphasia to fully participate in the informed consent processes.

This study will be carried out according to the Declaration of Helsinki, the NHMRC National Statement on Ethical Conduct in Research Involving Humans and the Notes for Guidance on Good Clinical Practice as adopted by the Australian Therapeutic Goods Administration and the ICH/ GCP (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines. Human ethics committee review shall be via the Central Adelaide Local Health Network, South Australia.

**Randomization**

The study aims to examine safety and feasibility of first in human use of autologous adult DPSC in stroke survivors with moderate to severe disability. It is an open label, non-randomized study. The selection of patients will be coordinated by the Participant Selection Committee.

**Interventions**

**Preparation of autologous DPSC from each person’s healthy tooth.** There are five steps in the generation of a person’s autologous DPSC, which include isolation from the dental pulp of a healthy tooth, initial growth, or expansion in defined DPSC medium, quality control of each participant’s own DPSC, cryopreservation until time of treatment and final expansion of DPSC for stem cell therapy. The generation of sufficient DPSC for stem cell therapy only requires one healthy

<table>
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<tr>
<td>1. Inclusion of both genders</td>
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<td>2. Age of the participant should be 18 years or over</td>
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<td>3. MCA ischemic stroke</td>
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<td>4. Moderate severity chronic disability – the participant would have a stable level (modified Rankin Score (mRS) of 2 to 4) of chronic motor, sensory, and/or language disability for at least 6 months prior to selection. Dominant hemisphere MCA stroke survivors with aphasia will need to attain an aphasia quotient score of 33–70 on the Western Aphasia Battery (WAB-AQ) to participate</td>
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<tr>
<td>5. Good cognitive function – the participant must achieve a Mini Mental State Examination (MMSE) score of 24 or more. Participants with aphasia must score above 23 on the Raven’s Colored Progressive Matrices (RPM)</td>
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<tr>
<td>6. All participants must pass Mini International Neuropsychiatric Interview (MINI); in those with aphasia a score of &lt;17 on the Stroke Aphasia Depression Questionaire-21 (SADQ-21)</td>
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<td>7. Healthy teeth to grow sufficient autologous DPSC</td>
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<td>1. The participant will be ineligible if they are at substantial risk under general anesthesia, have epilepsy, have had cancer diagnosed within the last 5 years, or have a coagulation disorder</td>
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<td>2. The participant will be ineligible if they have claustrophobia or other significant psychological issues, which may restrict their ability to undergo the required neuro-imaging investigations, rehabilitation, and clinical assessments</td>
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<td>3. Previous stem cell therapy</td>
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tooth. Two academic dentists on our team will coordinate dental procedures (pulpectomy or the whole tooth extraction), dependent upon the participant’s wish, at the Adelaide Dental Hospital. The isolated dental pulp will be transported to a good manufacturing practices (GMP) accredited laboratory in Adelaide for further processing. Rigorous standards of quality control will be used to exclude all hazardous infections throughout all five steps. Recently, it has been found that fetal calf serum used in defined DPS cell media can be replaced with human platelet lysate, which does not alter DPS cell biology, growth, and proliferation and is a preferred alternative to fetal calf serum, which cannot be used in clinical trials. Each person’s DPS will be processed in accordance with rigorous standard operating procedures (SOPs) to test critical biological properties of DPS prior to stem cell therapy. These include DPS immunophenotype using flow cytometry (CD75+/105+/146+/p75−), DPS protein expression profile (SDF-1, GDNF, VEGF, and MMP2), and their neurogenic potential using our short-term Ikaros assay.41,43,57,58

Neurosurgical procedure for DPS cell transplantation. Patients will have preoperative magnetic resonance imaging (MRI) studies to select peri-infarct target and trajectory sites for implantation of DPS cell. On the day of surgery, a Leksell stereotactic frame will be applied to the participant’s head using local anesthetic and intravenous sedation. A stereotactic computed tomography (CT) head scan will be performed and the images fused to the planning MRI study images using the BrainLab® software system. Access to the brain for stem cell therapy will be via a burr hole and corticotomy with a Pittsburgh Cell Implantation Cannula inserted into the peri-infarct region to administer DPS cell in Isolyte® using a Hamilton syringe and delivered by the neurosurgeon at multiple pre-selected target sites.59,60 The participant will be awakened during DPS injection to provide assessment of functional capacity, a technique routinely used in deep brain stimulation, as the brain parenchyma is anesthetic and this is the best technique to monitor surgery. Postoperatively, the patient will be observed in recovery and the neurosurgical high-dependency ward.

Rehabilitation. Each participant will be asked to identify specific motor, sensory, and/or language disabilities, which will become the focus for intensive task-specific rehabilitation schedules. Content, dosage, and evaluation will be determined by rehabilitation researchers and will be evidence-based.61-64 Initial baseline measurements before and after stem cell therapy will track baseline stability in critical behavioral domains of sensation, limb function, walking, language, and communication, overall stroke severity and functional independence. Modules of rehabilitation based on the identified goals will then be delivered in 3-week epochs and blinded measures of function will be probed to track individual response to therapy and rate of acquisition of specific skills. A maximum of three epoch will be delivered (9 weeks total) and measures will be followed up over the subsequent 6 months.

Measurements. Each participant will be required to undergo periodic neurological and imaging follow up as outlined in Table 2.

Clinical assessment. Measures of function will be continually probed to track individual response to therapy and rate of acquisition of specific skills. Outcome measures will be measured immediately prior to stem cell therapy, immediately following stem cell therapy and subsequently at 6, 9, and 12 months after stem cell therapy, to measure changes in clinical outcomes over time.

Imaging with MRI/PET. Each participant will have a total of five MRI scans over the duration of the trial: at the time of participant enrolment, within 1 month prior to and following stem cell therapy, and at 6 and 12 months following stem cell therapy. There are four sub-aims to our imaging schedule: (a) characterization of the infarct lesion, (b) planning peri-infarct neurosurgical injection sites, (c) monitoring for tumor or other pathologies (e.g. hemorrhage), and (d) quantifying any change in connectivity following stem cell therapy further to rehabilitation. To answer sub-aims (a), (b), and (c) standard 2D FLAIR sequences and high-resolution T1 weighted anatomical 3D MP R age images (1 x 1 x 1 mm3) will be acquired. In relation to sub-aim (d), we will quantify the integrity and structural and functional connectivity of brain networks that support the targeted sensorimotor or language function using high angular resolution diffusion imaging (measurement of white matter tracts) and resting state functional MRI (fMRI; measure of connectivity).65 Diffusion-weighted imaging protocols for fiber tract integrity and for white matter tract estimation will be acquired to map bio-logically reliable brain networks.65,66 Intrinsic (resting-state) connectivity data will be acquired over 7 min of continuous fMRI to provide information about functional connections, i.e. whether or how surviving tissue is working.

Patients will undergo PET scan of the brain using 18F-fluorodeoxyglucose (FDG), a radiopharmaceutical generated at a research-dedicated cyclotron located at SAHMRI. Studies have reported that PET can reveal changes in metabolic and vascular activity in the local
### Table 2. Study schedule of assessments

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<sup>a</sup>Tests to be performed in patients with aphasia.
tissue environment before and after treatment. Meltzer et al. reported that metabolic changes observed at 6 and 12 months may provide preliminary evidence for potential mechanisms associated with observed therapeutic outcomes with intra-cerebral stem cell transplantation. Each participant will have a total of four PET scans, the first two scans within 1 month prior to and 1 month following stem cell therapy, and then at 6 and 12 months following treatment. PET performed before (baseline) and after (1 month) treatment may provide useful information on any regional damage or any inflammation associated with intra-cerebral implantation and subsequent retention and biodistribution in the brain, which will be important for assessing safety of the transplantation procedure used in the study.

**Monitoring and blinding.** A Stroke Fellow will be aware of the therapeutic status of each participant and provide neurological follow-up to accurately and safely document any adverse events to be reported to the Data Monitoring Committee. Potential DPSC-related adverse event that the committee will investigate include the incidence of any tumors, seizures, and chronic pain syndrome associated with aberrant neuroregeneration. Rehabilitation and assessment will be performed in a single-blinded manner, such that the therapist involved in rehabilitation and assessment will be blinded to the therapeutic status of the patient.

**Outcomes**

**Primary outcomes.** Determine the MTD of autologous DPSC therapy. Define whether DPSC therapy at the MTD is safe and feasible in chronic stroke.

**Secondary outcomes.** Estimate the parameters of efficacy required to design a future Phase 2/3 clinical trial.

**Data monitoring committee**

An independent and internationally renowned Data Monitoring Committee will oversee safety monitoring for adverse events and compliance to protocol.

**Sample size estimates**

Stage 2, to answer Aim 1, is based on a cumulative 3 3 statistical design with a low starting stem cell dose and subsequent dose escalation, on the assumption that an acceptable probability of dose limiting complications is between 1 in 6 (17%) and 1 in 3 (33%) of patients. We anticipate that nine participants will be required to progress to a MTD of 10 million DPSC.

In Stage 3, to answer Aims 2 and 3, we have conservatively proposed a further 18 participants, which is in keeping with the numbers (range 5–18) used in previous Phase 1 clinical trials of stem cell therapy administered by intracranial injection for ischemic stroke.

Based on preclinical meta-analysis data with an effect size of 40%, at least 50 participants would be needed to investigate efficacy of stem cell therapy with 80% power at a significance level of 0.05. We propose that a first-inhuman Phase 1 clinical trial on large numbers of participants is unethical until we determine treatment safety and feasibility. If the proposed study is successful, this can be considered in a future Phase 2/3 study.

**Statistical analyses**

A combination of group and single subject design analyses will be employed to probe any change afforded by stem cell therapy further to that of rehabilitation. Safety and feasibility outcomes will be estimated and reported with corresponding 95% confidence intervals to answer objectives 2 and 3. Clinical and biomarker data will be analyzed and reported with the corresponding 95% confidence intervals to inform the choice of the most appropriate outcome and power analysis for the subsequent Phase 2/3 clinical trial.

**Limitations of the study.** The proposed study aims to investigate the safety and MTD of autologous adult DPSC in patients with post stroke disability. The limited number of participants recruited in the interest of patient safety may not provide sufficient power to evaluate efficacy. However, the clinical and imaging outcomes evaluated through the course of this study will yield valuable information to define endpoints for future studies.

**Study organization and funding**

The research team at the Stroke Research Programme, University of Adelaide, will organize the study. Funding shall be provided in the first instance by the Royal Adelaide Hospital Research Foundation and two philanthropic organizations, the Peter Couche Foundation and Stroke SA Inc. with subsequent funding for the remainder of the protocol requested through the National Health and Medical Research Council.

**Summary**

Therapies currently available for stroke are all initiated in the acute phase of ischemic stroke. Up to 80% of patients with stroke return home after their acute hospitalization. The lifetime costs of first-ever cases of stroke alone exceed $3.15 billion in Australia. There is an enormous and urgent unmet medical need to discover new therapies that will overcome chronic
disability following stroke. Stem cell therapy presents an attractive treatment option for stroke with research ongoing to achieve clinical translation to early phase human trials across many countries. There is promising evidence from a preclinical and clinical perspective that autologous adult DPSC treatment is safe and feasible in the majority of stroke survivors who are over the age of 65 years, in Australia, and retain their teeth.

TOOTH aims to investigate the safety and feasibility of autologous human adult stem cell therapy and estimate the parameters of efficacy in participants with chronic stroke disability. The findings from TOOTH would allow progression to Phase 2/3 clinical trials to investigate efficacy of this stem cell therapy in combination with rehabilitation.

Acknowledgments

TOOTH Trial Investigators: Chief Investigators: Professor Simon Koblar, The University of Adelaide; Professor Christopher Levi, John Hunter Hospital; Professor Songtao Shi, University of Pennsylvania, Philadelphia; Professor Leeanne Carey, Florey Institute of Neuroscience and Mental Health; Associate Professor Susan Hillier, University of South Australia; Associate Professor Miranda Rose, La Trobe University; Professor Leonid Churilov, Florey Neuroscience Institutes; Associate Professor Andrew Zacest, Central Adelaide Local Health Network Incorporated; Dr Karlea Kremer, The University of Adelaide; Mr Parabjit Takhar, South Australian Health and Medical Research Institute. Associate Investigators: Dr Aaron Tan, The Queen Elizabeth Hospital, Adelaide; Mr Austin G. Milton, The Queen Elizabeth Hospital, Adelaide; Dr Coralie English, University of South Australia; Professor Jane Mathias, University of Adelaide; Associate Professor Jim Jannes, University of Adelaide; Associate Professor Monica A. Hamilton-Bruce, The Queen Elizabeth Hospital, Adelaide; Dr Nikhil Sharma, University College, London; Associate Professor Peter Cathro, University of Adelaide; Professor Stan Grontous, University of Adelaide; Associate Professor Timothy Kleinig, Royal Adelaide Hospital, Adelaide.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References


neuronal stem cells from human wisdom teeth (tNSC) and the potential of tNSC for stroke therapy. Cytotherapy 2009; 11: 606–617.


5.4 Results

The PERSPECTIVES study manuscript in submission reported that patients considered outcomes such as recovery in social participation and decreased dependence on carers as most meaningful to them. Whilst improved motor function was important, they communicated that the impact on cognition, memory, mood, pain and fatigue were important determinants of their perception of benefit from a given therapy. The perception of risk versus benefit was likely influenced by the time elapsed since stroke, with patients being more willing to accept a higher level of risk early in the post-stroke disease course. Study participants indicated that an opportunity to participate in research should be available to all stroke survivors, irrespective of their cognitive capacity and researchers should ensure that appropriate mechanisms of consent are available for participants with varied cognitive capacity.

The findings of this study were synthesized into a manuscript for submission to *Health Expectations* in 2018.
### PERSPECTIVES: Stroke survivors' views on the design of an early phase cell therapy trial for patients with chronic ischemic stroke

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**Publication Details**

Health Expectations

### Principal Author

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**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.

**Date:** 07/09/2018

### 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 to 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.

iv. Please cut and paste additional co-author panels here as required.

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Title

PERSPECTIVES: Stroke survivors’ views on the design of an early phase cell therapy trial for patients with chronic ischemic stroke

Abstract

Background

Stem cell research holds the potential for a paradigm shift in the management of diseases such as stroke. Patient and public involvement in research (PPIR) can bring a focus to issues of clinical relevance and accelerate translation to real world clinical practice.

Objective

A qualitative thematic analysis of the perspectives of stroke survivors regarding the conduct and design aspects of a proposed Phase I clinical cell therapy study in stroke.

Design

Twelve stroke survivors were purposively recruited in July 2016-August 2017 and participated in semi-structured, face-to-face interviews for input into the design of a proposed Phase I clinical study of autologous dental pulp stem cells. Concurrent thematic analysis was conducted until data saturation was achieved.

