Published in final edited form as:

Gut. 2012 April ; 61(4): 613–621. doi:10.1136/gut.2010.235614.

Stem cell transplantation in neurodegenerative disorders of the gastrointestinal tract: future or fiction?

Subhash Kulkarni, Laren Becker, and Pankaj Jay Pasricha

Division of Gastroenterology and Hepatology, Stanford University, School of Medicine, Stanford, California, USA

Abstract

Current advances in our understanding of stem and precursor cell biology and in the protocols of stem cell isolation and transplantation have opened up the possibility of transplanting neural stem cells for the treatment of gastrointestinal motility disorders. This review summarises the current status of research in this field, identifies the major gaps in our knowledge and discusses the potential opportunities and hurdles for clinical application.

INTRODUCTION

The principal activities of the gut, including motility and secretion, are largely controlled by the intramural enteric nervous system (ENS), which is capable of performing most of its functions autonomously from the central nervous system (CNS).¹ The ENS is composed of 80–100 million phenotypically diverse enteric neurons² and many more enteric glial cells³ that are developmentally derived from migratory vagal and sacral neural crest cells.⁴ Enteric neurons and glial cells interact with various other cell types in the gut, such as the interstitial cells of Cajal, to produce coordinated contraction and relaxation of smooth muscle, resulting in effective gut motility and secretion.⁵

As with the CNS, the ENS can be affected by a variety of diseases that can be congenital or acquired, localised or diffuse and can affect one or more cell types.^{6–13} The treatment for many of these disorders is far from satisfactory. Most current therapies are at best palliative.¹⁴ Although this partially reflects our lack of understanding of the underlying pathophysiology in many instances, there are also intrinsic limitations to pharmacological approaches, particularly if the effector elements targeted by drugs are missing or non-responsive. Hence, similar to CNS disorders such as stroke, a true cure for at least some ENS disorders may involve replacing missing or restoring dysfunctional neurons or, alternatively, stimulating a local regenerative response. In this review we describe the potential for such therapies for neurodegenerative disorders of the gut.

Correspondence to: Pankaj Jay Pasricha, Division of Gastroenterology and Hepatology, M211, Stanford University, School of Medicine, Stanford, CA 94305, USA; pasricha@stanford.edu.

Competing interests None.

Provenance and peer review Commissioned; externally peer reviewed.

OVERVIEW OF STEM AND PRECURSOR CELLS

Stem cells can be classified in many ways but, broadly, are either both pluripotent and multilineage (giving rise to many cells of diverse developmental origin) or multipotent (generating different cell types within a single developmental lineage). In addition, precursor cells can be defined as more committed derivatives of stem cells that are capable of differentiating into only a single type of cell. Each of these types theoretically can be used to generate elements of the ENS (figure 1), and their characteristics are briefly summarised below.

Embryonic stem cells (ESC)

Embryonic stem cells (ESC) are a pluripotent multilineage stem cell population capable of differentiating into all three germ layers and hence give rise to any cell type. Numerous protocols exist to drive ESC to a particular fate in vitro.^{15–17} A major advantage of this stem cell population is their ease of propagation and expansion.^{18–21} However, there are both ethical and scientific concerns associated with the use of ESC.²² The intrinsic pluripotency of these cells presents a challenge in terms of limiting their differentiation to a specific cell fate, resulting in a tendency to form teratomas in vivo.²³ However, some of these limitations may be overcome by ‘committing’ ESC towards a desired lineage prior to transplantation.¹⁷ This ‘controlled’ pluripotency, along with the ease of propagation of ESC, may maintain them as attractive candidates for transplantation therapies.^{24–26}

Although there are established protocols for deriving general neural precursors from ESC,^{27–31} it is only recently that investigators have succeeded in inducing ESC towards a neural crest lineage from which the ENS is derived.^{32,33} These ESC-derived neural crest stem cells are capable of colonising explanted gut tissue and differentiating into enteric neuronal and glial cells,³² but we do not yet know whether they can develop functional neuromuscular connections in vivo.

