Cell-based therapies for amyotrophic lateral sclerosis/motor neuron disease (Protocol)

Abdul Wahid SF, Law ZK, Lai NM, Ismail NA, Azman Ali R

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Cell-based therapies for amyotrophic lateral sclerosis/motor neuron disease

S Fadilah Abdul Wahid1, 2, Zhe Kang Law3, Nai Ming Lai4, 5, Nor Azimah Ismail1, Raymond Azman Ali6

1 Cell Therapy Center, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia. 2 Clinical Haematology & Stem Cell Transplantation Services, Department of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia. 3 Department of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia. 4 Department of Paediatrics, University of Malaya, Kuala Lumpur, Malaysia. 5 School of Medicine, Taylor’s University, Kuala Lumpur, Malaysia. 6 Neurology Unit, Department of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia

Contact address: S Fadilah Abdul Wahid, Cell Therapy Center, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Kuala Lumpur, 56000, Malaysia. sfadilah@ppukm.ukm.edu.my.

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ABSTRACT

This is the protocol for a review and there is no abstract. The objectives are as follows:

To evaluate the efficacy and safety of cell-based therapy in people with ALS/MND compared with a placebo or no additional treatment

BACKGROUND

Description of the condition

Motor neuron disease (MND) is a rare neurodegenerative disorder with an annual incidence of approximately 2 per 100,000 population. MND affects both males and females of all ages, with a peak incidence at 50 to 70 years of age (Logroscino 2005; Logroscino 2008). The cause of MND is unknown, but up to 10% of cases are familial (Murray 2004). The clinical features of MND are attributable to the degeneration of neurons and corticospinal tracts from the primary motor cortex in the brain to the anterior horn cells in the spinal cord and brainstem nuclei (Rabin 1999). Four major categories of MND are recognized, namely, amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS) progressive muscular atrophy (PMA) and progressive bulbar palsy (PBP). When the person presents with both upper and lower motor neuron signs, the disease is known as ALS, which is the most common form of MND. The terms PLS and PMA are applied when the initial presentation reflects only upper motor neuron involvement or only lower motor neuron involvement, respectively. PBP presents with weakness of bulbar muscles. Common clinical features of MND include wasting and weakness of the muscles for mastication, speech articulation and swallowing, and intrinsic muscles of the hands. Respiratory failure due to respiratory muscle weakness is a late feature, leading to death. Rarely, ALS/MND may present with acute respiratory failure (Chen 1996). The disease is virtually always fatal. Approximately half of people with ALS/MND die within three years from onset of symptoms, although 10% of people with ALS/MND may live longer than 10 years (del Aguila 2003; Turner 2003).

The exact mechanism leading to selective cell death of motor...
neurons is not well understood and is likely to be multifactorial involving genetic and environmental factors. Several genes have been identified as the cause of familial ALS, including mutations in Cu²⁺/Zn²⁺ superoxide dismutase 1 (SOD1), TAR DNA-binding protein 43 (TARDBP), fused in sarcoma (FUS) and chordin. However, the genetic defect in sporadic ALS is still unknown. The neurodegenerative process of MND may involve a complex interplay between genetic factors, oxidative stress, glutamatergic excitotoxicity, protein aggregation, mitochondrial dysfunction and impairment of axonal transportation. The surrounding glial cells have also been implicated in the pathogenesis via the release of inflammatory mediators, impaired neuronal metabolic support and dysfunctional signalling pathways (Lunn 2014; Shaw 2005). All these processes eventually lead to apoptosis of motor neurons. To date, there is no effective treatment for MND. Current treatment regimens focus on relieving symptoms to improve the quality of life of those affected. Riluzole, an antiglutamate agent, is the only available pharmacological treatment for ALS but it has minimal effect on survival and no effect on muscle strength, functional capacity or quality of life (Bensimon 1994; Goodall 2006; Miller 2012). Many other pharmacological agents have been tried but generally without clear benefit.