Discussion and Conclusions

Participants conveyed that the most relevant outcomes to them were regaining participation, decreased dependence on caregivers and improvement in cognition, memory, mood, pain and fatigue. The perception of risk vs. benefit was likely influenced by the time elapsed since stroke, with participants being more willing to accept a higher level of risk early in the post-stroke disease course. They believed that all stroke survivors
should be given an opportunity to participate in research, irrespective of their cognitive capacity. A relatively small sample population of 12 stroke survivors was studied as thematic saturation was achieved.

PERSPECTIVES study applied principles of PPIR to early phase cell research. Incorporation of outcomes relevant to patients’ need within the study design are critical to generate data that will enable personalised application of regenerative medicine in stroke.

**Key words:** *stroke, patient participation, stem cell research, qualitative research, informed consent, survivors*
Main Text

1. Introduction

The Global Burden of Disease Study (2015) reported that stroke was the second highest cause of years of life lost globally.\(^1\) While the age-standardized mortality from ischemic stroke in the past decade has declined, approximately 33 million ischemic stroke survivors worldwide continue to experience lifelong disability and nearly 80% of patients with ischemic stroke return home with residual impairment.\(^1\)

Regenerative medicine represents a paradigm shift in approach to disease management with the possibility of potential cure or long-lasting remission for many disease conditions with high-unmet need. Early clinical studies with stem cell therapies support a novel approach for neuro-regeneration and repair following stroke with a potentially longer window of opportunity.\(^2\) Cell therapy comprises a composite of different cell types being investigated in different phases of stroke, with use of different dose and delivery regimens.

Clinical translation in stroke has been riddled with a disappointing failure of numerous promising preclinical therapeutic candidates, over the last few decades. Currently available therapies are limited in application to the acute phase of stroke.\(^3\) Stroke represents a diverse set of disease trajectories defined by distinct temporal patterns of neurovascular injury unique to a given patient.\(^4\) The heterogeneity in patient and disease characteristics has contributed to challenges in choosing the appropriate trial design as well as population and efficacy parameters. This, in turn, makes it difficult to assess the effect size of an intervention and its clinical relevance and validity.
Recognition of patients as key partners/stakeholders in research has been increasing over the past decade. It represents a promising approach to generate evidence that is relevant and trustworthy for patients and their families as well as clinicians. This is likely to contribute to a greater sense of empowered participation in patients who are the eventual users of the outcomes of such research.

Research evidence reporting facilitators and barriers to clinical trial participation and patient experiences with clinical research is increasing, particularly in areas such as cancer and stroke. Studies have investigated the relative importance of issues regarding research, in people with stroke. These reported that there was a discrepancy between priorities and relevance attributed to different outcomes by different stakeholders in research, such as patients, caregivers and researchers.

Early patient engagement is likely to be associated with increased recruitment and retention of study participants; development of research methods that are contextualized to patients’ experiences with the disease and utilization of relevant research questions and outcome measures. There is growing evidence for the value of ‘patient and public involvement in research’ (PPIR) in facilitating more patient-focused research by offering insights into prioritization, design and implementation and making trials more effective and credible. PPIR is increasingly being mandated for publicly-funded trials in many developed countries.

Active participation of potential participants is likely to provide a sense of empowerment to people with chronic stroke. Their engagement as ‘lay experts’ to provide their perspective on clinical relevance of different aspects of study design is
likely to improve the eventual study design. The increase in transparency and credibility of research associated with such partnership is specifically critical in innovative areas of research such as stem cell therapies. This was a key issue raised by stroke survivors who participated in the Stroke Survivors’ Forum held at the South Australian Health and Medical Research Institute (SAHMRI), in Adelaide in 2014.

In response to this advice, the PERSPECTIVES study sought to formatively collect insight into the beliefs and perspectives of people with chronic stroke through their involvement in the design of the TOOTH study (The Open study Of dental pulp stem cell (DPSC) Therapy in Humans). The TOOTH study aimed to investigate the effectiveness of autologous administration of adult dental pulp stem cells in people with chronic ischemic stroke.12

The study aimed to explore the views of people with chronic stroke on:

- the relevance and importance of an early phase clinical study such as TOOTH, with adult human dental pulp stem cells in chronic ischemic stroke
- the relevance and acceptability of the planned outcome measures and study design of the TOOTH study and
- issues with consent to participate in the TOOTH study.

2. Methods

2.1. Study Design

The study involved a naturalistic design, adapting from a participatory action-research approach to explore stroke survivors’ perspective on early clinical research design with
cell therapy (TOOTH). The study methodology fits within a constructionist epistemology paradigm, utilizing an inductive thematic analysis.

### 2.2. Study Population, Sampling and Participant Recruitment

Ethics approval for conducting this study was granted by the Human Research Ethics Committees of the University of Adelaide (Ref: H-2016-089) and the University of South Australia (Ref: 00035776).

The study recruited people with chronic ischemic stroke who were residents of Adelaide and likely to fulfil the proposed selection criteria for participation in the TOOTH trial as listed below:

**Inclusion Criteria**

- Inclusion of both genders.
- Age of the participant 18 years or over.
- History of chronic ischemic stroke with a stable level of disability.
- Sufficient cognitive and language ability to participate in an interview.

**Exclusion Criteria**

- Impaired cognition or significant psychological issues.
- Inability to communicate in the absence of a caregiver.
- Inability to travel to the interview location.

### 2.3. Study Enrolment

Eligible participants were recruited using purposive sampling from the research database of people with stroke, maintained by the Stroke and Rehabilitation Research Group (SRR) in the Sansom Institute for Health Research, University of South Australia. The people
included in this database had previously consented to be contacted for future research and were invited to participate (SH). Participants were enrolled on an ongoing basis during the period: July 2016 to August 2017, until the concurrent thematic analysis suggested that data saturation was achieved.

Following an expression of interest in participation, all potential study participants received a participant information pack containing the participant information sheet for the PERSPECTIVES study and the summary information sheet on the TOOTH trial. AN followed up with the participants by telephone to address any queries regarding the study information provided and obtain verbal consent to participate. Following written informed consent, the individuals participated in a semi-structured interview at SAHMRI. The interview was conducted in line with key areas of inquiry defined in the interview guide (Table 1), regarding the research design of the TOOTH trial. The sub-questions were adapted to lines of response provided by the participant. All interviews were audio-recorded and professionally transcribed verbatim by OutScribe Pty Ltd, Adelaide, South Australia.
Table 1: Interview Guide: Key areas of inquiry

<table>
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<td>What has been the impact of stroke on your daily life?</td>
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<td>What are your views on using stem cell therapies for managing stroke?</td>
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<tr>
<td>What are your thoughts on the usefulness of a study like TOOTH?</td>
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<tr>
<td>What are the effects that are important to measure in a study like TOOTH?</td>
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<td>Are there any specific risks that you feel should be measured in the TOOTH?</td>
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<tr>
<td>Would it be appropriate for participants with impaired thinking or understanding to participate in a study like TOOTH?</td>
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2.4. Data Analysis

Analysis of audio transcripts was carried out immediately after every interview and data were coded by AN using NVivo software Version 11 (QSR International, Melbourne Australia). AN read and re-read the transcripts and constructed an index of multiple emerging codes. This index was discussed and cross-checked with SK, SH and AHB. Coding was an iterative process that proceeded concurrently with ongoing interviews. As new codes were added, previous transcripts were re-coded to further refine the coding framework analysis of the data. The inductive thematic analysis continued until no new code emerged from subsequent interviews, i.e. data saturation was achieved. Subsequent analysis crystallized the key themes that represented the aspects emerging from refining of codes from the data.
3. Results

3.1. Participant Disposition

SH contacted 31 patients with stroke following review of their functional status. Following their indication of interest in participation, they were provided with study information by AN. Nineteen patients declined to participate, mostly due to time or mobility constraints. Twelve patients participated in face-to-face interviews, following provision of written consent. Patients were asked to complete a patient profile to understand the overall impact of stroke on their lives along with their age and stroke latency. The population was diverse concerning these parameters, but representative of the potential target population for stroke trials (Table 2).

Data saturation was achieved after twelve interviews with no new themes emerging after eight interviews.
Table 2: Study Participants' Characteristics

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<th>Age</th>
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<td>Range= 0.5-14 years</td>
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<tr>
<td>Impact of stroke on activities of daily living and ability to function independently</td>
<td>VAS* Scale\textsuperscript{16}</td>
</tr>
<tr>
<td>1-5</td>
<td>3</td>
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<tr>
<td>6-10</td>
<td>9</td>
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<tr>
<td>Interest in participation in TOOTH</td>
<td>Response</td>
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<td>Yes</td>
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<tr>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>Not sure</td>
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\textsuperscript{16}Visual Analogue Scale of 1-10: 1 being no/minimal impact to 10 being significant impact

3.2. Themes

The themes described below represent themes identified, even though some elements may overlap (Fig. 1).
3.2.1. **Real-life relevance of study outcomes-are all equally meaningful?**

The participants conveyed that the most meaningful change for them would be a change in their ability to participate in life interactions and their ability to get back to doing activities of interest, i.e. ‘being more normal’ with lessened dependence on significant others (caregivers) in their lives.

*Participant 001:* “I can’t play my music, I can’t sew, I can’t knit, I can’t sing anymore. It’s changed it a lot. I guess I would like the use of the things I’ve said that I can’t do.”

*Participant 002:* “Oh just being human. Just being able to get up in the morning and get a, do the things by yourself, without needing help.”
Participant 008: “It affects your personal life and you’re sitting there like an inanimate lump and so it has an adverse effect on your intimacy too.”

They conveyed that tests that can measure and track changes over time, in the impairments specific to a given patient, would be more meaningful to pursue as markers of therapy benefit.

Participant 005: “Whilst I understand why there are certain tests that you do at the beginning of therapy, and at the end of the therapy range, I guess that understanding a person’s day to day life and how they do things, and being conscious of it, what improvements it could mean to the individual.”

Participant 008: “So putting pegs on a board and things like that. If they can measure that, and they should be, they’ve got the measuring devices now, say you got 40% one day, six months later you got 45%, that’d be brilliant.”

Patients reported impairment of cognition and memory, especially in terms of the difficulty encountered in information processing and new learning situations. This was critical for patients as it jeopardized their ability to effectively participate in social and work-related interactions.

This in turn decreased their sense of self-worth and contributed to depression reported by the participants. They reported that depression adversely impacted quality of life after their stroke.

Participant 002: “And basically that stage, because you can’t, can’t do your normal things. And basically, everything, every, little things add up… call it
frustration, call it what you want, but the more things don't go right for you, the worse you become. Yeah and it happens every day.”

Measuring the impact of cell therapy on mood changes would be useful to monitor in any prospective study evaluating their effectiveness.

Participant 002: “Yep. If they can help that out that would be a major step, seriously. Yep. Because I reckon, I reckon your figure would be up over 90% of people who get very depressed. And depression leads to sort of not wanting to do normal things. And of course, while you're not doing them, your body’s shutting down even further, so.”

The interviewees also expressed their interest in measuring change in pain and fatigue, which they associated with a significantly adverse quality of life experienced post-stroke.

Participant 005: “I've met so many people who've had strokes ... but I think the only real common theme is the fatigue and, possibly, the pain.”

While improvement in speech was acknowledged as a relevant outcome to be measured, the issue of interest was the impact of this impairment on ease of communication and confidence in social interactions.

3.2.2. Risk-benefit-perception

Interviewees were quite pragmatic in their expectation of recovery of function, accepting that full recovery to pre-stroke level of functioning may not be achievable.
Participants believed that perhaps it was not realistic to expect complete recovery of function and they would consider any positive change in functional ability to be meaningful.

Participant 006: “Obviously, I would love to have the use of everything perfect again, but I know that’s probably something that’s not going to happen. But just, for me, just not to have the pain so much.”

Interestingly, the perception of possible benefit was impacted by the time since stroke irrespective of the extent of present disability. Participants expressed that their willingness to participate in studies such as TOOTH would have been higher ‘earlier in their disease’ course and defined that period to be within the first year following the stroke event.

The interviewees accepted that potential safety issues could be expected, given the early stage of the research. The perception of risk was consistent across the participant group. Participants expressed concern regarding risk of further functional impairment.

Participant 010: “I would worry that I could end up, worse off. That something unforeseen could happen and, maybe another part of my brain could die off.”

Participants also expressed concerns with the transplantation procedure, related to the extent of hospital stay required and risk of complications (e.g. infections) associated with the procedure. Interestingly, these concerns were related to risk associated with the surgical procedure per se and not with the issue of stem cell implantation under MRI guidance.

Death or cancer derived from cell transplantation was not cited as the most critical concerns, which is interesting, given that these adverse events are considered the most
important events to investigate and report by the research community. However, participants expressed their desire to know about any available information with regards to cancer risk.

3.2.3. **Attitude towards trial participation**

The interviewees expressed varied interest in participation ranging from no interest, to unsure, to a very keen interest to participate—often this related to their perceived level of current disability.

*Participant 002:* “Basically I got my body as good as it’s going to get. If I had stem cells put in me, and something went wrong, and it brought me back even 5%, then I’ve done all those years for nothing. But if I got offered stem cells right at the start, I would’ve thought, yeah go for it.”

Participants conveyed having very limited knowledge about stem cell research, particularly in the field of stroke, even though quite a few of them were aware of research with stem cells for other diseases such as Parkinson disease and multiple sclerosis.

*Participant 005:* “I’ve heard about stem cell research, with the likes of Parkinson’s, and other heart problems, etc. I haven’t heard of anything regarding stroke.”

Interviewees expressed an opinion that the proposed TOOTH study was relevant for people with disability following stroke. The predominant driver of this belief seemed to be an altruistic thought process regarding this research contributing to a potentially beneficial therapy to answer a current lack of meaningful therapy options and consistent rehabilitation.
3.2.4. Attitude towards participant selection

Participants communicated that study participation should be available to any patient with existing disability and that it was inappropriate to exclude patients with cognitive incapacity. Proxy consent in such situations was deemed acceptable but only in situations where consent was provided by a close member of the family, who would likely be aware of the patient’s wishes and likely preferences. However, such consent should not be provided by a professional caregiver.

Participant 002: “I’d like to see everybody involved in the study, it's probably an ethical thing. But in the case of (carer), if you've got full trust in the carer, yes, but, I mean that’s putting a lot on the individual carer. I mean if it is a family person, something like that, it's probably better. Like, someone of course had to care for me for a while and all that sort of stuff.”

Participants expressed the view that it was important to ensure that relevant rehabilitation and secondary prevention strategies such as control of hypertension, lipid levels and weight are optimized for any patient selected in the study, as these are likely to influence the eventual study outcome.