Induced pluripotent stem cells (iPSC)

The reprogramming of differentiated adult cells to become what are in effect embryonic stem cells is one of the most exciting scientific discoveries in this field in recent history. These cells, termed induced-pluripotent stem cells (iPSC), offer an attractive alternative to ESC for cell-based therapies³⁴ and theoretically can overcome some of the ethical and medical issues related to regular embryonic stem cells. iPSC were first generated by retroviral transduction of mouse and human fibroblasts with a selection of four key pluripotency genes: *Oct-3/4*, *Sox2*, *c-Myc* and *Klf4*.^{34,35} Like ESC, iPSC can be directed towards neural and glial lineage^{36,37} and have been successfully transplanted into a rat model for Parkinson’s disease with functional improvement.³⁸ Although they have much in common in terms of pluripotency, recent studies have made it clear that ESC and iPSC are not identical, with genomic and epigenetic differences that have potentially far-reaching implications.³⁹ Further, not all iPSC are the same, with gene expression patterns varying with the somatic cell of origin (and reflecting their epigenetic lineage), although these differences appear to become attenuated with increasing cell passage.^{39–43}

Since iPSC are host-derived, issues with immune tolerance are expected to be less than with allogenic cell transplants. However, although the exclusion of *c-Myc* from the set of genes and the use of non-viral transfection methods is a welcome and reassuring development,⁴⁴ concerns about the tumorigenicity of iPSC may be even greater than with ESC.⁴⁵ As reviewed recently, the genes required for reprogramming somatic cells to iPSC are also associated with many cancers. Furthermore, chromosomal aberrations—either inherited from somatic parent cells or acquired during the reprogramming and culturing process—are not uncommon and heighten the risk for the development of somatic tumours, in addition to that of teratomas.⁴⁵ Thus, although the discovery of iPSC has great scientific and clinical potential, there are major hurdles to be overcome before this can be translated into therapy.

Induced neuronal (iN) cells

The iPSC strategy for deriving neurons consists of first reprogramming fibroblasts (or other somatic cells) to a primordial ESC-like state and then differentiating them towards a neural fate.³⁸ Recently, investigators have been able to directly convert both mouse and human fibroblasts to neurons termed induced neuronal cells (iN cells) rather than going through an intermediate ESC phase using a combination of just three genes: *Ascl1*, *Brn2* (also called *Pou3f2*) and *Myt1l*.^{46,47} Since this technology bypasses the need for a multilineage pluripotent cell, it also theoretically reduces the carcinogenic potential. A similar approach to directly generate enteric neuronal precursor cells would be an important development and is eagerly awaited.

Neural stem cells (NSC)

Neural stem cells (NSC) are multipotent stem cells derived from nervous tissue that, unlike ESC, are already poised for a neural lineage even before transplantation. While adult stem cells may be propagated in vitro, they do not demonstrate similar long-term self-renewal as ESC and eventually undergo senescence due to lack of telomerase activity.⁴⁸ Thus, NSC have lower tumorigenic potential than ESC and iPSC but require an accessible source tissue for easy isolation of numerous cells. Here we summarise different populations of NSC that can be used for transplantation therapies for ENS regeneration and repair.

CNS-DERIVED NEURAL STEM CELLS (CNS-NSC)

CNS-derived NSC (CNS-NSC) have been identified and isolated from several sites in rodent brains, particularly the hippocampus and subventricular zone.⁴⁹ Despite their origin, CNS-NSC have remarkable plasticity. Neurons derived from CNS-NSC can form functional connections with colonic smooth muscle cells, thereby enabling them to translate neuronal signal into enteric smooth muscle cells (figure 2).¹⁴ We have also demonstrated that CNS-NSC can be nudged towards an enteric-like phenotype, as assessed by neurotransmitter expression as well as by neuronal behaviour, by co-culturing them in vitro with dissected gut tissue.⁵⁰ Similar enteric-like behaviour can be induced in differentiating CNS-NSC by activation of the RET pathway^{51,52} or by treatment with drugs such as ROCK (ρ kinase) inhibitors^{51,52} which are known to induce a neural crest phenotype.⁵³

