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White paper on guidelines concerning enteric nervous system stem cell therapy for enteric neuropathies ☆

Alan J. Burns [a,b,*], Allan M. Goldstein [c], Donald F. Newgreen [d], Lincon Stamp [e], Karl-Herbert Schäfer [f,g], Marco Metzger [h], Ryo Hotta [c], Heather M. Young [e], Peter W. Andrews [ac], Nikhil Thapar [a], Jaime Belkind-Gerson [i], Nadege Bondurand [j,aa], Joel C. Bornstein [k], Wood Yee Chan [l], Kathryn Cheah [m], Michael D. Gershon [n], Robert O. Heuckeroth [o,ab], Robert M.W. Hofstra [a,p], Lothar Just [q], Raj P. Kapur [r], Sebastian K. King [s], Conor J. McCann [a], Nandor Nagy [t], Elly Ngan [u], Florian Obermayr [v], Vassilis Pachnis [w], Pankaj J. Pasricha [x], Mai Har Sham [y], Paul Tam [u], Pieter Vanden Berghe [z]

[a] Stem Cells and Regenerative Medicine, UCL Great Ormond Street Institute of Child Health, London, UK
[b] Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands
[c] Department of Pediatric Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[d] Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville 3052, Victoria, Australia
[e] Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria 3010, Australia
[f] University of Applied Sciences, Kaiserlautern, Germany
[g] Clinic of Pediatric Surgery, University Hospital Mannheim, University Heidelberg, Germany
[h] Fraunhofer-Institute Interfacial Engineering and Biotechnology IGB Translational Centre – Würzburg branch and University Hospital Würzburg – Tissue Engineering and Regenerative Medicine (TERM), Würzburg, Germany
[i] Division of Gastroenterology, Hepatology and Nutrition, Massachusetts General Hospital for Children, Harvard Medical School, Boston, USA
[j] INSERM U955, 51 Avenue du Maréchal de Lattre de Tassigny, F-94000 Créteil, France
[k] Department of Physiology, University of Melbourne, Parkville, Victoria 3010, Australia
[l] School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong
[m] School of Biomedical Sciences, The University of Hong Kong, Hong Kong
[n] Department of Pathology and Cell Biology, Columbia University, New York 10032, USA
[o] Department of Pediatrics, The Children's Hospital of Philadelphia Research Institute, Philadelphia, PA 19104, USA
[p] Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands
[q] Institute of Clinical Anatomy and Cell Analysis, University of Tübingen, Germany
[r] Department of Pathology, University of Washington and Seattle Children's Hospital, Seattle, WA, USA
[s] Department of Paediatric and Neonatal Surgery, The Royal Children's Hospital, Melbourne, Australia
[t] Department of Anatomy, Histology and Embryology, Faculty of Medicine, Semmelweis University, Budapest, Hungary
[u] Department of Surgery, The University of Hong Kong, Hong Kong
[v] Department of Pediatric Surgery and Pediatric Urology, University Children's Hospital Tübingen, D-72076 Tübingen, Germany
[w] The Francis Crick Institute, Mill Hill Laboratory, London, UK
[x] Johns Hopkins Center for Neurogastroenterology, Baltimore, MD, USA
[y] Department of Biochemistry, The University of Hong Kong, Hong Kong
[z] Laboratory for Enteric NeuroScience (LENS), TARGID, University of Leuven, Belgium
[aa] Université Paris-Est, UPEC, F-94000 Créteil, France
[ab] Perelman School of Medicine at the University of Pennsylvania, Abramson Research Center, Philadelphia, PA 19104, USA
[ac] Centre for Stem Cell Biology, Department of Biomedical Science, University of Sheffield, Sheffield, UK

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ABSTRACT

Over the last 20 years, there has been increasing focus on the development of novel stem cell based therapies for the treatment of disorders and diseases affecting the enteric nervous system (ENS) of the gastrointestinal tract (so-called enteric neuropathies). Here, the idea is that ENS progenitor/stem cells could be transplanted into the gut wall to replace the damaged or absent neurons and glia of the ENS. This White Paper sets out experts' views on the commonly used methods and approaches to identify, isolate, purify, expand and optimize ENS stem cells, transplant them into the bowel, and assess transplant success, including restoration of gut function. We also highlight obstacles that must be overcome in order

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* Corresponding author at: Stem Cells and Regenerative Medicine, UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.
E-mail address: alan.burns@ucl.ac.uk (A.J. Burns).

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to progress from successful preclinical studies in animal models to ENS stem cell therapies in the clinic.

0. Introduction

This white paper, authored by 30 members of the enteric nervous system (ENS) basic science and clinical field, sets out their opinions on efforts to establish novel stem cell therapies for enteric neuropathies of the gastrointestinal tract. Such enteric neuropathies remain some of the most challenging clinical disorders to manage. Arguably the best understood enteric neuropathy is the congenital disorder Hirschsprung disease (HSCR) in which the neural crest-derived intrinsic ENS is absent in a variable length of the distal gut (called "aganglionosis"). The only treatment currently available for HSCR is surgical removal of the aganglionic bowel segment, and although life saving, chronic gastrointestinal problems, including faecal incontinence and enterocolitis, significantly reduce the quality of life for many people with HSCR even after surgery. Due to these problems, a novel treatment, whereby stem cells are transplanted into the aganglionic segment to replace the missing ENS, has been proposed and over the last 10–15 years numerous international groups have been, and are currently, involved in preclinical studies aimed at developing such a cell replacement therapy.