4. Discussion

4.1. Key findings and implications for research

The unique challenges posed by personalized application of stem cell therapies arise as a question of whether the research designs can be optimized to facilitate meaningful progress in this field.²
This study reports on the views and perspectives of ischemic stroke survivors on the study design of a proposed Phase 1 open label cell therapy trial. The study explores the key outcomes (effectiveness and safety) of interest to them. The insights generated are likely to have an important impact on the design of such proposed studies and to be a useful reference to guide trial design for similar stem cell studies in the future. Importantly, it highlights that active participation of patients in research design can result in trials that generate data, which are more meaningful for the patient community. This provides additional specific evidence to support the value of PPIR in optimizing research quality.

The critical focus of early phase research has always been to gather evidence for safety while establishing proof of concept. In the context of stem cell therapies, it is universally accepted that early phase studies in healthy volunteers is ethically unacceptable. The early clinical trials in stroke published to date, have confirmed feasibility of use in varied phases of stroke. This presents an interesting opportunity to engage these individuals and tap into their lived experience of stroke, which can inform research design to produce clinically relevant data that enables efficient decision making in clinical practice. Patient involvement in early phase research is likely to help researchers understand what potential safety concerns are important/relevant to them. The study reports that patients are more concerned about the risk of losing their current level of functioning, rather than the potential risk listed in study materials, based on preclinical and postulated biological mechanisms, such as tumorigenicity, or conventional risks such as mortality. This highlights the need for researchers to ensure that research data specifically addresses this identified patient need as well as clinician and legal requirements.
Early clinical studies seek to collect exploratory evidence of benefit. The heterogeneity in presentation and recovery trajectory of stroke has always presented a challenge in terms of defining study designs that can generate scientifically rigorous yet clinically meaningful data.\textsuperscript{21} Our study indicates that the patient community has a significant depth of insightful about this conundrum. Stroke survivors suggested that the selection criteria of patients should include optimization of secondary prevention strategy and patient-specific rehabilitation strategies. Emerging research postulates that the recovery trajectory in most patients can be predicted based on existing integrity of neural pathways and current level of brain atrophy are likely to be involved.\textsuperscript{22} It might be interesting to consider whether the suggestion by our community for optimization post-stroke is an intuitive exercise in enrichment for ‘responders’ on the proportional recovery prediction rule, thereby selecting individuals that are likely to have most benefit with additional investigational interventions. Finally, patients suggested that measurement of change in clinical outcomes needs to be personalized to patient specific impairment. Using this approach can potentially lead to identification of homogenous patient clusters, which may enable a more efficient assessment of effect size and appropriate target population.

A continuing debate in the research and clinician community is whether the outcome measures currently utilized are valid and relevant to the patients' life experience following stroke.\textsuperscript{23,24} The National Institutes of Health Stroke Scale (NIHSS) is an established measure of stroke severity and literature supports its use in the prognosis of post-stroke recovery.\textsuperscript{25} The Modified Rankin Score (mRS) is the most commonly selected primary endpoint in drug and rehabilitation studies.\textsuperscript{26} While it assesses a range of outcomes, including severe disability and death, it is not adequately sensitive to assess cognition,
mood or return to social and occupational functioning.\textsuperscript{23} The use of these endpoints on their own to define success or failure of studies, particularly those involving personalized therapy options such as stem cell therapies, may not adequately measure the range of outcomes found to be critical from a patient perspective. The World Health Organisation (WHO) proposed the International Classification of Functioning (ICF) \textsuperscript{27} and recommend outcomes evaluation within dimensions of body function impairments, activity limitations and participation restrictions. In the context of stroke, a very small percentage of pharmacological or rehabilitation studies have to date examined impact on participation restriction, as is also the case for cognition and mood outcomes.\textsuperscript{23} Evidence for widespread prevalence of issues in these domains reported by patients has been steadily increasing in recent years.\textsuperscript{24} Research involving patient-reported outcomes has described persistent and significant impact on patients’ lives even for those that fully recover their pre-stroke functional level. The present study reiterates the importance of these outcomes and their measurement to the patients. The International Consortium for Health Outcomes Measurement (ICHOM) conducted an iterative Delphi process that included diverse stakeholders such as clinicians, patients, stroke registers and stroke societies.\textsuperscript{28} The study suggested a ‘Stroke Standard Set’—a minimum dataset of outcomes and risk adjustment variables to collect for all patients hospitalized with stroke. The categories recommended within the ICHOM standard set for assessment were survival and disease control, acute complications, and patient-reported outcomes (PROM). PROM included assessment at 90 days for pain, mood, feeding, self-care, mobility, communication, cognitive functioning, social participation, ability to return to usual activities, and health-related quality of life, along with data on mobility, feeding, self-care, and communication, collected at discharge. Collecting data on these parameters using validated tools at
different phases of stroke targeted in early clinical studies, would build the quantum of data available on the magnitude of effect that is plausible with cell therapies. The increased understanding of anticipated effect can better inform decisions on minimal clinically important difference (MCID) that are acceptable and meaningful to clinical practice. It stands to reason that such informed decision making would contribute to increased efficiency in later phases of development and more informed and relevant study size determination and design.

Participants indicated an acceptance of their current level of functioning and that the return of functioning over time, was due to consistent effort on their part to engage with the rehabilitation options available. This drove the heightened concern for the potential risk of loss of this functional improvement that they worked very hard to achieve. They indicated that they would have been more accepting of this potential risk if an opportunity to participate in a clinical study such as TOOTH had arisen ‘earlier’ in their disease course, defined as within a year of their stroke occurrence. This insight has important implications for study recruitment for stem cell studies in chronic stroke. A recently reported study also highlighted these as important determinants of interest in participation.\textsuperscript{13} Allowing for stabilization of patient’s general medical condition and spontaneous post-stroke recovery to take place are reasonable postulates.\textsuperscript{29} Our study suggests that targeting a narrower window for recruitment (up to 1 year after the stroke event) might facilitate recruitment and provide a more favorable risk-benefit proposition to potential participants. Most researchers in stem cell research in stroke accept that stem cell therapies in practice are likely to be co-delivered with rehabilitation.\textsuperscript{30} Application of stem cell therapies at a time
point following stroke where rehabilitation has achieved maximum possible benefit, may enable clearer distinction of incremental change with stem cell therapies.

A long-standing debate in stroke trials is the issue of consent particularly for patients with cognitive deficit. Proxy consent by next of kin is now well accepted in the context of acute trials. Participants indicated that proxy consent might be considered in stem cell studies, even in the chronic phase of stroke. However, they highlighted that this was likely to place a significant psychological burden on the caregivers. Cunningham et al. reported similar findings as a potential ‘care conflict’ between patients and caregivers in such research situations.

Our study reports low levels of awareness about ongoing research with stem cell therapies in stroke and limited understanding of postulates regarding their mechanism of action. A recent study by Aked et al. also reported similar findings regarding the low level of awareness regarding stem cell research. Patient advocacy groups constitute a promising though still underutilized means of increasing this awareness. The participants in our study, who reported prior knowledge of stem cells, attributed this to information they received from such groups. Patient involvement in research from an early phase can also help build awareness and knowledge in the stroke patient community. This is likely to enable patients to become more informed and empowered participants in research.

In a broader societal and medical practice context, the increased awareness of current state of regenerative neurology in stroke and evidence-based estimation of risk and benefit to be expected can become a deterrent to unscrupulous use of unproven stem cell therapies for commercial use and stem cell tourism.
4.2. **Strengths and Limitations**

The study utilized face-to-face interviews with stroke survivors, as the method of qualitative enquiry. This enabled a relaxed and supportive environment in which they shared their individual preferences, contextualized to their unique lived experience with stroke. The study findings highlighted key outcomes considered important from patient perspectives, and that need to be measured within study design.

This approach also minimized the dilution of information likely with other modalities such as combined focused groups with other stakeholders such as caregivers. Our study is a part of a wider exercise that will also explore views from different stakeholders, particularly caregivers, in separate studies. The rationale behind this strategy is based on the growing body of evidence for disconnect in the perception/acceptance of risk and benefit, between patients and caregivers.\(^{13,14,19,21,33}\)

The requirement to travel and engage in an in-depth interview meant that the study did not include patients with very severe disability, cognitive deficits and severe aphasia. The participants were therefore, not fully representative of the overall stroke survivor community. However, the perspectives shared by them in the context of preferences for study design components were largely agnostic to the degree of severity of post-stroke disability and may well be more relevant to the broader group. The severity of present disability has been shown previously to impact on motivation to participate in other studies\(^ {8,32}\) and this was corroborated in our study. However, it did not appear to influence the relative importance assigned to different outcome and design elements. In addition, the eventual number of participants may appear rather small. Previous studies with similar research methodology have reported this to be possible.\(^ {34-36}\) The intention of our study
was to provide rich thematic description of our qualitative enquiry. The research team conducted constant comparison of emerging themes to ensure that data saturation was confirmed to ensure validity of study findings.

5. **Conclusions**

The PERSPECTIVES study applied principles of patient and public involvement in research in early clinical stem cell research in stroke. Engagement of stroke survivors as 'lay experts' to provide input into study designs can provide critical insights that can enable more targeted research. In an evolving field such as cell therapy in stroke, this partnership can potentially help researchers to efficiently address the challenges posed by the inherently 'personalized' field of regenerative medicine.

**Authors' contributions**

AN: Ethics application, Study design and conduct (participant consent/recruitment/interviews), data collection, analysis, manuscript preparation.

SH: Supervision of study design and conduct; provided access to the patient database for study recruitment; critical review of data analysis and manuscript.

AGM: Ethics application; critical review of manuscript.

MAHB: Supervision of study design and conduct; critical review of data analysis and manuscript.

SAK: Supervision of study design and conduct; critical review of data analysis and manuscript.
References


5.5 Conclusions

Our findings highlight insights from stroke survivors regarding their perspectives on cutting-edge medicines such as cell therapies. Incorporation of patient-centric outcomes in clinical trial protocols, targeting information on perceived risk associated with CTs and delivery mechanisms and conveying balanced information on the present state of knowledge regarding efficacy are key areas that need attention whilst planning future clinical studies with CTs.
CHAPTER 6: HEALTH ECONOMICS CONSIDERATIONS

6.1 Background

Early phase research in the use of CTs in stroke, Alzheimer’s disease, Parkinson’s disease and progressive multiple sclerosis has indicated a potential for meaningful benefit (419). As research with CTs progresses from bench to the bedside, both reimbursement and broad patient access are likely to represent key challenges (420, 421). The assessment of health outcomes and clinical effectiveness in the context of a personalised nature of the clinical application of CTs, presents a challenge to their value proposition (422).

Economic evaluation encompasses comparative analysis of alternative courses of action, in terms of both their costs and consequences (423). This analysis can take many methodological approaches including full analysis (both the costs and consequences of alternatives are examined), as well as partial approaches, which examine only some of these components (423). Full economic evaluations are preferred in the healthcare setting, as they provide a greater understanding of the potential costs and benefits of healthcare interventions to assist decision-makers considering alternative paths of action (423). Three approaches form most of the full economic evaluations undertaken in healthcare settings: cost-benefit analysis, cost-effectiveness analysis and cost-utility analysis (423). Cost-benefit analysis measures both costs and benefits in monetary terms, to determine the net benefit of the intervention of interest (423). However, several limitations to the application of cost-benefit analysis within healthcare exist, including difficulty in valuing the outcomes of healthcare (such as improvements in physical health, mental health and wellbeing) in monetary terms (423). On the other hand, cost-effectiveness analysis (CEA) compares the
incremental costs associated with alternative health interventions, with the incremental benefits of those interventions measured as changes in clinical and/or patient focused outcomes (424). CEAs usually report their findings as an incremental cost-effectiveness ratio (ICER) which provides an indication of the incremental cost per unit change in clinical effect (423). This allows decision-makers to compare alternative courses of action that result in similar benefits. However, it becomes difficult to compare benefits across different disease groups and treatments in which benefits are logically measured using different outcomes (423). To assist, a specialized form of cost-effectiveness analysis, cost-utility analysis (CUA) has been developed (423). A CUA compares the incremental costs associated with alternative interventions with a standardized measure of benefits such as a quality adjusted life year (QALY) (423).

The QALY incorporates a measure of quantity of additional survival, assessed in life years associated with an alternative intervention, with a calculation of the quality of those years or ‘utility’ (424). This provides a standardized measure of benefits that maintains its meaning across different health interventions and across different patient age groups. The quality of the years is measured using standardized generic preference-based instruments, designed to measure quality of life, which include a standard health state descriptive system, and off-the-shelf scoring weights usually based on the preferences for the health states of members of the general population (424).

Healthcare budgets are already facing a ballooning of long-term costs of illness. This represents a risk to research and development (R&D) investment in high technology products such as CT with unclear benefit at present, as the value of such an investment is continually challenged in the wake of more pressing current needs (425). Generation of economic evidence early in the development of new therapies is becoming increasingly important to ensure development costs are in line with the potential benefits to consumers from these interventions (425). There is a recent argument for
early health technology assessment in parallel with phase I/II clinical research (426). Generating economic evidence at an early stage can accelerate clinical translation by enabling strategic R&D decisions (427). These include preclinical market assessment, portfolio decisions, clinical trial design, market access and pricing strategies (427). Therefore, there is an imperative to explore health technology assessment (HTA) methods that are more specific to early stages of product development and used by industry and academia alike to inform decision making that will accelerate productive translational research (412). In recent years, the evidence base has built in support of the efficacy and safety of use of CTs (419) in neurological diseases, but there has been no formal assessment of economic outcomes of CTs in terms of cost-effectiveness and the value to patients or healthcare systems to date.

6.2 Research Objective

A systematic review was conducted to evaluate potential cost-effectiveness measures that are critical to CT research in stroke.

6.3 Methods

This systematic review aimed to assess the breadth of current economic evaluation undertaken on the use of CTs in stroke. The scope of this review was broadened to include neurological disorders, following a preliminary search of literature that yielded minimal data.

An appraisal of the quality of evidence generated by these studies was undertaken to determine the current state of knowledge in this fast-growing area of research, which is likely to become a critical determinant of success in the translation of regenerative medicine as a viable and broadly available clinical strategy. The protocol for the review was defined a priori and published in PROSPERO: International prospective register of systematic reviews in 2017.
# Statement of Authorship

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□ Unpublished and Unsubmitted work written in manuscript style |
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## Principal Author

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## 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 to 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.