CNS-NSC-derived neurons are also capable of expressing functional neuronal nitric oxide synthase (nNOS), an enzyme required to produce the inhibitory neurotransmitter nitric oxide, in vitro and in vivo on transplantation into rodent stomach.⁵⁴ In addition, CNS-NSC transplanted into the stomach of nNOS-deficient (gastroparetic) mice in vivo partially restored gastric function after 1 week (figure 3).⁵⁵ However, for reasons that are unclear, there was no sustained engraftment with significant cell loss 2–4 weeks after transplantation. It can be hypothesised that CNS-NSC do not possess the signalling machinery for long-term survival in a relatively alien environment. Our studies suggest that intrinsic apoptotic programming is more important than host immune responses in their survival.⁵⁶ Whether we can overcome the former by strategies such as activation of RET or inducing anti-apoptotic pathways is the subject of ongoing research in our laboratory.

An increasing concern with CNS-NSC is the difficulty in accessing them, particularly if autologous transplantation is contemplated. To bypass the need for self-derived CNS-NSC, some commercial companies in the USA and UK have made significant progress in standardising protocols for culture and propagation of allogeneically-derived CNS-NSC in ongoing phase 1 clinical trials for the treatment of CNS disorders such as ischaemic stroke, chronic spinal cord injury and amyotrophic lateral sclerosis.⁵⁷ If progress can be made in manipulating the CNS-NSC to form stable, integrated and functional ENS tissue, these cells could also be tested for the treatment of ENS disorders.

ENTERIC NEURAL STEM CELLS (ENSC) AND ENTERIC NEURAL PRECURSORS

Despite the plasticity of CNS-NSC, intrinsic differences between NSC populations may influence the exact phenotypic fate following transplantation into a particular tissue.⁵⁸ Recently, neural stem cells/precursors have been isolated directly from the gut.^{59–67} Thus, this population of cells may be more appropriate for regenerating the ENS than CNS-NSC, and the remainder of this review will focus on these cells. However, it is important to mention that non-neural stem cells may also be important in regenerative approaches to neuromuscular disorders of the gut, as many of these conditions are associated with loss of other cell types such as interstitial cells of Cajal that may be required for normal motility.¹²⁶⁸ Progenitors of interstitial cells of Cajal have recently been discovered⁶⁹⁷⁰ and could be attractive candidates for transplantation therapy of selective gastrointestinal disorders.

TRANSLATING ENSC BIOLOGY INTO TREATMENT

Metzger *et al*⁶³ reviewed reports from several groups that describe isolation of neural stem cells/precursors from embryonic and postnatal guts in rodents and humans. Since many of these cells have been inadequately characterised for their ‘stemness’, some of them are more appropriately termed neural precursors. However, for the sake of convenience, we will use the term enteric neural stem cells (ENSC) for the remainder of this discussion. Table 1 summarises recent reports on these cells, describing the methods of isolation and maintenance and their plasticity in vitro, ex vivo and, in some instances, in vivo circumstances. As can be seen, there are very few studies in which ENSC or enteric neural

precursor cells have actually been transplanted *in vivo*. Along with an as yet incomplete characterisation of the ENSC population (see below), this is reflected in our uncertainty about the answers to many fundamental questions¹⁴⁸⁰ about how best to use them for therapy, as discussed in subsequent sections. In this context it is worth noting that, although the classical paradigm teaches us that stem cells exert their beneficial effects by replacing or replenishing dead or dying neurons, other mechanisms may also be involved. The transplanted NSC may have ‘bystander effects’, serving as a source of essential trophic and other biological factors that stimulate a local regenerative response, provide scaffolding or other physical means for facilitating host regeneration and may favourably modulate host immune/inflammatory responses.^{81–83}

HOW CAN ENSC BE IDENTIFIED?

Until now, most investigators have relied on a surrogate marker for ‘stemness’-the ability to generate neurospheres as evidence for the presence of a self-renewing multipotent ENSC. Neurospheres are characteristically spherical but an extremely heterogeneous mixture of precursor and non-precursor cells in various phases of growth and differentiation.⁸⁴ In most studies these neurospheres are then transplanted either intact or after brief dissociation. However, this is clearly not ideal for several reasons. It cannot be reliably determined that every neurosphere derives from a single stem cell, multiple stem cells of the same or different type or even whether non-stem cells are an integral constituent (since most studies start with a heterogeneous cell population) (table 1). It is possible that the positive effects of ‘stem cell transplantation’ observed in preclinical studies actually result from more than one cell type, but not knowing exactly what you ‘put in’ makes it difficult to interpret what ‘comes out’.