Description of the intervention

The use of multipotent stem cells may provide an attractive therapeutic option because of their ability to migrate into damaged neural tissues and promote regeneration of neurons (neurogenesis). These multipotent stem cells produce neurotrophic (growth-stimulating) factors, thus provoking the transdifferentiation of stem cells into neurons (Karussis 2010).

To date, there have been numerous clinical trials on the treatment of ALS/MND with cell-based therapy utilising cells isolated mostly from autologous bone marrow and peripheral blood, thus minimising the risk of rejection. The types of cells used for implantation have been bone marrow mononuclear cells (BM-MNCs; Blanquer 2012; Dedé 2009; Prabhakar 2012), bone marrow-derived mesenchymal stem cells (BM-MSCs; Baek 2012; Blanquer 2010; Karussis 2010; Martinez 2012; Mazzini 2003; Mazzini 2006; Mazzini 2008; Mazzini 2010; Mazzini 2012), granulocyte-colony stimulating factor (G-CSF)-mobilised-peripheral blood mononuclear cells (M-PBMNCs; Cashman 2008; Chio 2011; Nefussy 2010; Tarella 2010), olfactory ensheathing stem cells (OESCs; Chen 2007; Chen 2012; Chew 2007; Giordana 2010; Huang 2008; Piepers 2010), and neural stem cells (NSCs; Feldman 2014; Glass 2012; Riley 2012; Riley 2014). BM-MNCs are usually separated from bone marrow aspirate obtained from the individual’s hip bone by a density gradient method. Mesenchymal stem cells (MSCs) can be easily isolated from bone marrow, placenta, muscle and fat and subsequently cultured for three to five weeks to provide large numbers of cells for therapeutic application. These cells can be expanded in vitro with no risk of malignant transformation (Bernardo 2007). M-PBMNCs can be obtained by administration of G-CSF to increase the number of stem cells in the patient’s circulation and subsequently removing the stem cells from the patient’s blood using a blood cell separation machine. OESCs are extracted from human fetal olfactory bulb tissue and cultured for two to three weeks. The NSCs used in the clinical studies are cultured human neural stem cells derived from a single source human fetal spinal cord tissue of approximately eight gestational weeks and expanded serially by epigenetic means only (Glass 2012).

Implantation of cells into patients has been performed via several routes. The common methods of implantation include intrathecal (into the subarachnoid space via spinal canal), intracortical (into the cerebral cortex) and direct transplantation of autologous MSCs into surgically-exposed spinal cord under general anaesthesia. It has been shown that direct transplantation of autologous cells into the spinal cords of people with ALS is well tolerated and feasible (Glass 2012; Mazzini 2010; Mazzini 2012).

A number of clinical trials have provided important insights into the safety and feasibility of stem cell mobilisation and transplantation in people with ALS/MND. There remain uncertainties, however, regarding its effectiveness to achieve functional improvement and its long-term safety profile, in particular whether this mode of therapy is associated with acceleration of disease progression (Lunn 2014).

How the intervention might work

There are two possible mechanisms by which stem cell therapy may help in the treatment of ALS/MND. Firstly, by replacing dying neuronal cells with progenitor cells that have been generated ex vivo. Experimental observations have shown that transplanted stem cells and mononuclear cells have the capacity to generate new neurons and stimulate regenerative processes (Mazzini 2003). In animal models of ALS, stem cell transplantation can significantly slow the progression of the disease and prolong survival (Mazzini 2003). Increasing numbers of preclinical studies have shown that transplanted stem cells are capable of migrating to regions of experimentally induced nerve injury, where they are able to proliferate and differentiate into neurons and glial cells (Jiang 2002; Liu 2000; McDonald 1999; Terada 2002; Woodbury 2000). The types of stem cells that have been tested in preclinical models include bone marrow stem cells, MSCs, cord blood stem cells, embryonic stem cells, neural stem and progenitor cells, human glial restricted progenitors and induced pluripotent stem cells. Secondly, stem cells promote the survival of existing neurons. MSCs are very attractive candidates for cell therapy in MND because of their great plasticity (Chen 2008) and immunomodulatory properties (Mazzini 2010). MSCs can induce a neuroprotective microenvironment via their anti-inflammatory and immunosuppressive effects on astrocytes and microglial cells (Uccelli 2008). MSCs release soluble molecules such as cytokines
and chemokines and express immune-relevant receptors such as chemokine receptors and cell adhesion molecules that ameliorate inflammation and stimulate the survival of neuronal cells (Uccelli 2008). Preclinical data have shown that MSCs are capable of transdifferentiation into neurons and glial cells both in vitro and in vivo (Black 2001; Kim 2002; Sanchez-Ramos 2000). In addition, neural stem cells have the ability to generate immunomodulatory cells, growth-factor releasing cells and functional support cells to modify motor neuron survival and activity (Gowing 2011).