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Economic evaluation of stem cell therapies in neurological diseases: a systematic review

ANJALI NAGPAL, RACHEL MILTE, SUSAN W KIM, SUSAN HILLIER, JULIE RATCLIFFE, MONICA ANNE HAMILTON-BRUCE, SIMON ANDREAS KOBLAR

Citation
ANJALI NAGPAL, RACHEL MILTE, SUSAN W KIM, SUSAN HILLIER, JULIE RATCLIFFE, MONICA ANNE HAMILTON-BRUCE, SIMON ANDREAS KOBLAR. Economic evaluation of stem cell therapies in neurological diseases: a systematic review. PROSPERO 2017 CRD42017072937 Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42017072937

Review question
To identify, describe, appraise and summarize economic evaluation studies of stem cell therapies in neurological disorders, particularly in stroke.

To provide an overview of the quality of the economic evidence available on this topic.

Searches
Systematic searches will be undertaken in MEDLINE, CINAHL, Embase, PsycINFO, Scopus, Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, EconLit, Database of Abstracts of Reviews of Effects (DARE), Cochrane Database of Systematic Reviews (CDSR), NHS Economic Evaluation Database (NHS EED), the Health Technology Assessment (HTA) database and the Cost-Effectiveness Analysis (CEA) Registry for the period: 2007-2017.

Additional material will be obtained by scanning the reference lists of included studies and from grey literature sources.

Types of study to be included
Eligible studies will include cost-utility analyses, cost-effectiveness analyses, or cost-benefit analyses. In addition, partial economic evaluations will also be included (e.g. those reporting costs associated with the intervention alongside clinical trials). Both analyses conducted alongside clinical trials, as well as those modeled studies based on empirical data, will be included. Studies of effectiveness/outcomes which do not report costs, and studies purely reporting the burden of disease without including the intervention will be excluded.

Condition or domain being studied
Neurological diseases, particularly in stroke.

Participants/population
Inclusion Criteria: Studies performing economic evaluation of use of stem cell therapy in adult patients with any neurological diseases.
Exclusion Criteria: Studies of effectiveness/outcomes which do not report costs, and studies purely reporting the burden of disease without including the intervention will be excluded.

Intervention(s), exposure(s)
Any stem cell therapy intervention applied in the management of a neurological disease in adults.

Comparator(s)/control
Any comparison – active, alternate, sham/placebo, usual care or no intervention, if feasible.

Context
The review will evaluate studies involving adult patients reported between 2007-2017 in English databases.

Primary outcome(s)
The primary outcomes of interest will include cost analyses that report the costs of the stem-cell therapy and economic evaluations that report both costs and outcomes.

**Timing and effect measures**
There will be no restrictions on study outcomes because the purpose of the review is to document what outcomes have been or could be estimated in the context of economic evaluation of the use of stem cell therapies in neurological diseases, particularly in stroke.

**Secondary outcome(s)**
To provide an overview of the quality of the economic evidence available on this topic.

**Timing and effect measures**
There will be no restrictions on study outcomes because the purpose of the review is to document what outcomes have been or could be estimated in the context of economic evaluation of the use of stem cell therapies in neurological diseases, particularly in stroke.

**Data extraction (selection and coding)**
Two reviewers will independently screen titles and abstracts against the inclusion criteria. Subsequently, the full texts of all studies deemed to be potentially relevant will also be assessed independently by two reviewers. Any disagreements will be resolved through discussion or by recourse to a third reviewer. A data extraction form will be used to extract relevant information from the studies in a consistent format. Both data extraction and quality assessment will be undertaken independently by both reviewers. Any disagreements will be resolved through discussion or by recourse to a third reviewer. Extracted data will include: study details (author, year of publication country and setting); study population; disease indication; intervention and comparator (if relevant), main analytical approaches for the economic analysis; view point, sources of effectiveness and resource use estimates, prices and currency for reporting economic outcomes, any sensitivity analyses undertaken; main economic outcomes reported.

**Risk of bias (quality) assessment**
The quality of reporting within the cost-effectiveness studies will be assessed using the revised CHEERS checklist developed by Drummond et al (2013). The checklist can be accessed at: http://www.equator-network.org/wp-content/uploads/2013/04/Revised-CHEERS-Checklist-Oct13.pdf. All studies will be included in the review regardless of the quality of the individual study, although commentary will be made on the quality and risk of bias of individual studies and the available evidence as a whole, and recommendations for future research will be provided.

**Strategy for data synthesis**
A narrative synthesis, based on the preceding data extraction and critical appraisal, will provide a descriptive overview of all studies included. The evidence will be mapped according to patient population and methods for economic analysis used. This exercise will assist decision-making for future economic evaluations; for example, the analysis approach, view point, population groups, and any gaps in the evidence where further research would be beneficial.

**Analysis of subgroups or subsets**
If feasible, a subgroup analysis of all studies conducted in a stroke population will be undertaken.

**Contact details for further information**
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**Organisational affiliation of the review**
University of Adelaide; University of South Australia; South Australian Health & Medical Research Institute (SAHMRI)

**Review team members and their organisational affiliations**
Anticipated or actual start date
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None known

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Stage of review
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Subject index terms status
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Subject index terms
Cost-Benefit Analysis; Genetic Therapy; Humans; Nervous System Diseases; Stem Cell Transplantation

Date of registration in PROSPERO
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Date of publication of this version
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Details of any existing review of the same topic by the same authors

Stage of review at time of this submission
The review has not started
**Stage** | **Started** | **Completed**
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Preliminary searches | No | No
Piloting of the study selection process | No | No
Formal screening of search results against eligibility criteria | No | No
Data extraction | No | No
Risk of bias (quality) assessment | No | No
Data analysis | No | No

**Versions**

25 July 2017
6.4 Results

The published review reported that there is sparse evidence for economic evaluation at early stages of research in CT. Only three economic evaluation studies have been published to date, of which only one was in stroke. All studies undertook a cost-utility analysis of CT versus the current standard of care using decision analytical modelling. The studies reported that CTs could provide meaningful cost savings in terms of direct costs of disease management accrued to the government (healthcare bodies and social services). The findings of this systematic review were published in Value in Health in 2018.
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ABSTRACT

Objectives: To examine economic evaluation studies of stem cell therapies (SCTs) in neurological disorders and to provide an overview of the quality of the economic evidence available on this topic. Methods: The review examined studies that performed an economic evaluation of the use of stem cells in adult patients with neurological diseases and that were published in English during the period 2007 to 2017. Data analyzed and reported included study population, disease indication, main analytical approaches for the economic analysis and perspective, key assumptions made or tested in sensitivity analyses, cost outcomes, estimates of incremental cost effectiveness, and approaches to quantifying decision uncertainty. Results: A total of three studies reporting on the findings of the economic evaluation of the use of SCT in stroke, Parkinson disease, and secondary progressive multiple sclerosis, respectively, were identified. All three studies conducted a cost-utility analysis using decision-analytic models and reported an incremental cost per quality-adjusted life-years gained (incremental cost-effectiveness ratio) versus standard care. These studies reported meaningful cost savings in stroke, Parkinson disease, and secondary progressive multiple sclerosis in the base-case scenarios. Conclusions: Despite significant progress in clinical research in the use of SCT in neurological diseases, economic evaluation of these therapies is still at a nascent stage. Given the early stage of research inputs (clinical and cost outcomes data) into the models per se, further research is urgently needed to enable meaningful assessment of the cost-effectiveness of these advanced therapies and to ensure sustainable access for population groups most likely to benefit in clinical practice.

Keywords: economic evaluation, neurological disease, stem cells, stroke.

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Introduction

In recent decades, improved nutrition and health care have led to decreasing mortality across the entire spectrum of diseases. This has been associated with an increasing gap between life expectancy and healthy life expectancy. This gap has recently been reported to be more than 10 years, indicating increasing years of life with suboptimal health and disability [1]. Neurological diseases such as stroke [2], Parkinson disease (PD), Alzheimer disease, and progressive multiple sclerosis [3] have been increasingly contributing to this emerging pattern. Deterioration in quality of life associated with these conditions has either risen or stayed stable despite inroads made in terms of mortality. The situation is further worsened by the fact that, for most of these conditions, research into novel therapeutic options over the last few decades has met with multiple and costly failures [4].

In this context, regenerative medicine (cell/gene/bioengineering products) offers an exciting option for delivering a meaningful solution to the current unmet need from a patient and public health perspective [5]. Stem cell therapies (SCTs) potentially replace or regenerate diseased human cells, tissues, or organs to restore or establish normal function. Early-phase research in the use of SCT in stroke [6], Alzheimer disease [7], PD [8], and progressive multiple sclerosis [9] has indicated a potential for meaningful benefit. Despite the increased number of regulatory approvals for SCT in different countries, reimbursement and broad patient access remain challenges [5,10]. The personalized nature of their clinical application and assessment of health
outcomes and measures of clinical effectiveness pose a challenge to an assessment of their value proposition [11].

The present global explosion in health care budgets has resulted in fiscal tightening of spending on research and clinical translation of innovative therapies [12]. In recent years, the evidence base has expanded to support the efficacy and safety of SCT in neurological diseases [6-9]. Generating economic evidence at an early stage can accelerate clinical translation by enabling strategic research and development decisions, preclinical market assessment, portfolio decisions, clinical trial design, and market access and pricing strategy arrangements [12-15]. Nevertheless, there has been no formative assessment of literature reporting economic outcomes in terms of cost effectiveness and value to patients or health care systems of SCT to date.

This systematic review presents an overview of the quality and quantity of economic evaluations of the use of SCT in neurological diseases. This is likely to become a critical determinant of successful translation of regenerative medicine as a viable clinical strategy.

**Methods**

**Protocol and Registration**

The protocol for the review was prepared and registered on PROSPERO (International Prospective Register of Systematic Reviews; Ref-CRD42017072937) [16].

**Eligibility Criteria**

All studies that performed an economic evaluation of the use of stem cells in adult patients with neurological diseases and that were published in English during the period 2007 to 2017, which reflects the period of maximum publications in the field of stem cell research, were eligible for inclusion. Although there were no restrictions on the types of study, design eligible, studies of effectiveness that did not report costs and studies purely reporting the burden of disease or the cost of illness without including the intervention were excluded.

**Search and Study Selection**

Systematic searches were undertaken in MEDLINE, PubMed, CINAHL, Embase, PsycINFO, Cochrane Central Register of Controlled Trials, Database of Abstracts of Reviews of Effects, Cochrane Database of Systematic Reviews, National Health Service Economic Evaluation Database, the Health Technology Assessment database, and the Cost-Effectiveness Analysis Registry. The reference lists of included studies were scanned for any additional studies. The database search used primary search terms on cell therapy, health economics, and neurological diseases (see Appendix 1 in Supplemental Materials found at https://doi.org/10.1016/j.jval.2018.07.878).

**Data Extraction**

The screening and data extraction for this review was conducted using the Covidence platform [17].

Two of the reviewers independently conducted screening against the inclusion/exclusion criteria and subsequent full-text review of all potentially relevant studies. Data extraction included study details (author, year of publication, country, and setting), study population, disease indication, main analytical approaches for the economic analysis and perspective, key assumptions made in the base case or tested in sensitivity analyses, costs, estimates of incremental cost-effectiveness, and approaches to quantifying uncertainty (e.g., deterministic and/or probabilistic sensitivity analysis). Any disagreements were resolved through consensus or by recourse to a third reviewer.

**Quality Assessment**

The quality was assessed using the revised checklist proposed in the International Society for Pharmacoeconomics and Outcomes Research Consolidated Health Economic Evaluation Reporting Standards (CHEERS) Task Force Report [18]. One of the reviewers provided additional specific input on the quality assessment of analytical methods (item no. 17). Any disagreements were resolved through discussion or by recourse to a third reviewer. Each item was rated as “fully satisfied,” “partially satisfied,” or “not satisfied” or “not applicable.”

**Data Synthesis**

A narrative synthesis, based on the preceding data extraction and quality appraisal, was undertaken. A meta-analysis was not feasible because of the low number of studies overall and the fact that eligible studies involved different diseases, SCT types, and input variables in the models. The costs were converted to a common currency (US dollars) and inflated to the price level of 2016 using the CCEMG-EFPI-Centre Cost Converter (version 1.4) for reporting [19].

**Results**

In total, 12,840 titles were identified in the preliminary search. Removal of duplicates resulted in 7116 potentially relevant articles. Of these, 6888 articles were excluded after screening of title and abstract, and 228 articles underwent a review of the full text. Of these articles, 225 were excluded for reasons provided in Figure 1. The remaining three studies were included in the review. Key descriptions of included studies are presented in Table 1. The quality assessment of these studies is presented in Table 2.

**Study Characteristics: Disease Indication, Intervention, Design, Time Horizon, Discount Rate, and Perspective**

The studies differed in terms of type of SCT used and neurological diseases studied: stroke [20], PD [21], and secondary progressive multiple sclerosis (SPMS) [22]. The PD study compared the use of embryonic neural stem cells with standard care. The study on SPMS looked at the use of hematopoietic stem cell transplantation (HSCT) versus mitoxantrone therapy. The stroke study model did not define the SCT type and compared assumed effect to existing standard care, on the basis of expert opinion.

The stroke and SPMS studies evaluated cost outcomes over a lifetime [20,22], whereas the PD study reported them over a 25-year horizon [21].

The SPMS study compared a patient cohort with SPMS who received HSCT to a matched comparator group receiving mitoxantrone as part of standard care [22]. The target population in the PD study included patients with motor impairment (Hoehn and Yahr [HY] stages III–IV) who received SCT [21]. The HY staging captures increasing severity (stage I [minimal impairment] to stage V [confined to bed]) of progressive motor impairment in PD [23].

The data on the comparator population (standard care) were sourced from a clinical practice cohort [24]. The stroke study defined its target population as patient cohorts of age 55, 65, and 75 years with a modified Rankin Scale (mRS) score of 1 to 4 at discharge [20]. The mRS is commonly used to measure disability (0 indicates no disability and 6 is death) in patients with stroke [25]. The data for comparator population, that is, standard of care, were derived from a previous randomized multicenter study [26].

The stroke model allowed for subgroup analysis according to age at stroke onset, functional status at hospital discharge,
assumed effectiveness of SCT, mode of stem cell administration, risk of recurrent stroke, or death caused by intervention. The PD study evaluated subgroups according to age (64 and 70 years). By comparison, the SPMS study did not report on any specified subgroups. All three studies used discounting for future costs and benefits: the PD and stroke studies used a 3% discount rate for all calculations, as recommended in Sweden, whereas the SPMS study used 3.5%, in line with existing recommendations in the United Kingdom. The PD and SPMS studies examined the costs from a government perspective. The stroke study adopted a societal perspective.