To overcome this problem, some investigators have attempted to sort putative ENSC using molecular markers (table 1). The neurotrophin receptor p75 and $\alpha 4$ integrin have both been used in this context but are clearly not exclusive for stem cells, as only some of the positive cells proliferate and not all of the proliferating colonies are equally multipotent on differentiation.⁵⁹⁶⁰ Others have suggested the transcription factor Sox2 as a marker for enteric neural precursor cells that can proliferate and differentiate into neurons and glial cells.⁶⁶ However, Sox2 is not expressed on the surface and therefore cannot be used for sorting experiments.⁸⁵ Further, it is also expressed in glial cells.⁶⁶ Clearly, there is still an unmet need to identify an ENSC marker, and the ability unequivocally to define the ENSC will be of tremendous value in furthering this field.

WHERE SHOULD ENSC BE OBTAINED FROM?

In mice, BrdU staining has demonstrated the presence of enteric neural precursors in areas between the myenteric ganglia and longitudinal muscle whence they may migrate to the plexi to differentiate into new mature neurons.⁶⁷⁷⁴⁷⁶ However, unlike the situation in the CNS, regional localisation of stem cells in the ENS has not yet been described. On the contrary, it appears that, in humans, ENSC-like cells may be found in both the small and large intestines (in the muscle as well as the subepithelial layers) in embryonic, neonatal and adult gut (table 1). However, what is not clear is whether these ENSC are identical

regardless of their origin or, instead, whether there are fundamental differences in the phenotype and behaviour of these cells determined by their microenvironment and/or the passage of time.

Nevertheless, the finding that ENSC are widely distributed in the gut is quite surprising and has several potential implications. First, ENSC may be readily accessible and harvested easily by endoscopic or minimally invasive surgical techniques. Indeed, table 1 lists recent studies which show that ENSC can be derived from mucosal biopsies taken during endoscopy.^{61,62} Second, if stem cells are so ubiquitous in the gut, why does neuronal loss occur at all in some diseases? This could be due to a defect in the stem cells themselves or a disturbance in the host environment that prevents them from either generating new neurons during the development of the ENS or replenishing degenerating neurons. In this case, pharmacological stimulation of in situ ENSC or modulation of local tissue factors may represent an alternative to transplantation. Experiments from the Gershon laboratory using BrdU pulse-chase experiments have shown that enteric neurogenesis not only occurs in adult mice but that it can be stimulated by 5-HT₄ agonists.⁷⁸ However, whether 5-HT₄ agonists have the same effect in humans remains unclear.

WHAT DOES IT TAKE TO PRODUCE LONG-LASTING AND ROBUST INTEGRATION OF TRANSPLANTED CELLS IN THE HOST?

The experience with CNS-NSC provides sobering lessons for those of us working in the ENS where poor survival and limited differentiation after transplantation are major factors in the lack of a robust or durable clinical response.⁸⁶ Both host and graft factors may be important in this equation, and understanding these is an important prerequisite for future progress in this field. The ‘soil versus seed’ consideration may be particularly important in disorders where the environment is known to be incapable of supporting NSC during development, such as in certain forms of Hirschsprung disease caused by deficiency of glial-derived neurotrophic factor or endothelin signalling. Compensation for the loss of these factors is therefore likely to be an important therapeutic adjunct in these conditions, although it is possible that the requirements for proper ENSC engraftment and differentiation in the postnatal or adult state may be different.