Most studies on the pathogenesis of ALS thus far have been animal studies. There are many limitations when extrapolating the findings observed in animal models into humans. Firstly, there are interspecies differences in neuronal physiology and specific gene splicing patterns (Hardingham 2010). Secondly, there is an overemphasis on rat superoxide dismutase 1 (SOD1) based models when most cases of human sporadic ALS may not have a SOD1 defect. In this respect, stem cells could be used to model disease, allowing us to further explore the pathophysiological process of ALS.

Why it is important to do this review

The lack of effective pharmacologic treatment for ALS/MND and compelling preclinical data have provided a supportive rationale for the therapeutic application of stem cells for this devastating incurable disease. Early clinical trials have suggested that stem cells may have the potential to replace and repair the damaged motor neurons in people with ALS/MND (Martinez 2009; Mazzini 2003; Mazzini 2010). Moreover, the procedures of expansion and transplantation of these cells into people with ALS/MND are safe, well tolerated and feasible. However, as most of these clinical trials involved small numbers of participants, the results have been inconsistent; combining the available data in a systematic review may allow meaningful conclusions to be drawn. It is also important to determine whether cells derived from different sources may have different impacts on clinical outcomes of people with ALS/MND. For example, stem cells obtained from different sources may possess different biological (plasticity, self renewal, differentiation, homing, migration, secretion of trophic factors) and immunological (modulating immune response) properties. These may be attributed to the inherent biological properties of the stem cells or changes to the cells that may have occurred during enrichment and processing of the cells. Moreover, questions regarding the optimal treatment regimen including the cell dose, phenotype, preparation and delivery system remain to be answered (Abdul 2013).

This systematic review sets out to determine the effectiveness and safety of cell-based therapy in people with ALS/MND. The findings of this systematic review may facilitate design of the optimal cell-based therapy program for people with ALS/MND as well as identify critical areas for improvement and recommendations for future clinical trials.

O B J E C T I V E S

To evaluate the efficacy and safety of cell-based therapy in people with ALS/MND compared with a placebo or no additional treatment

M E T H O D S

Criteria for considering studies for this review

Types of studies

We will include randomised controlled trials (RCTs), quasi-RCTs and cluster RCTs. Quasi-random methods of assignment to interventions are systematic methods that are not truly random, such as allocation using alternation, date of birth, day of visit or medical record number.

Types of participants

We will include people of any age with a diagnosis of definite or probable ALS/MND according to the revised El Escorial World Federation of Neurology criteria (Brooks 2000).

Types of interventions

We will include trials that assess the use of mononuclear cells or stem cells compared with i) a placebo or ii) no additional treatment. We will permit the use of co-interventions including standard treatment such as riluzole and other symptomatic treatment provided that they are administered to each group equally.

Types of outcome measures

Primary outcomes

1. Change in Expanded Disability Status Scale (EDSS) or Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) at 6 and 12 months.

Secondary outcomes

1. Change in manual muscle testing of the upper and lower limbs (Medical Research Council (MRC) grade) at 6 and 12 months.
2. Change in forced vital capacity (FVC) at 6 and 12 months.
3. Change in compound muscle action potential (CMAP) and neurophysiological index (NI) at 6 and 12 months.
4. Change in mood state and quality of life using the Profile of Mood State (POMS) and quality of life scale questionnaires at 6 and 12 months.
5. Structural changes in serial magnetic resonance imaging (MRI) at 6 and 12 months.
6. Overall survival at 6 and 12 months.
7. Adverse events.