Quality
All studies differed in the type of SCT used. The evidence from clinical studies to date does not indicate a significant difference in clinical effect of different cell types. None of the studies has examined SCT type as a potential variable in the economic analysis model. The time horizon for model application was reported in all studies, but justification for the choice was missing. The selection of target and comparator populations is well described for all the studies. Only the SPMS study, however, reported matching the SCT cohort and comparator group on the basis of baseline diagnosis of SPMS and Expanded Disability Status Scale (EDSS) values. The PD and stroke studies did not report whether the SCT and comparator had been matched. None of the studies explained in detail why the chosen perspective provides the most appropriate economic viewpoint of analysis. Two of three studies did not report on potential conflicts of interest.

Estimating Resources and Costs: Model-Based Evaluations and Currency
The PD study (Table 1) was limited to direct medical (hospital care, pharmaceuticals, and investigations) and direct nonmedical (transportations and home help) costs. The cost data for management of PD, SCT transplantation, and associated complications were sourced from previous clinical studies. Calculations were made in euros (€) according to the price level of 2002. The SPMS study included intervention costs (treatment costs for mitoxantrone and autologous HSCT and any related adverse events) and other costs (Table 1) associated with the management of multiple sclerosis. Hospital resource use and pharmaceutical costs were calculated in pounds using the National Health Service and the British National Formulary reference costs for the year 2006 to 2007.

The stroke study included direct health care costs for initial and recurrent events (Table 1), sourced from a previous study. Long-term costs included social services and indirect costs owing to disability and productivity losses, sourced from a study on the cost of stroke in Sweden. Calculations were expressed in US dollars for the year 2009.

Quality
The PD study used resource costs from a previous study but provided limited details about where unit costs were derived from. No opportunity costs were included. The stroke study drew from assumptions based on expert opinion and previously published literature but did not clearly describe where some unit costs were drawn from. Opportunity costs were not included. The SPMS study described the sources for cost estimations in detail. Nevertheless, indirect costs such as lost productivity and out-of-pocket expenses were excluded from the analysis.

Outcomes: Choice of Health Outcomes, Assumptions, and Valuation of Preference-Based Measures of Effectiveness
All the studies used quality-adjusted life-years (QALYs) as a health outcome. The PD model used effectiveness data from a
Table 1 – Summary of included studies.

<table>
<thead>
<tr>
<th>Study characteristic</th>
<th>Hjelmgren et al. [21]</th>
<th>Tappenden et al. [22]</th>
<th>Svensson et al. [20]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease indication</td>
<td>PD</td>
<td>SPMS</td>
<td>Stroke</td>
</tr>
<tr>
<td>Country</td>
<td>Sweden</td>
<td>United Kingdom</td>
<td>Sweden</td>
</tr>
<tr>
<td>Design</td>
<td>Markov state transition model</td>
<td>Markov state transition model</td>
<td>Decision tree model</td>
</tr>
<tr>
<td>Intervention</td>
<td>Embryonic neural stem cells</td>
<td>HSCT</td>
<td>Intracerebral stem cell implantation</td>
</tr>
<tr>
<td>Comparator</td>
<td>Standard pharmacological therapy</td>
<td>Mitoxantrone</td>
<td>Standard poststroke care</td>
</tr>
<tr>
<td>Base-case population</td>
<td>Idiopathic patients with PD (HY stage III–IV) aged 64 y</td>
<td>Patients with SPMS in two MS registries: the Lyon Clinique Neurologie MS Registry and the EBMT MS registry</td>
<td>Cohort aged 55 y at stroke onset, with mRS 2 at hospital discharge, and an assumed increase in the probability to improve 1 mRS grade of 50% with SCT</td>
</tr>
<tr>
<td>Method</td>
<td>CUA</td>
<td>CUA</td>
<td>CUA/CBA</td>
</tr>
<tr>
<td>Time horizon Perspect</td>
<td>25 y</td>
<td>Government (direct and nonmedical costs)</td>
<td>Lifetime</td>
</tr>
<tr>
<td>Base-case scenario</td>
<td>Patients aged 64 years with early onset PD (HY stage III–IV) with an assumed</td>
<td>Patients with a baseline EDSS score Z3 and ±8; modeled with three scenarios with different interpretations of the disease progression</td>
<td>Patients aged 55 y at stroke onset, with mRS 2 at hospital discharge</td>
</tr>
<tr>
<td>Base-case assumptions</td>
<td>Initial progressive improvement during first 2 y, followed by a stationary period up to 5 y after transplantation, and return to preoperative rate of disease progression thereafter</td>
<td>Transitions between EDSS states may be progressive or regressive</td>
<td>Relative effectiveness of intracerebral SCT transplantation of 50% and no associated side effects</td>
</tr>
<tr>
<td>Comparator costs (value in US $ inflated to 2016)</td>
<td>HY stage III: €158,943 (US $22,557) HY stage IV: €186,279 (US $26,437)</td>
<td>Scenario 1*: £107,126 (US $181,938) Scenario 2*: £107,126 (US $181,938) Scenario 3*: £107,126 (US $181,938)</td>
<td>Scenario 1*: £221,956 (US $246,040)</td>
</tr>
<tr>
<td>QALYs gain</td>
<td>HY stage III: 0.873 HY stage IV: 1.133</td>
<td>Scenario 1*: –1.02 Scenario 2*: 0.23 Scenario 3*: 1.40</td>
<td>1.34</td>
</tr>
<tr>
<td>Cost-effectiveness threshold (value in US $ inflated to 2016)</td>
<td>€38,000 (US $5,393) €70,000 (US $9,934)</td>
<td>US $110,400 (US $122,379)</td>
<td></td>
</tr>
<tr>
<td>Sensitivity analysis</td>
<td>Univariate analysis: time horizon (10–20–30 y); discount rate (0%–5%); treatment efficacy (±50%); occurrence of complications (±100%); analytical perspective (direct medical costs only vs. including other direct costs); method of determining utilities</td>
<td>Univariate analysis: transplant-related mortality rate (0/1.3%); relative PFS hazard ratio between HSCT and mitoxantrone; tariff cost of HSCT (±25%); costs of managing MS (±25%); discount rate (0/3.5%); Scenario analysis: effectiveness duration</td>
<td>Univariate analysis: relative efficacy of SCT, mode of transplantation; age at stroke onset; annual risk of recurrent stroke; SCT procedure risk of death; intervention on mRS 3/4; extended leave period</td>
</tr>
</tbody>
</table>

continued on next page
Table 1 - continued

<table>
<thead>
<tr>
<th>Study characteristic</th>
<th>Hjelmgren et al. [21]</th>
<th>Tappenden et al. [22]</th>
<th>Svensson et al. [20]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impact of variables on cost effectiveness</td>
<td>The results were sensitive for patients in HY stage III to changes in time horizon, discount rate, treatment effect, and health utility method, but were stable for patients in HY stage IV.</td>
<td>Shorter treatment effect persistence resulted in HSCT not being cost-effective in optimistic scenario; decreased intervention cost and mortality risk associated with HSCT improved cost effectiveness.</td>
<td>SCT remained cost-effective but societal value decreased — decreased QALY gain and increased incremental costs with decrease in relative efficacy, higher age at stroke onset; intervention in patients with higher disability (mRS 4)</td>
</tr>
<tr>
<td>Variable ranges included in analysis</td>
<td>No explanation provided</td>
<td>No explanation provided</td>
<td>Variable ranges based on expert opinion</td>
</tr>
<tr>
<td>Study findings</td>
<td>Long-term cost savings in most instances in early onset PD patients in HY stages III–IV</td>
<td>A potential to achieve a level of cost effectiveness that is acceptable to policymakers and health care purchasers, but is largely determined by the interpretation of available clinical effectiveness data and the duration over which such effects may be observed.</td>
<td>A potential for long-term cost savings by reducing the disability after stroke; societal value up to US $166,500 (US $184,567), particularly in younger patients with stroke with moderate disability, with possible cost effectiveness estimated down to relative efficacy of 14%</td>
</tr>
<tr>
<td>Generalizability</td>
<td>Enables cost-effectiveness analysis based on real-world progression using a clinical surrogate end point (HY stages)</td>
<td>Focus on the potential cost effectiveness of autologous HSCT in the management of SPMS only</td>
<td>Enables CBA for patients with stroke under a wide range of assumptions</td>
</tr>
<tr>
<td>Limitations</td>
<td>Small number of patient-level data; clinical effectiveness data based on open-label transplantation trials.</td>
<td>The absence of direct RCT evidence to input into the model</td>
<td>Effectiveness of SCT in humans was based on expert opinion; did not include differential costs on early vs. late administration poststroke; limited standard care data reflecting survival, treatment patterns, and transition probabilities for mRS.</td>
</tr>
</tbody>
</table>

CBA, cost-benefit analysis; CUA, cost-utility analysis; EDSS, Expanded Disability Status Scale; HSCT, hematopoietic stem cell transplantation; HY, Hoehn and Yahr stages of PD; ICER, incremental cost-effectiveness ratio; mRS, modified Rankin Scale; MS, multiple sclerosis; NHS, National Health Service; PD, Parkinson disease; PFS, progression-free survival; QALY, quality-adjusted life-year; RCT, randomized controlled trial; SCT, stem cell therapy; SPMS, secondary progressive multiple sclerosis.

* Scenario 1: Strict 6-mo sustained progression from baseline EDSS.
† Scenario 2: Next-visit sustained progression from baseline EDSS.
‡ Strict 6-mo sustained progression from any EDSS.

clinical practice [21] and an SCT study cohort [28] at their institution. Health state utilities were generated for every HY stage of PD, using the generic EuroQol five-dimensional questionnaire (EQ-5D), measured by the time trade-off method [29]. QALYs were obtained by multiplying the number of mortality-adjusted life-years spent in each HY stage with its health utility weight.

The stroke model used assumptions regarding effectiveness of stem cells on the basis of expert opinion. The change in function and quality of life after a stroke was classified into distinct health states in accordance with the different mRS scores. A previously published algorithm that translates the mRS into EQ-5D utility was used to generate QALYs for each mRS score [30].

The SPMS model sourced individual patient-level disease progression data from a real-world clinical practice registry (mitoxantrone) [31] and an HSCT patient registry and estimated relative effectiveness based on Kaplan-Meier progression-free survival curves [32]. Discrete health states, defined by the EDSS state (a measure of disability in SPMS), were associated with unique health-related quality of life. The time spent in each EDSS state weighted by its respective health-related quality-of-life level (from previous literature) provided an estimate of the number of QALYs gained in each treatment group.

Quality

All the studies reported QALYs as the health outcome. None of the studies, however, justified its choice in the study context. With respect to reporting study parameters, all the studies missed reporting variability around mean estimates of effectiveness.

Model-Based Economic Evaluations: Choice of Model and Analytical Method

All three studies conducted a cost-utility analysis of SCT versus standard care. In addition, the stroke study reported a cost-benefit analysis in terms of societal value expressed as the value of health benefits (QALYs x willingness to pay per QALY gain) less the incremental cost of SCT over standard care [20–22].
stroke study used the decision tree model [20], but the PD and SPMS studies [21,22] used the Markov state transition model.

**Quality**

All the studies justified that their analytical method and choice of model, along with the input parameters used in the model, were appropriate, given the early stage of research with SCT. The models were explained using a schematic in the stroke and SPMS studies.

**Incremental Costs and Outcomes**

The PD study reported overall cost savings ($351,40 [HY stage III]; $322,462 [HY stage IV]) and gains in QALYs (0.873 [HY stage III]; 1.133 [HY stage IV]) with the use of SCT in the base-case scenario: patients with early onset PD (HY stages III-IV) with an assumed initial improvement during the first 2 years, after a stationary period of up to 5 years after grafting, and return to preoperative rate of disease progression thereafter [21]. These were evaluated against the cost-per-QALY thresholds acceptable to UK ($5593) and Swedish ($9934) payers. This predicted that a price premium ($5109–$9083) was available for recovering investment on development.

The stroke study reported a QALY gain of 1.34. Although the SCT intervention increased costs by $70,960, these were offset by decrease in productivity losses to result in an overall saving of $21,122. SCT dominated standard care in terms of incremental cost-per-QALY gain in base-case population: patients aged 55 years at stroke onset, had an mRS score of 2 at hospital discharge, and were given intracerebrally transplanted SCT with an assumed relative effectiveness of 50% and experiencing no side effects [20].

The study reported that the societal value of SCT in stroke was $184,567, assuming a Swedish willingness to pay for a QALY of $122,379. This represented a potential headroom of $21,122 per treatment for developers to realize a return on investment [20].

The SPMS study presented the central estimates of cost effectiveness for autologous HSCT versus mitoxantrone across three base-case scenarios incorporating different methods of measuring disease progression (EDSS) [22]. The study reported an incremental cost per QALY gained (incremental cost-effectiveness ratio) of $4726/QALY in the scenario in which disease progression was measured as EDSS progression sustained for 6 months from any EDSS [22]. Nevertheless, HSCT may be dominated (costlier and less effective) by mitoxantrone in the scenario in which confirmation of disease progression required sustained increase over 6 months since baseline. In the scenario requiring next-visit sustained progression from baseline, the incremental cost of $125,679/QALY gained was not cost-effective as per the UK threshold of $33,967 to $50,090/QALY [22].

**Quality**

All the studies reported on incremental costs and outcomes adequately in terms of costs per QALYs or incremental cost-effectiveness ratio as appropriate.
Characterizing Uncertainty

All three studies undertook univariate sensitivity analyses to test for the impact of changes in parameters included in the model on the study results. In addition, the SPMS study undertook a scenario analysis involving different durations of persistence of clinical benefit.

The PD study undertook a univariate sensitivity analysis to test whether changes in model specifications had an impact on the robustness of the results, focusing on the time horizon, discount rate, treatment efficacy, occurrence of complications, and changes in the analytical perspective (only direct medical costs vs. other direct costs as well). The outcome was varied from the EQ-5D health state-based utilities to the time trade-off visual analogue scale method. The results were found to be sensitive to changes in time horizon, discount rate, treatment effect, and health utility method for patients in HY stage III, but were stable for patients in HY stage IV.

The stroke study undertook univariate sensitivity analysis when model assumptions and specifications regarding effect size, age of onset, aspects of the therapy provided, risk of stroke, and procedure-related mortality risk were varied to see their impact on the robustness of the results. SCT remained cost-effective, but there was a decrease in QALY gain with increased incremental costs associated with decrease in relative efficacy, higher age at stroke onset, and intervention in patients with higher disability (mRS score of 4), leading to lower societal value. SCT, however, remained cost-effective down to a relative efficacy size of 14%.

The SPMS study undertook a scenario analysis including optimistic, pessimistic, and middle-ground scenarios according to the assumption of the duration of sustained benefit and the method of measuring EDSS progression. The interpretation of clinical effectiveness (on the basis of different scenarios for measuring EDSS) had a significant impact on cost effectiveness. Univariate sensitivity analysis was also undertaken with varying model inputs such as the mortality rate associated with the intervention, treatment effect duration, cost of the intervention, cost of managing multiple sclerosis, and the discount rate.