Various modalities can be explored for the delivery of ENSC to the site(s) of transplantation, depending in part on the nature of the underlying condition. Local injection, whether by endoscopic or surgical means, is the most direct for localised diseases. ENSC can also be delivered intraperitoneally and may be able to home in to the gut by as yet unidentified cues.⁸⁷ Recent studies show that transplantation of ENSC embedded in artificial or bioengineered materials, scaffolds or tissues can also offer viable alternatives to injection of cells into the diseased tissue.⁸⁸ Finally, intravascular delivery of NSC, which has been tried in the treatment of CNS disorders, is also a feasible route of delivery for the treatment of ENS disorders.⁸⁹ However, physical transplantation of NSC in the gut is just the beginning of a long and poorly understood process to functional colonisation of the host tissue by transplanted ENSC. The process of successful integration into host tissue by transplanted cells has been likened to a military campaign, where injected cells will initially face space constraints and injury and immune responses from the host, but the success of the graft will

depend on the cross-talk with the host tissue and a functional integration with the native tissue (box 1).⁸⁰ Further research is needed to optimise this process and to answer important questions such as the method of delivery (direct injection, intravascular or intraperitoneal), the appropriate number of cells needed, the form in which these cells are to be delivered (neurospheres or dissociated cells) and the requirement for both insulating and trophic factors (both of which could theoretically be incorporated along with the cells using ‘smart’ biomaterials). An interesting variation is to bioengineer integrated neuromuscular units in vitro containing both muscle and neural components for subsequent transplantation, as has been shown in recent ‘proof of principle’ studies to create an artificial anal sphincter.⁹⁰ Finally, it is not completely clear what immunosuppressive regimen, if any, is necessary for allogenic transplants. We have previously shown, using caspase inhibitors, that intrinsic apoptotic programmes are more important in determining short-term survival than modulation of the host immune response.⁵⁶ However, experiments observing the long-term survival of the graft and graft-derived cells are needed.

Box 1

Post-transplantation phases and challenges

Phase I: Expeditionary

- Space constraints.
- Early inflammation and injury response.

Phase II: Engagement

- Target homing and contact.
- Trophic deprivation and adaptation.
- Host immune response.

Phase III: Integration

- Functional interaction with neighbours.
- Phenotypic stability.
- Long-term survival and/or capacity for self-renewal.

Reproduced from Schäfer *et al*⁸⁰ with permission of the publisher.

SAFETY ISSUES

We have already discussed the risk of tumorigenesis from pluripotent cells. However, this risk does not disappear with the use of more committed precursors as donor-derived brain tumours have been reported following NSC transplantation into the CNS in a human.⁹¹ As observed above, a significant number of long-term transplantation experiments in animal models need to be performed to better understand the safety risks associated with the transplantation of committed stem cells and precursor cells in vivo. Even if the risk of tumorigenicity as assessed from such animal model transplantations is deemed to be low, monitoring of transplanted tissue will continue to be required in patients.

CONCLUSIONS: ENS REGENERATION—FUTURE OR FICTION?

The answer to this question may involve an affirmative to both choices. For instance, NSC transplantation represents an ideal way to treat achalasia in which there is a clearly defined neuronal loss and the symptoms predominantly result from a functional defect (ie, loss of relaxation) in a small readily accessible region of the oesophagus. Even though other neurons may be affected, replacement of a single neurotransmitter (nitric oxide) in the vicinity of the muscle may be sufficient to attenuate symptoms even if it does not restore the ENS to its former condition.⁹² Further, because ENSCs in other regions of the gut such as the colon may not be affected, there is a real potential for autologous transplantation that would further alleviate safety concerns and the need for immunosuppression. On the other hand, widespread loss or dysfunction of neurons and associated elements may be too formidable to overcome by simple transplantation of NSC (either autologous or allogenic), because of the requirement to replace multiple cell types in a regionally appropriate manner and to provide the complex connectivity essential for normal motility across the length of the affected segment. Stimulation of local NSC or modulation of their environment may be a better approach in such instances. It is clear, however, that further research into the biology of ENSC will provide hope for either scenario and holds great promise to finding a lasting cure for ENS disorders.

Acknowledgments

Funding NIDDK (1R01DK080920).