Search methods for identification of studies

Electronic searches
We will identify trials from the Cochrane Neuromuscular Disease Group Specialized Register, and search the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE and EMBASE, without applying any language restrictions, using the search strategy in Appendix 1. We will include studies reported as full-text publications as well as those published as abstracts and proceedings.

We will also conduct a search of the US National Institutes for Health Clinical Trials Registry, ClinicalTrials.gov (www.clinicaltrials.gov) and the WHO International Clinical Trials Portal (ICTRP; apps.who.int/trialssearch/) to identify other ongoing and unpublished studies. In addition, we will search the National Institute for Health Research Database of Abstracts and Reviews of Effects (DARE) and Health Technology Assessments (HTA) database to identify reviews and assessments for inclusion in the 'Discussion' section. We will search National Health Service Economic Evaluation Database (NHS EED) for any available cost information and include any information on costs in the 'Discussion' section. We will request the help of the Cochrane Neuromuscular Disease Group Trials Search Co-ordinator in executing the electronic searches.

Searching other resources
We will search reference lists of all primary studies and review articles for additional references. We will contact the authors of randomised controlled trials and other experts in the field to obtain any additional published or unpublished studies. We will search relevant manufacturers’ websites for trial information to identify further relevant studies.

Data collection and analysis

Selection of studies
Two review authors (SFAW and ZKL) will independently screen titles and abstracts of all studies identified from the first round of searching. We will code potentially relevant studies or studies that require further assessments as 'retrieve'. We will code studies that are clearly not relevant as 'do not retrieve'. Two review authors (SFAW and ZKL) will inspect the full-text versions of the studies that are coded as "retrieve" to further identify trials to be included in our meta-analysis. Among studies that are retrieved but excluded, we will record reasons for exclusion. We will resolve any disagreement through discussion or, if required, we will consult a third person (RAA). We will identify and exclude duplicates and collate multiple reports of the same study so that each study rather than each report is the unit of interest in the review. We will record the selection process in sufficient detail to complete a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram and 'Characteristics of excluded studies' table.

Assessment of risk of bias in included studies
Two review authors (NML and SFAW) will independently assess risk of bias for each study according to the domains listed below.
as outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We will resolve any disagreements by discussion or by involving another author (RAA).

1. Random sequence generation.
2. Allocation concealment.
3. Blinding of participants and personnel.
5. Incomplete outcome data.
6. Selective outcome reporting.
7. Other bias, such as premature termination and extreme baseline imbalance.

We will accord a judgment of low or high risk of bias if there is sufficient information in the study report, and justify our grade with a quote from the study in the 'Risk of bias' table. If there is insufficient information available from the study to enable a judgment, we will grade the risk of bias as unclear. We will consider blinding separately for clinical and laboratory outcomes where necessary. Where information on risk of bias relates to unpublished data or correspondence with the study authors, we will note this in the 'Risk of bias' table.

**Measures of treatment effect**

We will analyse dichotomous data as risk ratios and continuous data as mean differences, or standardised mean differences if conceptually similar outcomes are measured on different scales. In this case, we will adjust all the scales to achieve a consistent direction of effect.

We will undertake meta-analyses only where the participants, intervention, comparison and outcomes are similar enough for pooling to be meaningful.

We will narratively described skewed data reported as medians and interquartile ranges.

**Unit of analysis issues**

For cluster-RCTs (in other words, trials in which the assignment to intervention or control group was made at the level of the unit/ward rather than the individual participant), we will assess whether the study authors have made appropriate adjustments for the effects of clustering, using appropriate analysis models such as the Generalized Estimating Equation model. We will inspect the width of the standard error (SE) or 95% confidence interval of the estimated treatment effects to double-check the possible unit of analysis in the study. If we find an inappropriately small SE or a narrow 95% CI, we will ask the authors of the study to confirm the unit of analysis.