Quality

Although all the studies performed univariate sensitivity analyses, the choice and range of variables examined were not explained in detail. Only the SPMS study explained why some variables were included. Although univariate sensitivity analysis is a valid approach, more sophisticated approaches can provide an indication of the impact of the overall uncertainty in the model when uncertainty around the model parameters exists (such as in the included studies). Approaches such as multiway analysis, threshold analysis, scenario analysis, and probabilistic sensitivity analysis can account for differences in multiple parameters at the same time and provide a more comprehensive picture of the total uncertainty in the findings [33].

Discussion

Main Findings

This is the first systematic review of published studies conducting economic evaluation of the use of SCT in neurological diseases. The studies included in our review differed in terms of disease indications, SCT types, clinical measures, evaluation perspectives, and cost outcomes included, which makes it difficult to compare results across the studies. All the included studies conducted a cost-utility analysis using early-stage health economic modeling. The models estimated the value proposition of SCT in disease populations over a long-term time horizon. All the studies reported potential cost savings over long-term and ongoing benefit in terms of decreased rate of disease progression and disability.

The individual studies examined different types of stem cells. The evidence from clinical research with SCT to date does not indicate that differences in cell types have a significant impact on effectiveness of therapy or safety. Having said that, the impact of using different cell types on potential clinical benefit (in terms of relative efficacy and safety) and thereby on cost effectiveness has not been specifically examined. This may reflect the early stage at which clinical research was at the time of publication of these studies [27]. As more studies are being conducted with distinctly characterized cell types, it may be possible in future to determine whether cell type should be a variable to examine in the cost-effectiveness analysis. There are uncertainties regarding the effect size of SCT, given the early stage of research and the inherent heterogeneity of disease characteristics, which make assessment of cost effectiveness complicated. Nevertheless, as newer data emerge regarding longer term clinical benefit with SCT, these models can be reworked with more substantive data to assess economic value and identify patient groups that are likely to maximally benefit.

Diseases such as stroke and PD contribute substantially to the health care budget [34,35]. Assessing effectiveness from this limited perspective may, however, underestimate the value of personalized interventions such as SCT. It fails to consider the potential gains in terms of decreased disability for the patients and increased participation in work and society. This is important to consider while assessing negotiated pricing strategies.

The quality of study methods and reporting is important because economic evaluations in this field are likely to grow. We assessed the quality of the reviewed studies using the CHEERS checklist [18]. None of the included studies fully satisfied all the criteria listed in the CHEERS checklist, with only 50% of the items being rated as “fully satisfied.” Even though these studies were published before the formulation of the CHEERS checklist, the review highlights the need for incorporating these requirements in the conducting of and reporting for future economic evaluations of SCT.

Study Limitations

The included studies differed in terms of disease populations and SCT types. In addition, heterogeneity in terms of analytical methods, cost, and effectiveness measures used meant that only a narrative synthesis of findings is presented, because a formal meta-analysis was not appropriate.

Two of the three studies examined cost effectiveness from a government perspective only. Although this is key for determining future access strategy, economic evidence from a broad societal perspective may provide directions to optimize value by targeted research in specific patient groups and health care delivery pathways.

All the studies have sourced their effectiveness data from either single-arm studies, registry, or expert opinion. Although this may be acceptable for rare disease indications, effectiveness data in the more common neurological diseases such as stroke or PD may be more informative if adequately powered for the clinical outcome end points. A methodologically sound meta-analysis of smaller studies in future with appropriate sensitivity analysis may provide a reasonable early estimate of cost effectiveness. This, however, requires these studies to collect data on resource use in terms of effectiveness of the therapies in their study design. The models proposed in the studies included in the review may then be able to incorporate data from such analysis appropriately. The use of models is appropriate to the stage of
research, and these models are amenable to more extensive data inputs as they become available. Nevertheless, it is currently difficult to provide a detailed assessment of bias and generalizability of the findings of this review.

**Potential Value of Health Economics Data at Early Drug Development**

As research in regenerative medicine for neurological diseases approaches an exciting juncture, it becomes imperative to explore the associated cost outcomes [36]. This is critical to inform a targeted development strategy that maximises chances of an eventual product that resonates with the value expectations for patients in need as well as for payers [37,38]. The focus on development efficiency is heightened by the constant shortening of the window of opportunity to realize returns on investments; research and development teams are faced with complex trade-offs in terms of developing an efficient product development strategy [39]. Early health economics data can provide useful input into defining these strategies.

**Recommendations for Future Practice for Economic Evaluation in Regenerative Medicine**

SCTs have been investigated in an increasing number of phase I and II trials across the globe for different neurological indications [6–9]. Incorporating cost outcomes into the research protocol at this stage will enable formative evidence to be generated and maximize the unique opportunity that stem cell research provides, in that these therapies are often researched in patient populations from the earliest stage rather than in healthy volunteers. The long-term persistence of the effects of SCT is a key consideration in choosing appropriate clinical and cost out-comes to calculate cost-effectiveness [39,40]. A patient or societal perspective may be preferred because the high initial costs of SCT could be justified by ongoing cost savings because of sustained clinical improvement, improved independence, and participation in activities of daily life. This represents a more comprehensive measure of value. Participants in early-phase clinical studies with SCT are increasingly being followed over a longer time duration via extension studies or registries [40]. Collection of resource use data in these settings represents a useful means to examine cost-effectiveness over longer durations. This will enable credible analysis of economic evidence and the complex trade-offs between investments during development and potential returns and help manage access to these innovative therapies in a sustainable way.

**Conclusions**

Economic evaluation of SCT in neurological diseases is still at a nascent stage. Nevertheless, the recent progress in terms of clinical research underlines the urgent need to advance this field in tandem. Research to build economic evidence for cost-effectiveness of these innovative therapies can potentially accelerate their clinical translation and provide channels for providing sustainable access to these therapies to patients in clinics.

**Acknowledgment**

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**Supplementary Materials**

Supplemental material accompanying this article can be found in the online version as a hyperlink at https://doi.org/10.1016/j.jval.2018.07.078 or, if a hard copy of article, at www.valueinhealthjournal.com/issues (select volume, issue, and article).

**REFERENCES**

6.5 Conclusions

Economic evidence for cost-effectiveness of these innovative therapies is critical to accelerate their clinical translation and provide channels for providing sustainable access to these therapies for patients. Early CT research is carried out in stroke patients rather than in healthy volunteers as is more common in early phases of research with other therapies and this provides a unique opportunity to evaluate ways to collect economic evidence for CTs. Future studies need to consider collecting cost and resource use data within the trial design.
CHAPTER 7: DISCUSSION

7.1 Discussion

This thesis describes a compendium of projects that assessed the different considerations in design and implementation of early phase clinical studies investigating the use of CTs in stroke. The research applied a mixed method approach to identify and describe the study design, regulatory policy, ethical concerns and health economic considerations that are likely to be critical to the quality of early phase CT study execution in patients with ischaemic stroke. This research may potentially lead to the development of a framework including suggested standards for early phase CT research in ischaemic stroke.

Significant advances have been made in our understanding of brain injury and recovery following stroke at molecular, cellular and systems levels, due to the availability of sophisticated applications that use various ‘omics’ techniques and advanced structural and functional brain imaging. This research has yielded numerous promising preclinical therapeutic candidates in the past years. However, except for thrombolytics, none of the biological or pharmacological candidates have succeeded in clinical trials so far (298). Whilst there could be multiple reasons for this to happen, the complexity of the disease and the repair mechanisms operational in stroke are certainly important contributors (146). The preclinical research in CTs across different cell types indicates a two-way responsive mechanism of action that modulates the brain microenvironment towards optimization of neuroinflammatory cues and facilitates neurogenic and angiogenic processes (156). Early phase studies of CTs in human stroke subjects indicate functional benefit and credible safety to date (428). As clinical testing moves into a more definitive assessment of efficacy with numerous CT candidates moving into Phase II or larger Phase I/II trials, it is imperative to examine various
aspects of trial conduct in stroke, which have been likely contributors to translational setbacks in the past. Furthermore, it is important to examine these considerations in the specific context of CTs, as these innovative therapies bring with them unique challenges in terms of trial execution, regulatory oversight mechanisms, ethical debates and demonstrating cost effectiveness.

The first clinical study with CTs in stroke, published in the year 2000, was sponsored by Layton BioScience Inc. and investigated pre-differentiated neuronal cells from the NT2/D1 human precursor cell line (429). Since then, academic research groups, predominantly, have carried the research effort forward. In 2014, 70% of CT trials across different disease indications were led by academic groups and 30% were led by industry (430). This may perhaps be because the clinical development of these products is likely to be characterised by a high degree of uncertainty and risk compared with conventional drug development (425).

7.1.1 Is Research Design Letting CT Research Down?

Evidence based medicine has long established that randomised clinical trials are the ‘gold standard’ for generation of data for supporting the clinical use of investigational modalities (431). By reducing bias and enhancing the rigor of investigation, RCTs have contributed significantly to improving the quality of health research outcomes by clarifying the benefits and drawbacks of countless interventions (431). RCTs determine average treatment effects in a target population and rely on the deliberate selection of a homogenous population to study and compare, to reduce the effect of bias, confounding and effect modification (431). This is achieved by establishing appropriate inclusion and exclusion criteria, standardizing interventions and selecting the most relevant outcomes. However, the suitability of RCTs has often been challenged in areas of clinical research where the disease characteristics are inherently heterogeneous and therefore therapeutic strategies are intuitively ‘personalised’ and defy standardization of trial elements (a key
requirement of RCTs) (432). Some examples have been fields such as surgical and psychotherapy interventions (432). In the context of stroke, rehabilitation is one such area of research, which has struggled with applying aspects of RCT design (139). Despite requiring a long duration to recruit and complete, most RCTs remain relevant to a very selective patient population (432). This understandably limits the real-world clinical applicability of their eventual findings (432).

CTs research in stroke represents a similar conundrum. The effect of a stroke injury is a sum of individualized patterns of injury that are influenced by numerous patient and management factors. In this context, the application of CTs that inherently rely on the cross-talk with an injured brain environment to facilitate recovery, poses challenges with artificially fitting diverse patients into a group and analysing global functional outcomes (433). **Chapter 3** summarised our review which reported that the bulk of clinical studies (20/26 studies) with CTs have so far used alternative methodologies to RCT (mostly single-arm open label studies or case series with historic and contemporary controls). Meta-analysis to derive the magnitude of effect on outcomes indicates a potential benefit in these studies although the effect size in the subgroups of studies with comparator arm was marginal (428). The high level of heterogeneity across these studies weakened the overall strength of evidence (428). Future effectiveness studies may be more efficient if designed to identify a benefit in a specific subpopulation within the broader stroke population. This would require *a priori* selection of a specific nature of impairment within the stroke population and choice of outcome measures that specifically measure relevant domains of impairment, activity and participation. Methodologies to modify the conventional RCT design have been proposed in the literature to enable this (434) and regulators have recently expressed their openness to engage with developers to examine the value of these methods (435).
One of these methodologies is the use of randomised trial designs that adaptively change enrolment criteria during a trial, called adaptive enrichment designs (436). Adaptive designs can potentially provide clinically relevant information about which subpopulations are likely to benefit from the investigational therapy. The intended subpopulations, enrolment modification rules and statistical analyses need to be pre-specified in the study protocol. These subpopulations could be defined, based on baseline characteristics such as functional or prognostic phenotype or the use of structural, functional or imaging biomarkers. The advantage of using this approach is that the trial population could be enriched by a greater proportion of subjects with a potential for benefit from the treatment (434). Furthermore, it allows for the early stopping of exposure in subpopulations that do not demonstrate any benefit. In the context of early phase CT research, this approach can enable researchers to have increased power to detect and measure true effect size in the subpopulations that show benefit and improve efficiencies in designing subsequent confirmatory trials. From a safety standpoint, the ability to limit exposure in suboptimal populations wherein potential long-term adverse events need to be prevented is an important advantage, especially relevant to CTs (437). This methodology can also prove useful for adaptively choosing the patient subgroups, focused on data on baseline characteristics of interest and stratified randomization of study participants according to those subgroups of interest. The recent DIFFUSE 3 study used this methodology to evaluate endovascular treatment in ischaemic stroke patients with imaging-perfusion mismatch (438).

The other methodology to consider is the use of cluster randomization. Cluster randomization has been used in rehabilitation trials to evaluate the treatment impact of interventions in different patient groups clustered by site or patient characteristics (439). The key premise is that clusters share common characteristics and that observations on individuals in the same cluster tend to be correlated (348). This can be accounted for in study planning by incorporating the design effect
into sample size calculations. Design effect depends on the average cluster size and the degree of correlation within clusters (intraccluster correlation coefficient or ICC). ICC for outcome measures such as BI, mRS and NIHSS have been calculated previously and can be used in planning future studies that use these models (368). Rehabilitation is recommended as a necessary concomitant therapy that should be included in all CT clinical studies (328). There is evidence that CT and rehabilitation may have a mutually facilitative biological effect (440). Clinical guidelines recommend the delivery of rehabilitative interventions individualized to specific patient needs (137). In the context of designing future effectiveness studies with CT, it may be useful to consider CT in combination with rehabilitation as an integrated therapeutic package. While this may have labelling implications, it makes intuitive clinical sense. Randomizing patients into clusters with similar rehabilitative needs, based on similar functional deficits, may enable delivery of rehabilitation that is targeted, yet can be standardized enough to fulfil the requirements of an RCT. This will potentially help in understanding the clinical relevance of any changes seen in chosen outcome measures after CTs administration.

In addition to the overall study population disposition, the choice of outcome measures assessed can have a critical impact on research output. The systematic review indicated that the majority of CT studies enrolled patients with middle cerebral circulation infarction. Most patients were therefore likely to have had predominantly sensory-motor deficits. It is interesting to note that only six of the 26 studies used outcome measures that specifically measured change in motor impairment. In most studies, the change in disability following CTs administration was evaluated using either a global measure of impairment such as NIHSS, or a measure of activity dependency such as mRS or BI. While these studies followed conventional advice from stroke trials to use established (hard) global disability endpoints, future effectiveness studies may be more efficient if the primary outcome measure chosen is domain-specific and aligned to specific impairment in the
selected study population. This endpoint could be supported by secondary endpoints such as changes in domains of activity and participation. Regulatory agencies have communicated their willingness to accept such choices in effectiveness studies as these would hopefully provide clearer data guidance for eventual clinical use (441).