References

1. Wood JD, Alpers DH, Andrews PL. Fundamentals of neurogastroenterology. *Gut*. 1999; 45(Suppl 2):II6–16. [PubMed: 10457039]
2. De Giorgio R, Camilleri M. Human enteric neuropathies: morphology and molecular pathology. *Neurogastroenterol Motil*. 2004; 16:515–31. [PubMed: 15500508]
3. Ruhl A. Glial cells in the gut. *Neurogastroenterol Motil*. 2005; 17:777–90. [PubMed: 16336493]
4. Heanue TA, Pachnis V. Enteric nervous system development and Hirschsprung's disease: advances in genetic and stem cell studies. *Nat Rev Neurosci*. 2007; 8:466–79. [PubMed: 17514199]
5. Ward SM, Sanders KM. Involvement of intramuscular interstitial cells of Cajal in neuroeffector transmission in the gastrointestinal tract. *J Physiol*. 2006; 576:675–82. [PubMed: 16973700]
6. Kraneveld AD, Rijnierse A, Nijkamp FP, et al. Neuro-immune interactions in inflammatory bowel disease and irritable bowel syndrome: future therapeutic targets. *Eur J Pharmacol*. 2008; 585:361–74. [PubMed: 18417115]
7. Hunt RH, Tougas G. Evolving concepts in functional gastrointestinal disorders: promising directions for novel pharmaceutical treatments. *Best Pract Res Clin Gastroenterol*. 2002; 16:869–83. [PubMed: 12473296]
8. Ben-Horin S, Chowers Y. Neuroimmunology of the gut: physiology, pathology, and pharmacology. *Curr Opin Pharmacol*. 2008; 8:490–5. [PubMed: 18675937]
9. Wingate D. Motility disorders of the small intestine in functional intestinal disorders (Place des troubles de la motricité du grêle dans les troubles digestifs fonctionnels). *Presse Med*. 1989; 18:290–3. [PubMed: 2522225]
10. Wedel T, Busing V, Heinrichs G, et al. Diverticular disease is associated with an enteric neuropathy as revealed by morphometric analysis. *Neurogastroenterol Motil*. 2010; 22:407–14. e93–4. [PubMed: 20040058]