To put this work in context, it is important to know that the human ENS contains approximately 500 million neurons and four times as many glia distributed along the entire bowel in two interconnected layers called the submucosal and myenteric plexus (Furness, 2006). These neurons and glia control bowel motility, respond to sensory stimuli, regulate blood flow, support epithelial function and modulate local immunity (Furness, 2012). To perform these roles, there are at least 14 enteric neuron subtypes (Furness, 2000) that express every neurotransmitter in the CNS and there are several types of enteric glia. All cells of the ENS are neural crest-derived and migrate into the bowel during week three to eight of human gestation (or day 9.5–13.5 of mouse fetal development) and then must differentiate and establish a sophisticated regulatory network (Sasselli et al., 2012b). The goal of stem cell therapy is to repair or replace defective or missing enteric neurons and/or glia to improve bowel function.

At the fourth international meeting "Development of the enteric nervous system; cells, signals, genes and therapy" held in Rotterdam, The Netherlands (April 2015), a multidisciplinary group of basic scientists and clinicians, including surgeons, gastroenterologists, and pathologists, decided that a White Paper should be written to clearly set out methods and approaches to identify, isolate, purify, expand and optimize ENS stem cells and progenitors, transplant them into the bowel, and assess transplant success as a way to restore gut function. By reviewing published studies on ENS stem cell therapy, we identified specific areas to help direct future research, gaps in knowledge, and strategies to address these challenges, taking advantage of knowledge gained from central nervous system (CNS) stem cell biology. Although there are no "gold standard" approaches to isolate and propagate ENS stem cells, published studies delineate many methods commonly used in this field. This White Paper aims to form a consensus and provide the ENS and stem cell biology communities with protocols for working with ENS stem cells, for their transplantation into the bowel, and for their subsequent analysis.

Considering the steady advance and success of several preclinical trials in animal models, we now need to consider "first in man" studies of stem cell therapy for enteric neuropathies. Here we also discuss obstacles that must be overcome to move ENS stem cell therapy to the clinic. This includes a discussion of the "best" diseases to initially treat, the accompanying safety studies that will need to be performed, and an outline of what "first in man" studies should include. With the emergence of new techniques, including approaches to label stem cells for transplantation and new gene editing technology, we are optimistic that ENS stem cells, capable of reforming enteric neuronal networks, will be obtained more reproducibly and with higher efficiency in near future. Here we aim to define standard methodologies that can be adapted to provide the necessary safety, regulatory and good manufacturing practice protocols required for eventual clinical application.

1. What are the target diseases for stem cell transplantation?

Neurogastrointestinal diseases are congenital or acquired disorders that affect the GI tract focally or diffusely and may involve all enteric neurons or only a subpopulation. Etiologies for neurointestinal diseases include genetic, inflammatory, degenerative, or paraneoplastic processes. Given this complexity, one needs to consider the underlying defect and its etiology in choosing the most reasonable targets for cell transplantation in animal models and, ultimately, for human clinical trials. Here we describe the pathophysiology of several neurogastrointestinal diseases that represent promising targets for cell-based therapy.

1.1. Hirschsprung disease (HSCR)

HSCR results from failure of enteric neural crest-derived cells to complete colonization of the distal intestine during fetal development. The uncolonized distal bowel remains aganglionic and tonically contracted, causing functional obstruction. Short-segment HSCR, in which the rectosigmoid colon lacks ganglion cells, affects 80% of patients, while the remainder have more extensive aganglionosis proximal to the rectosigmoid. Current treatment involves surgical removal of the aganglionic segment, but functional outcome is variable and many patients suffer life-long complications (Conway et al., 2007; Laughlin et al., 2012; Ludman et al., 2002; Pini Prato et al., 2008; Tsuji et al., 1999). This may reflect dysfunction of the so-called "normo-ganglionic" segment (Di Lorenzo et al., 2000; Kohno et al., 2007), abnormal anal sphincter function, retention of aganglionic distal bowel, or the sequelae of proctectomy. Enteric neuronal stem/progenitor cell (ENSC) transplantation provides a potential therapy to replace absent ganglia. For this purpose, ENSCs have been successfully isolated from ganglionic and aganglionic bowel of human HSCR patients and expanded in culture. These cells migrate and differentiate into neurons and glia following transplantation into embryonic hindgut (Almond et al., 2007; Metzger et al., 2009b; Wilkinson et al., 2015). Furthermore, studies using murine ENSCs from embryonic and postnatal intestine showed that transplanted ENSCs differentiate into neurons with processes that project into the gut muscle and form functional, synaptic connections (Hotta et al., 2013).

Identifying the optimal source of ENSCs for transplantation is a priority (discussed in Section 3). For clinical application, autologous cells avoid the issue of immunologic rejection. HLA-matched human embryonic stem cells or patient-specific induced pluripotent stem (iPS) cells also represent potential sources, but driving them along the correct lineage to generate functional enteric neurons and, if necessary, "correcting" the inherited genetic mutation present in those cells, remain major challenges. Choosing the right animal model of