7.1.2 The Regulatory Maze on the Way to Market?

The pace of clinical translation with CTs in different disease indications (Fig. 2) has increased in the last decade (430). Regulatory agencies across the globe are cognizant of a paradigm change in medicine that CTs potentially represent in terms of being able to generate replacements for cells that are lost to injury or disease. The agencies are increasingly aware of the unique challenges to researchers and healthcare providers and of the potential for harm if adequate oversight mechanisms are not in place to ensure patient safety. Chapter 4 examined key developments in the regulatory field over the last decade that have come forth in response to this acceleration and provide guidance to researchers in the field of regenerative medicine. These guidelines have created certain requirements that developers in academia and industry must fulfil to have a successful path to market. Our review analysed guidelines from regulatory jurisdictions that have been at the forefront of research and development in CTs and included the USA, Europe, Canada, Japan and Australia. We analysed these different guidelines to identify key commonalities and differences, to define the critical considerations that developers need to address in future effectiveness studies with CTs, for these products to have a successful transition to the treatment clinic.
CTs are technically challenging to develop, when compared to conventional pharmaceutical products. Their clinical use is likely to be more akin to cell and organ transplant products making their development fit better with academic institutes than conventional manufacturers. Multiple early phase exploratory clinical trials have resulted in a rising number of high impact publications. However, about 98% of clinical cell therapy trials have not yet resulted in licensed products, as most of these successful exploratory studies have not been followed by well-designed (controlled) efficacy trials (442).

Academic centres with their scientific expertise in diseases with high unmet need and relatively easy access to patient cohorts and their clinical samples and imaging data, are well placed to lead
research in innovative areas like CTs (443). However, they are inherently limited in taking this research all the way to market due to key issues regarding systems, funding and capacity (443). Academic groups, driven by scientific principles, are often limited in their capability to analyse an investigational product in terms of its ‘target product profile’ (TPP) and to implement required standardization and quality control processes essential to medicinal product development (444). TPP is a term commonly used by commercial drug developers and regulators but may be relevant in the context of CTs, even at early phases of research, due to their unique characteristics (322, 445). Establishing TPP in the early phases of development is essential for targeted research with complex interventions such as CTs. In addition, academic groups often lack regulatory expertise and most academic institutions have sparse resources available to enable access to such expertise for a research group (444). In this scenario, CTs tested in early phase studies cannot be taken further as they do not meet the product quality assurance requirements for GMP and Investigational Medicinal Product Dossier compliance.

The key themes to emerge from our review that are relevant to address in the course of research planning are:

1. Clear articulation of the TPP for the CTs in development is critical. This will potentially ensure that the development plan results in generation of data that justifies the suitability of the structural and functional profile of CTs for the intended clinical use. Furthermore, the research groups should articulate the intended clinical use in terms of regulatory terminology for use. This is an essential component of clinical trial planning and regulatory approval process, as the pathways for assessment and oversight differ substantially, depending upon the exact conditions of clinical use.

2. The clinical use should be linked to the level of processing and manipulation that the cells have undergone prior to administration in human subjects and the understanding of the
risks these manipulations may represent to CTs recipients. The development plans are required to justify how the selected clinical trial designs optimally assess and capture these often-unpredictable safety risks and ensure ongoing risk-benefit assessment.

3 Access to GMP compliant cell therapy processing services and expertise in product development has emerged as perhaps the most important rate-limiting step in successful translation in academic settings. Its significance is underscored by the fact that collaborative networks established for facilitating this have been established in countries that have been leading the CT research field, such as the UK (Cell Therapy Catapult) (446), Canada (Canadian Centre for Commercialization of Regenerative Medicine) (447) and the USA (Production Assistance for Cellular Therapies (PACT) program) (448).

7.1.3 What are Patients with Stroke Expecting from CT Research?

The current stage of research with CTs in stroke faces ethical issues that are also seen in early stage research in other clinical areas (400). However, the novelty of science with CTs in general and aspects of the evolving knowledge in this field (449), combined with challenges specific to stroke, raise some important considerations that are vital to address for efficient trial planning and implementation. Chapter 5 examined key ethical issues that can impact on the practical aspects of patient participation in CT clinical research. The ‘PERSPECTIVES’ study conducted thematic analysis of perspectives shared by a purposive sample of stroke survivors in Adelaide, regarding their expectations from research with CTs in stroke in general and provided inputs into the research design of a proposed early phase CT study developed by our research group (TOOTH study protocol and study materials). The study used qualitative research methods to collate patient experiences with stroke and issues and outcomes of importance to them in line with the principles of Patient and Public Involvement in Research (PPIR).
PPIR is an emerging field that aims to evaluate whether research is relevant to issues in disease management or therapy delivery that are considered important by patients and the public (399). PPIR seeks to ensure that the results of research investment represent value from a patient’s (the ultimate beneficiary) perspective (450). PPIR can be implemented at various levels such as: engaging patient or carer communities to define areas in which to prioritize research funding; to develop more patient-relevant research questions, study designs, and outcomes; to develop research materials to improve readability and recruitment; and the dissemination of study results (397). There is a worldwide push to establish PPIR as an essential element of research, especially with publicly-funded research projects.

The stroke survivors who participated in our study provided interesting insights about their perception of risk and benefit that are useful to understand in the context of the promise that CT research potentially offers. Previous studies have reported a general positive attitude towards participation in CT research but there was a wide divergence in efficacy expectations between study participants and research teams. Informed context in the context of CTs in stroke presents an argument to re-examine the process in terms of an ongoing dialogue rather than a one-off event at the start of the trial. This is due to the possibility of unpredictable but long-term effects of CTs that may be seen later in the course of the study or safety follow-up and consent may be required for management of these complications. Therapeutic misconception can arise for various reasons and appropriate information sharing is the key to addressing this. Participants in our study communicated a pragmatic realistic approach to anticipated benefits. This finding seems to differ from the findings from similar patient research done in the context of CT in haematological malignancies (405). It would be useful to explore the factors driving this difference. Perhaps the difference in anticipated survival may play a role in this. It was also interesting that patients were more worried about a loss of function as a possible risk as compared to more widely discussed risks.
such as tumourigenicity and death. The importance of measuring changes in often neglected outcomes such as cognition, mood and overall ability to restore ‘normal’ pre-stroke participation levels is a key insight that should inform future trial designs. This patient perspective about recovery expectation has been reported in previous qualitative research with stroke survivors (394,451). Thus, patient involvement in research design with future CT studies is likely to provide key insights that can result in more relevant trial conduct, increase motivation to participate and improve recruitment in these complex clinical studies.

7.1.4 Will CTs Eventually be Worth It?

Regenerative medicine potentially represents a paradigm shift due to the potential for modification of the underlying causes of disease by repairing, replacing, or regenerating damaged cells in the body. If successful, CTs may potentially reduce the burden of disease and improve the health-related quality of life of many patients with chronic diseases such as stroke. This may potentially translate into cost reductions to health services. The lack of cost outcomes data with CTs can make the demonstration of value proposition for these innovative, but high cost therapies, extremely challenging. CT researchers may be required to anticipate the access challenges that may represent the last component of the widely acknowledged ‘translation roadblock’.

Incorporation of health economic considerations in early stages of research with CTs can serve two purposes:

- inform researchers and developers about the regulatory and reimbursement strategy, using early-stage (or iterative) health economic modelling including headroom analysis
- estimate unknown effect sizes and beliefs using methods like stakeholder preference elicitation and multi-criteria decision analysis to refine trial designs and maximize the generation of acceptable cost-effectiveness data.
Chapter 6 presented the findings of the first systematic review of health economic evaluation of CTs at the early phase of research. This area of research is still in infancy as is borne out by the fact that only three studies have been published on a cost-effectiveness analysis of the use of CTs. The modelling study in stroke using expert opinion on probable effect size of CTs, reported overall societal value driven by long-term cost savings due to decreased cost of disability and productivity losses (414). Early health technology assessment (HTA) studies are required to generate evidence for the value proposition of CTs. Inclusion of measures relevant to the impact on costs associated with healthcare resource use and avoidance of productivity losses, as secondary endpoints in early phase trials, will generate data for input into early HTA studies. This will enable simultaneous generation of supportive evidence for efficacy and economic sustainability for CTs.

7.2 Contribution and Impact

There is a scientific imperative for a successful clinical translation of CT into relevant treatment strategies for patients with stroke-related disability. STEPS recommendations proposed high-level consensus based pragmatic concepts underpinning the development of CTs in stroke. However, academic groups engaged in CT research would benefit from an expanded and practical framework that can help in planning research and the development of investigational CT into viable medical products.

This thesis used a mixed methodology approach as relevant, to explore and analyse the critical quality determinants of early phase clinical studies investigating CT in stroke. Based on the findings of the subprojects of this thesis, a practical framework in a checklist form, incorporating study design, regulatory, ethical and health economic parameters is proposed. This checklist can potentially serve as a tool for research teams, particularly in academic settings, to efficiently plan operationalisation of early clinical studies in stroke. This can help the team to determine areas of
expertise gaps and enable them to allocate resources accordingly, for capacity building. The checklist is displayed on the following pages.
<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study design</td>
<td></td>
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<tr>
<td>Target population</td>
<td>Is the intended patient population clearly identified (from preclinical and other exploratory studies)?</td>
</tr>
<tr>
<td></td>
<td>Is the applicable patient population yet to be adaptively selected in the trial due to the potential patient and/or disease heterogeneity?</td>
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<tr>
<td></td>
<td>Are baseline characteristics of participants mapped to ensure alignment to primary impairment of interest, before randomization?</td>
</tr>
<tr>
<td></td>
<td>Is imaging required to support clinical examination for baseline assessment?</td>
</tr>
<tr>
<td>Trial design</td>
<td></td>
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<tr>
<td></td>
<td>Is the trial use group randomization by cohorts defined by impairment rather than lesion territory (clinical/imaging)?</td>
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<tr>
<td></td>
<td>Is there value in adopting adaptive trial design versus fixed RCT?</td>
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<tr>
<td></td>
<td>Does the trial design allow interim selections of design aspects (dose levels or sample size) or design parameters (effect threshold)?</td>
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<td></td>
<td>Is the interim analysis pre-specified in trial protocol?</td>
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</table>
Table 7: Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct (continued)

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Are rules for efficacy/futility prespecified in trial protocol?</td>
</tr>
<tr>
<td></td>
<td>Is MCID for selected primary outcome measure established <em>a priori</em> and used to decide on efficacy/futility?</td>
</tr>
<tr>
<td>Study endpoint</td>
<td>Is primary outcome measure appropriate to the primary impairment of interest?</td>
</tr>
<tr>
<td></td>
<td>Do secondary endpoints include domain specific outcome measures for activity &amp; participation?</td>
</tr>
<tr>
<td></td>
<td>PROM incorporated as secondary endpoints?</td>
</tr>
<tr>
<td>Timing of study after stroke</td>
<td>Which is the preferred phase of stroke for use of the candidate CT product?</td>
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<td></td>
<td>What additional logistics challenges does this phase pose for CT delivery?</td>
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<tr>
<td></td>
<td>Is CT processing feasible for time of delivery after stroke?</td>
</tr>
<tr>
<td></td>
<td>Is time window for delivery defined <em>a priori</em>?</td>
</tr>
<tr>
<td>Items</td>
<td>Response</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Intervention</td>
<td>Is CT (dose/route) selected based on extrapolation from preclinical research or deduced using adaptive design?</td>
</tr>
<tr>
<td></td>
<td>Is rehabilitation intervention targeted to primary impairment of interest</td>
</tr>
<tr>
<td></td>
<td>Is dose of rehabilitation defined \textit{a priori} in protocol</td>
</tr>
<tr>
<td></td>
<td>Is temporal sequencing of CT &amp; rehabilitation used in study protocol? – justification from available evidence?</td>
</tr>
<tr>
<td>Comparator</td>
<td>Is control arm receiving placebo delivery?</td>
</tr>
<tr>
<td></td>
<td>Is control arm undergoing sham procedure?</td>
</tr>
<tr>
<td></td>
<td>Is intervention and dose of rehabilitation in control arm matched to experimental arm?</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>Does statistical plan support adaptive design, if chosen, and pre-specify the interim analysis and study progress rules?</td>
</tr>
<tr>
<td></td>
<td>Does statistical plan justify the analytical methods used for selected endpoint analysis?</td>
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</tbody>
</table>
Table 7: Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct (continued)

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety reporting</td>
<td>Is there a mechanism for long-term safety follow-up through extension studies/registries?</td>
</tr>
<tr>
<td>Regulatory</td>
<td>Is it allogeneic or autologous?</td>
</tr>
<tr>
<td>Intended CT use</td>
<td>Is it homologous/non-homologous?</td>
</tr>
<tr>
<td></td>
<td>Is CT combined with tissue engineering products or devices</td>
</tr>
<tr>
<td></td>
<td>Would CT delivery need invasive procedure and/or use of delivery devices?</td>
</tr>
<tr>
<td>CT processing</td>
<td>Is the CT manipulation 'minimal'?</td>
</tr>
<tr>
<td></td>
<td>Will CT be processed on-site or sourced from cell processing facility/sponsor manufacturing site?</td>
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<tr>
<td></td>
<td>Does the site/outsourced facility have required manufacturing approvals?</td>
</tr>
<tr>
<td></td>
<td>Does the site/outsourced facility have GMP compliant cell-processing protocols?</td>
</tr>
<tr>
<td></td>
<td>Does the site/outsourced facility have CT specific release criteria?</td>
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<td></td>
<td>Do the release criteria include structural and functional characterisation using validated assays?</td>
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</table>
Table 7: Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct (continued)

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
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<tbody>
<tr>
<td>Do the release criteria include infection screening?</td>
<td></td>
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<tr>
<td>Does the site/outsourced facility have required quality control processes in place?</td>
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</tr>
<tr>
<td>Does the site/outsourced facility have required cryopreservation protocols to store and transport CT?</td>
<td></td>
</tr>
<tr>
<td>Expertise in submission of clinical trial applications to regulators</td>
<td>Does the team have access to expert support for regulatory submissions and negotiations?</td>
</tr>
<tr>
<td>Ethical Patient involvement in research (PPIR)</td>
<td>Has any PPIR been conducted/planned for the proposed CT study?</td>
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<tr>
<td></td>
<td>If yes, what aspects of study design were evaluated in PPIR?</td>
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<tr>
<td></td>
<td>Does the study protocol address the findings from the PPIR?</td>
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<tr>
<td></td>
<td>Is there patient/public representation on study steering committee?</td>
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<td></td>
<td>Were patient materials such as information sheet and other recruitment materials reviewed with patient/public representatives?</td>
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</tbody>
</table>
Table 7: Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct (continued)

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are endpoints measuring change in participation, independence of living, mood, pain and fatigue included in study protocol?</td>
<td></td>
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<tr>
<td>Has a lead-in period to optimise secondary prevention in participants prior to participant randomisation, been considered in study protocol?</td>
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<tr>
<td>Is formal cognitive assessment performed for all potential participants prior to consent?</td>
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<tr>
<td>If cognitively impaired patients are included in study, is process for proxy consent defined in protocol and approved by relevant ethics committee?</td>
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<tr>
<td>If implementing an adaptive research design, has the study team considered a dissemination strategy for changes in study conduct following prespecified interim analysis?</td>
<td></td>
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<tr>
<td>Study safety committee</td>
<td>Does the study committee include patient representation?</td>
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<tr>
<td>Health economic</td>
<td>Cost outcomes</td>
</tr>
<tr>
<td>Is resource use data for study and comparator groups defined in protocol?</td>
<td></td>
</tr>
<tr>
<td>Is data on direct costs of intervention/comparator defined and collected in protocol?</td>
<td></td>
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</tbody>
</table>
Table 7: Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct (continued)

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is data on direct costs of concomitant medications/interventions defined and collected in protocol?</td>
<td></td>
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<tr>
<td>What indirect costs data can be collected during the study period, and during study follow-up?</td>
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<tr>
<td>Is a PROM instrument that measures work productivity included in the study?</td>
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<tr>
<td>Justification for inclusion/exclusion of PROM?</td>
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</tbody>
</table>

7.2.1 Evaluation of TOOTH Study Protocol

The above checklist was used to analyse a Phase I/II study (TOOTH) proposed by our research team, the Stroke Research Programme at the University of Adelaide. It was used to identify key areas where the trial planning needed to be optimised and resources identified and secured. The completed checklist for this study is included as Appendix 1.