If no adjustment was made for the effects of clustering, we will perform adjustments by multiplying the SEs of the final effect estimates by the square root of the ‘design effect’, represented by the formula, $1 + (M-1) \times ICC$, where M is the average cluster size (number of participants per cluster) and ICC is the intracluster correlation. We will determine the average cluster size (M) from each trial by dividing the total number of participants by the total number of clusters. We will use an assumed ICC of 0.10, as we consider this to be a realistic general estimate that is derived from previous studies on implementation research (Campbell 2001).

We will combine the adjusted final effect estimates from each trial with their SEs in meta-analysis using generic inverse variance methods, as stated in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). If the determination of the unit of analysis is not possible, we will include the studies concerned in a meta-analysis using the effect estimates reported by the authors. We will perform sensitivity analyses to assess how the overall results are affected by the removal of the studies in which i) adjustment of unit of analysis is appropriate but not possible and ii) the unit of analysis is unknown.

**Dealing with missing data**

If key information is missing, such as study characteristics, methods or outcome data, we will contact investigators to obtain the relevant information. Where this is not possible, we will conduct a deterministic sensitivity analysis at the study level by adopting the “worst case scenario” approach. If the effect estimate of the study changes substantially following our sensitivity analysis, we will consider the study to be at high risk of attrition bias. At the review level, we will again conduct a sensitivity analysis to explore the impact of including such studies with high risk of attrition bias in the overall pooled estimates of the major outcomes.

**Assessment of heterogeneity**

We will use the I² statistic to measure heterogeneity among the trials in each analysis. If we identify substantial unexplained heterogeneity (as shown by an I² of greater than 50%) we will report it and explore possible causes by prespecified subgroup analyses.

**Assessment of reporting biases**

If we are able to pool more than 10 trials, we will create and examine a funnel plot to explore possible publication biases. If we find significant asymmetry in the funnel plot, which may indicate possible publication bias, we will report this with a note of caution in the discussion taking into account the area of the void in the funnel plot. We do not plan to further explore publication bias using statistical methods in view of the limitations of these methods in the presence of the relatively small number of studies in a typical systematic review (Higgins 2011).

**Data synthesis**

We will perform our meta-analysis in RevMan 5 , using a fixed-effect model. We will perform a sensitivity analysis to assess the change in the overall results with a random-effects model.
If there is more than one comparison, we will report the results for each comparison separately.

'Summary of findings' table

We will create a 'Summary of findings' (SOF) table comparing cell-based therapy versus placebo or no additional treatment using the following outcomes: EDSS and ALSFRS scales, manual muscle testing, FVC, survival rate, and adverse events at 12 months. Our judgment on the overall quality of the body of evidence will be guided by the five Grading of Recommendations Assessment, Development and Evaluation (GRADE) considerations, namely, limitations in study design, consistency of effect, imprecision, indirectness and publication bias. We will use methods and recommendations described in Chapter 12 of the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011) using the GRADE profiler (GRADEpro) software (GRADEpro 2008). We will justify all decisions to down- or up-grade the quality of studies using footnotes in the SOF table.

Subgroup analysis and investigation of heterogeneity

We plan to carry out a subgroup analysis based on the type of cell-based therapy received, i.e. either BM-MNCs, BM-MSCs, M-PBMCNs, OESCs or NSCs. We will also conduct a subgroup analysis based on delivery method, i.e. intrathecal, intracranial, intraspinal and intravenous. We will use EDSS and ALSFRS scales, FVC, quality of life scores, MRI changes, survival rate, neurophysiological index and adverse events as outcome measures. We will use the formal test for subgroup interactions in RevMan to assess differential effects of the intervention between the subgroups.

Sensitivity analysis

We plan to carry out the following sensitivity analyses if applicable:
1. Repeat the analysis excluding studies at high risk of selection and attrition biases.
2. Repeat the analysis excluding large studies to assess the effect of these studies on the overall results.
3. Repeat the analysis with a random-effects model.
4. Repeat the analysis excluding unpublished studies.