7.3 Limitations

The thesis examined different aspects critical to the development of innovative cell therapies and analysed the key factors that need to be addressed by development teams. Having said that, there are some limitations to the research conducted that are highlighted below.

First, since the predominant share of early clinical research in CTs is coordinated in academic institutions, this research focused on factors important in these settings. While we acknowledge
that these determinants are equally critical in the industry setting, our current research was focused on academic groups, as the need for the proposed framework is likely to be higher in those settings.

Second, access to patient level data proved to be a challenge whilst conducting the systematic review of clinical studies with CT in stroke. Access to such datasets is likely to be key in collating evidence from multiple smaller studies, which are likely to represent a major part of evidence in the early stages of translation. Access to this data would enable researchers to analyse whether heterogeneity in patient and disease characteristics have been adequately addressed. Database resources such as Virtual International Stroke Trials Archive (VISTA) database, established to serve as repository of anonymized data from completed clinical trials in stroke are already functional. Creating a similar resource for CT trials may be a way forward to collate data across numerous small studies.

Third, in the context of ethical considerations, our study specifically examined patients’ perspectives and there is a need to follow this up with a similar study conducted in stroke carers. This is important as carers are important stakeholders in PPIR. Simultaneous enquiry with both stakeholder groups in a single study is likely to limit insights as there is evidence from prior studies that there can be wide differences in patient and carer perspectives. Qualitative methods are best suited for evaluation of the needs and perspectives of different stakeholders in research and PPIR. Having said that, these methods are not well accepted in conventional medical literature, as evidenced by the very low numbers of published studies using these methods for PPIR in the context of CT use in stroke.

Fourth, health economic analysis was limited by the sparse availability of literature in this field. Our analysis and suggested framework will potentially highlight the need for the early generation of evidence for these outcomes. The critical importance of doing this is highlighted by the fact that
the majority of approved CTs are still struggling with reimbursement challenges and are not available to patients in need.

Lastly, our research underscores the urgent need for researchers to be trained in the requirements of medical product development. However, analysis of gaps in training curricula as a potential consideration in improving research efficiency, was not undertaken, given the time limitation. It would be important to understand the interdependencies across disciplines that underlie the present capacity gap in the research community.

**7.4 Future Directions**

The proposed checklist needs to be validated for use with key stakeholders such as clinicians, researchers, patient advocacy groups, patients, ethics committees, regulators and HTA experts. Future research could potentially involve this validation. This could be done using focus group discussions or using DELPHI methodology. The proposed checklist, if validated, can serve as a decision aid for academic research groups engaged in cell therapy research, to improve their research output and enable them to lead CT product development effectively for stroke. Some components of this checklist are relevant beyond stroke research to critical factors affecting wider CT research. Thus, this checklist can be adapted to requirements of other disease areas as well, in the future.
CHAPTER 8: CONCLUSIONS

Stroke represents one of the leading causes of disease burden in Australia and across the globe (10). The landmark success in the clinical translation of reperfusion strategies – thrombolysis and endovascular thrombectomy, represents significant mortality and morbidity benefit. However, these therapies are limited by a narrow window of opportunity. Despite declining mortality and morbidity due to general improvements in systems of stroke care, there is a present unmet need for impactful new therapeutic strategies in addition to rehabilitation.

Cell therapies represent an exciting option with demonstration of neurovascular repair and abrogation of neuroinflammation in preclinical stroke models. Despite a robust amount of exploratory clinical trial data published over the last decade, only a minimal percentage of these cell therapies have progressed further towards medical product development. Given a long history of expensive translational failures in stroke research and the fact that CTs research has been driven by academic research groups primarily, there is an urgent need to identify and address key factors that can enable efficient execution of clinical development of cell therapy products for stroke.

Firstly, innovative, adaptive study designs that identify specific homogenous subpopulations likely to benefit from a chosen cell therapy candidate and measure the impact of cell therapies in terms of domain-specific endpoints, along with conventional endpoints such as NIHSS and mRS, are critical. Secondly, the study team would need to address regulatory requirements for the development of cell therapies, which may vary depending on specific composition and conditions of use. Thirdly, patient and public involvement in early research with cell therapy can help make this research more relevant to real world practice. Lastly, researchers would do well to generate health economic data early in the course of cell therapy research. To ensure these aspects are
considered in a timely manner during early clinical study planning, this thesis proposes a practical framework that can help the research team to identify existing capacity gaps and build financial and human resources to address these gaps. This can enable academic institutes to improve their translational research programs with increased focus on cell therapy development, identifying existing skill gaps and allocating resources to address these gaps. This will potentially lead to enhanced efficiencies in early clinical research and accelerate clinical translation of these innovative therapies that hold a promise, if successful, of ushering in a paradigm change in stroke treatment and more widely in medical practice.
## APPENDICES

### APPENDIX 1

Analysis of TOOTH Study against Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study design</td>
<td></td>
</tr>
<tr>
<td><strong>Target population</strong></td>
<td></td>
</tr>
<tr>
<td>Is the intended patient population clearly identified (from preclinical and other exploratory studies)?</td>
<td>✓</td>
</tr>
<tr>
<td>Is the applicable patient population yet to be adaptively selected in the trial due to the potential patient and/or disease heterogeneity?</td>
<td>Specific impairment not defined</td>
</tr>
<tr>
<td>Are baseline characteristics of participants mapped to ensure alignment to primary impairment of interest, before randomization?</td>
<td>Not specifically defined</td>
</tr>
<tr>
<td>Is imaging required to support clinical examination for baseline assessment?</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Trial design</strong></td>
<td></td>
</tr>
<tr>
<td>Is the trial use group randomization by cohorts defined by impairment rather than lesion territory (clinical/imaging)?</td>
<td>×</td>
</tr>
</tbody>
</table>
APPENDIX 1: Analysis of TOOTH Study against Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct  

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is there value in adopting adaptive trial design versus fixed RCT?</td>
<td>✓</td>
</tr>
<tr>
<td>Does the trial design allow interim selections of design aspects</td>
<td>×</td>
</tr>
<tr>
<td>(dose levels or sample size) or design parameters (effect threshold)?</td>
<td></td>
</tr>
<tr>
<td>Is the interim analysis pre-specified in trial protocol?</td>
<td>×</td>
</tr>
<tr>
<td>Are rules for efficacy/futility pre-specified in trial protocol?</td>
<td>NA</td>
</tr>
<tr>
<td>Is MCID for selected primary outcome measure established <em>a priori</em> and</td>
<td>×</td>
</tr>
<tr>
<td>used to decide on efficacy/futility?</td>
<td></td>
</tr>
<tr>
<td>Study endpoint</td>
<td></td>
</tr>
<tr>
<td>Is primary outcome measure appropriate to the primary impairment of</td>
<td>Needs to be defined</td>
</tr>
<tr>
<td>interest?</td>
<td></td>
</tr>
<tr>
<td>Do secondary endpoints include domain specific outcome measures for</td>
<td>✓</td>
</tr>
<tr>
<td>activity &amp; participation?</td>
<td></td>
</tr>
<tr>
<td>PROM incorporated as secondary endpoints?</td>
<td>✓</td>
</tr>
</tbody>
</table>
### APPENDIX 1: Analysis of TOOTH Study against Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct (continued)

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timing of study after stroke</strong></td>
<td></td>
</tr>
<tr>
<td>Which is the preferred phase of stroke for use of the candidate SCT product?</td>
<td>✓</td>
</tr>
<tr>
<td>What additional logistics challenges does this phase pose for SCT delivery?</td>
<td>Patient recruitment from community</td>
</tr>
<tr>
<td>Is SCT processing feasible for time of delivery after stroke?</td>
<td>✓</td>
</tr>
<tr>
<td>Is time window for delivery defined <em>a priori</em>?</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Intervention</strong></td>
<td>Not specified</td>
</tr>
<tr>
<td>Is SCT (dose/route) selected based on extrapolation from preclinical research or deduced using adaptive design?</td>
<td>✓</td>
</tr>
<tr>
<td>Is rehabilitation intervention targeted to primary impairment of interest</td>
<td>✓</td>
</tr>
<tr>
<td>Is dose of rehabilitation defined <em>a priori</em> in protocol?</td>
<td>×</td>
</tr>
</tbody>
</table>
APPENDIX 1: Analysis of TOOTH Study against Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct (continued)

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is temporal sequencing of SCT &amp; rehabilitation used in study protocol? Justification from available evidence?</td>
<td>✓</td>
</tr>
<tr>
<td>Comparator</td>
<td></td>
</tr>
<tr>
<td>Is control arm receiving placebo delivery?</td>
<td>NA</td>
</tr>
<tr>
<td>Is control arm undergoing sham procedure?</td>
<td>NA</td>
</tr>
<tr>
<td>Is intervention and dose of rehabilitation in control arm matched to experimental arm?</td>
<td>NA</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td></td>
</tr>
<tr>
<td>Does statistical plan support adaptive design, if chosen, and pre-specify the interim analysis and study progress rules?</td>
<td>Not defined</td>
</tr>
<tr>
<td>Does statistical plan justify the analytical methods used for selected endpoint analysis?</td>
<td>✓</td>
</tr>
<tr>
<td>Safety reporting</td>
<td></td>
</tr>
<tr>
<td>Is there a mechanism for long term safety follow-up through extension studies/registries?</td>
<td>×</td>
</tr>
<tr>
<td>Items</td>
<td>Response</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Regulatory Intended SCT use</td>
<td></td>
</tr>
<tr>
<td>Is it allogeneic or autologous?</td>
<td>Autologous</td>
</tr>
<tr>
<td>Is it homologous/non-homologous?</td>
<td>Non-homologous</td>
</tr>
<tr>
<td>Is SCT combined with tissue engineering products or devices</td>
<td>×</td>
</tr>
<tr>
<td>Would SCT delivery need invasive procedure and/or use of delivery devices?</td>
<td>✓ MRI-guided intracerebral injection</td>
</tr>
<tr>
<td>SCT processing</td>
<td></td>
</tr>
<tr>
<td>Is the SCT manipulation 'minimal'?</td>
<td>✓</td>
</tr>
<tr>
<td>Will SCT be processed on site or sourced from cell processing facility/sponsor manufacturing site?</td>
<td>Need to be defined</td>
</tr>
<tr>
<td>Does the site/outsourced facility have required manufacturing approvals?</td>
<td>Need to be defined</td>
</tr>
<tr>
<td>Does the site/outsourced facility have GMP compliant cell processing protocols?</td>
<td>Need to be defined</td>
</tr>
<tr>
<td>Does the site/outsourced facility have SCT specific release criteria?</td>
<td>Need to be defined</td>
</tr>
</tbody>
</table>
APPENDIX 1: Analysis of TOOTH Study against Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct  
(continued)

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do the release criteria include structural and functional characterisation using validated assays?</td>
<td>Need to be defined</td>
</tr>
<tr>
<td>Do the release criteria include infection screening?</td>
<td>Need to be defined</td>
</tr>
<tr>
<td>Does the site/outsourced facility have required quality control processes in place?</td>
<td>Need to be defined</td>
</tr>
<tr>
<td>Expertise in submission of clinical trial applications to regulators</td>
<td>×</td>
</tr>
<tr>
<td>Does the team have access to expert support for regulatory submissions and negotiations?</td>
<td>✓</td>
</tr>
<tr>
<td>Ethical Patient involvement in research (PPIR)</td>
<td>✓</td>
</tr>
<tr>
<td>Has any PPIR been conducted/planned for the proposed SCT study?</td>
<td>Outcomes, consent</td>
</tr>
<tr>
<td>If yes, what aspects of study design were evaluated in PPIR?</td>
<td>✓</td>
</tr>
<tr>
<td>Does the study protocol address the findings from the PPIR?</td>
<td>✓</td>
</tr>
</tbody>
</table>
APPENDIX 1: Analysis of TOOTH Study against Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct (continued)

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is there patient/public representation on study steering committee?</td>
<td>✓</td>
</tr>
<tr>
<td>Were patient materials such as information sheet and other recruitment materials reviewed with patient/public representatives?</td>
<td>✓</td>
</tr>
<tr>
<td>Are endpoints measuring change in participation, independence of living, mood, pain and fatigue included in study protocol?</td>
<td>✓</td>
</tr>
<tr>
<td>Has a lead-in period to optimize secondary prevention in participants prior to participant randomization, considered in study protocol?</td>
<td>×</td>
</tr>
<tr>
<td>Is formal cognitive assessment performed for all potential participants prior to consent?</td>
<td>✓</td>
</tr>
<tr>
<td>If cognitively impaired patients are included in study, is process for proxy consent defined in protocol and approved by relevant ethics committee?</td>
<td>NA</td>
</tr>
</tbody>
</table>
APPENDIX 1: Analysis of TOOTH Study against Clinical Translation of Cell Therapies in Stroke: Checklist for efficiency in trial conduct  (continued)

<table>
<thead>
<tr>
<th>Items</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>If implementing an adaptive research design, has the study team considered a dissemination strategy for changes in study conduct following prespecified interim analysis</td>
<td>×</td>
</tr>
<tr>
<td>Study safety committee</td>
<td>Does the study committee include patient representation? ✓</td>
</tr>
<tr>
<td>Health economic</td>
<td>Cost outcomes</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
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</tbody>
</table>
APPENDIX 2

List of Conference Proceedings


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