If the overall results are affected substantially by the sensitivity analysis, we will place a note of caution in our discussion and conclusions regarding the certainty of our estimates, and propose a need for further research where appropriate to explore the possible sources of variation in the outcome estimates.

ACKNOWLEDGEMENTS

The authors developed this protocol using a template originally developed by the Cochrane Airways Group, and adapted by the Cochrane Neuromuscular Disease Group.

This project was supported by the National Institute for Health Research via Cochrane Infrastructure funding to the Cochrane Neuromuscular Disease Group. The views and opinions expressed therein are those of the authors and do not necessarily reflect those of the Systematic Reviews Programme, NIHR, NHS or the Department of Health. The Cochrane Neuromuscular Disease Group is also supported by the MRC Centre for Neuromuscular Disease and the Motor Neurone Disease Association.

The Cochrane Neuromuscular Disease Group Trials Search Coordinator, Angela Gunn, advised the review authors on the search strategy.

REFERENCES

Additional references

Abdul 2013

Baek 2012

Bensimon 1994

Bernardo 2007

Black 2001
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Blanquer 2012

Brooks 2000

Campbell 2001

Cashman 2008

Chen 1996

Chen 2007

Chen 2008

Chen 2012

Chew 2007

Chio 2011

Deda 2009

del Aguila 2003

Feldman 2014

Giordana 2010

Glass 2012

Goodall 2006

Gowing 2011
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Huang 2008

Jiang 2002

Karussis 2010

Kim 2002

Liu 2000

Logroscino 2005

Logroscino 2008

Lunn 2014

Martinez 2009

Martinez 2012

Mazzini 2003

Mazzini 2006

Mazzini 2008

Mazzini 2010

Mazzini 2012

McDonald 1999

GRADEpro 2008

Hardingham 2010

Higgins 2011
Miller 2012

Murray 2004

Nefussy 2010

Piepers 2010

Prabhakar 2012

Rabin 1999

RevMan 2014

Riley 2014

Sanchez-Ramos 2000

Shaw 2005

Tarella 2010

Terada 2002

Turner 2003

Uccelli 2008

Woodbury 2000

* Indicates the major publication for the study
APPENDICES

Appendix 1. MEDLINE (OvidSP) search strategy

Database: Ovid MEDLINE(R) <1946 to June Week 4 2014>
Search Strategy:

1 randomized controlled trial.pt. (377908)
2 controlled clinical trial.pt. (88775)
3 randomized.ab. (275946)
4 placebo.ab. (147416)
5 drug therapy.fs. (1714581)
6 randomly.ab. (195454)
7 trial.ab. (286121)
8 groups.ab. (1255003)
9 or/1-8 (3220367)
10 exp animals/ not humans.sh. (3964775)
11 9 not 10 (2741162)
12 exp Motor Neuron Disease/ (19947)
13 (moto$1 neuron$1 disease$1 or moto?neuron$1 disease).mp. (6433)
14 ((Lou Gehrig$1 adj5 syndrome$1) or (Lou Gehrig$1 adj5 disease)).mp. (98)
15 charcot disease.tw. (13)
16 Amyotrophic Lateral Sclerosis.mp. (16498)
17 or/12-16 (24159)
18 Leukocytes, Mononuclear/ (28270)
19 Mesenchymal Stromal Cells/ (15990)
20 Bone Marrow Transplantation/ (41445)
21 exp stem cells/ (138494)
22 exp Stem Cell Transplantation/ (54441)
23 (mononuclear adj5 cell$1).tw. (65283)
24 mesenchymal stem cell$1.tw. (16045)
25 (angiogenesis adj3 therap$).tw. (2167)
26 bone marrow.tw. (157621)
27 stem cells.tw. (85722)
28 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 (390100)
29 11 and 17 and 28 (77)
30 remove duplicates from 29 (75)

CONTRIBUTIONS OF AUTHORS

All authors wrote the protocol and approved the protocol in its final form.

SFAW, ZKL and NAI drafted the search strategy.
DECLARATIONS OF INTEREST
None known

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Internal sources
• No sources of support supplied

External sources
• No sources of support supplied, Malaysia.