11. Wedel T, Roblick U, Gleiss J, et al. Disorders of intestinal innervation as a possible cause for chronic constipation (In German). *Zentralbl Chir.* 1999; 124:796–803. [PubMed: 10544485]
12. Kashyap P, Gomez-Pinilla PJ, Pozo MJ, et al. Immunoreactivity for Ano1 detects depletion of Kit-positive interstitial cells of Cajal in patients with slow transit constipation. *Neurogastroenterol Motil.* 2011; 23:760–5. [PubMed: 21585622]
13. Camilleri M. Enteric nervous system disorders: genetic and molecular insights for the neurogastroenterologist. *Neurogastroenterol Motil.* 2001; 13:277–95. [PubMed: 11576388]
14. Micci MA, Pasricha PJ. Neural stem cells for the treatment of disorders of the enteric nervous system: strategies and challenges. *Dev Dyn.* 2007; 236:33–43. [PubMed: 17029286]
15. Kehat I, Kenyagin-Karsenti D, Snir M, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest.* 2001; 108:407–14. [PubMed: 11489934]
16. Vittet D, Prandini MH, Berthier R, et al. Embryonic stem cells differentiate in vitro to endothelial cells through successive maturation steps. *Blood.* 1996; 88:3424–31. [PubMed: 8896407]
17. Zhang SC, Wernig M, Duncan ID, et al. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol.* 2001; 19:1129–33. [PubMed: 11731781]
18. Sidhu KS, Ryan JP, Tuch BE. Derivation of a new human embryonic stem cell line, endeavour-1, and its clonal propagation. *Stem Cells Dev.* 2008; 17:41–51. [PubMed: 18271699]
19. Sidhu KS, Walke S, Tuch BE. Derivation and Propagation of hESC Under a Therapeutic Environment. *Curr Protoc Stem Cell Biol.* 2008; 6:1A.4.1–1A.4.31.
20. Kehoe DE, Lock LT, Parikh A, et al. Propagation of embryonic stem cells in stirred suspension without serum. *Biotechnol Prog.* 2008; 24:1342–52. [PubMed: 19194949]
21. Park Y, Choi IY, Lee SJ, et al. Undifferentiated propagation of the human embryonic stem cell lines, H1 and HSF6, on human placenta-derived feeder cells without basic fibroblast growth factor supplementation. *Stem Cells Dev.* 2010; 19:1713–22. [PubMed: 20201681]
22. Wobus AM, Boheler KR. Embryonic stem cells: prospects for developmental biology and cell therapy. *Physiol Rev.* 2005; 85:635–78. [PubMed: 15788707]
23. Reubinoff BE, Pera MF, Fong CY, et al. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nat Biotechnol.* 2000; 18:399–404. [PubMed: 10748519]
24. Xu C, Police S, Hassanipour M, et al. Efficient generation and cryopreservation of cardiomyocytes derived from human embryonic stem cells. *Regen Med.* 2011; 6:53–66. [PubMed: 21175287]
25. Alper J. Geron gets green light for human trial of ES cell-derived product. *Nat Biotechnol.* 2009; 27:213–14. [PubMed: 19270655]
26. Wadman M. Stem cells ready for prime time. *Nature.* 2009; 457:516. [PubMed: 19177087]
27. Zhang SC. Neural subtype specification from embryonic stem cells. *Brain Pathol.* 2006; 16:132–42. [PubMed: 16768754]
28. Pera MF, Andrade J, Houssami S, et al. Regulation of human embryonic stem cell differentiation by BMP-2 and its antagonist noggin. *J Cell Sci.* 2004; 117:1269–80. [PubMed: 14996946]
29. Koch P, Opitz T, Steinbeck JA, et al. A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. *Proc Natl Acad Sci U S A.* 2009; 106:3225–30. [PubMed: 19218428]
30. Gerrard L, Rodgers L, Cui W. Differentiation of human embryonic stem cells to neural lineages in adherent culture by blocking bone morphogenetic protein signaling. *Stem Cells.* 2005; 23:1234–41. [PubMed: 16002783]
31. Elkabetz Y, Panagiotakos G, Al Shamy G, et al. Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. *Genes Dev.* 2008; 22:152–65. [PubMed: 18198334]
32. Lee G, Kim H, Elkabetz Y, et al. Isolation and directed differentiation of neural crest stem cells derived from human embryonic stem cells. *Nat Biotechnol.* 2007; 25:1468–75. [PubMed: 18037878]
33. Kawaguchi J, Nichols J, Gierl MS, et al. Isolation and propagation of enteric neural crest progenitor cells from mouse embryonic stem cells and embryos. *Development.* 2010; 137:693–704. [PubMed: 20147374]

34. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–76. [PubMed: 16904174]
35. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131:861–72. [PubMed: 18035408]
36. Hu BY, Du ZW, Zhang SC. Differentiation of human oligodendrocytes from pluripotent stem cells. *Nat Protoc*. 2009; 4:1614–22. [PubMed: 19834476]
37. Hu BY, Weick JP, Yu J, et al. Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc Natl Acad Sci U S A*. 2010; 107:4335–40. [PubMed: 20160098]
38. Wernig M, Zhao JP, Pruszak J, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc Natl Acad Sci U S A*. 2008; 105:5856–61. [PubMed: 18391196]
39. Chin MH, Mason MJ, Xie W, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell*. 2009; 5:111–23. [PubMed: 19570518]
40. Urbach A, Bar-Nur O, Daley GQ, et al. Differential modeling of fragile X syndrome by human embryonic stem cells and induced pluripotent stem cells. *Cell Stem Cell*. 2010; 6:407–11. [PubMed: 20452313]
41. Polo JM, Liu S, Figueroa ME, et al. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol*. 2010; 28:848–55. [PubMed: 20644536]
42. Kim K, Doi A, Wen B, et al. Epigenetic memory in induced pluripotent stem cells. *Nature*. 2010; 467:285–90. [PubMed: 20644535]
43. Pick M, Stelzer Y, Bar-Nur O, et al. Clone- and gene-specific aberrations of parental imprinting in human induced pluripotent stem cells. *Stem Cells*. 2009; 27:2686–90. [PubMed: 19711451]
44. Okita K, Nakagawa M, Hyenjong H, et al. Generation of mouse induced pluripotent stem cells without viral vectors. *Science*. 2008; 322:949–53. [PubMed: 18845712]
45. Ben-David U, Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer*. 2011; 11:268–77. [PubMed: 21390058]
46. Vierbuchen T, Ostermeier A, Pang ZP, et al. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*. 2010; 463:1035–41. [PubMed: 20107439]
47. Pang ZP, Yang N, Vierbuchen T, et al. Induction of human neuronal cells by defined transcription factors. *Nature*. 2011; 476:220–3. [PubMed: 21617644]
48. Cheng LC, Tavazoie M, Doetsch F. Stem cells: from epigenetics to microRNAs. *Neuron*. 2005; 46:363–7. [PubMed: 15882632]
49. Alvarez-Buylla A, Lim DA. For the long run: maintaining germinal niches in the adult brain. *Neuron*. 2004; 41:683–6. [PubMed: 15003168]
50. Kulkarni S, Micci MA, Tiwari G, et al. An in vitro model to study the potential of neural stem cells of diverse origins to assume an enteric phenotype (abstract). *Gastroenterology*. 2010; 138:T1736.
51. Tiwari G, Micci MA, Kulkarni S, et al. Transient induction of RET activity pushes CNS neural stem cells towards enteric phenotype through activation of multiple signaling pathways (abstract). *Gastroenterology*. 2011; 140:S-4.
52. Tiwari G, Oh J, Becker L, et al. ROCK1/2 inhibitor induces enteric phenotype in CNS derived neural stem cells (abstract). *Gastroenterology*. 2011; 140:S-522.
53. Hotta R, Pepdjonovic L, Anderson RB, et al. Small-molecule induction of neural crest-like cells derived from human neural progenitors. *Stem Cells*. 2009; 27:2896–905. [PubMed: 19711454]
54. Micci MA, Learish RD, Li H, et al. Neural stem cells express RET, produce nitric oxide, and survive transplantation in the gastrointestinal tract. *Gastroenterology*. 2001; 121:757–66. [PubMed: 11606488]
55. Micci MA, Kahrig KM, Simmons RS, et al. Neural stem cell transplantation in the stomach rescues gastric function in neuronal nitric oxide synthase-deficient mice. *Gastroenterology*. 2005; 129:1817–24. [PubMed: 16344050]

56. Micci MA, Pattillo MT, Kahrig KM, et al. Caspase inhibition increases survival of neural stem cells in the gastrointestinal tract. *Neurogastroenterol Motil.* 2005; 17:557–64. [PubMed: 16078945]
57. Mack GS. ReNeuron and StemCells get green light for neural stem cell trials. *Nat Biotechnol.* 2011; 29:95–7. [PubMed: 21301419]
58. Mosher JT, Yeager KJ, Kruger GM, et al. Intrinsic differences among spatially distinct neural crest stem cells in terms of migratory properties, fate determination, and ability to colonize the enteric nervous system. *Dev Biol.* 2007; 303:1–15. [PubMed: 17113577]
59. Kruger GM, Mosher JT, Bixby S, et al. Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness. *Neuron.* 2002; 35:657–69. [PubMed: 12194866]
60. Bixby S, Kruger GM, Mosher JT, et al. Cell-intrinsic differences between stem cells from different regions of the peripheral nervous system regulate the generation of neural diversity. *Neuron.* 2002; 35:643–56. [PubMed: 12194865]
61. Metzger M, Caldwell C, Barlow AJ, et al. Enteric nervous system stem cells derived from human gut mucosa for the treatment of aganglionic gut disorders. *Gastroenterology.* 2009; 136:2214–25. e1–3. [PubMed: 19505425]
62. Metzger M, Bareiss PM, Danker T, et al. Expansion and differentiation of neural progenitors derived from the human adult enteric nervous system. *Gastroenterology.* 2009; 137:2063–73. e4. [PubMed: 19549531]
63. Metzger M. Neurogenesis in the enteric nervous system. *Arch Ital Biol.* 2010; 148:73–83. [PubMed: 20830970]
64. Schafer KH, Hagl CI, Rauch U. Differentiation of neurospheres from the enteric nervous system. *Pediatr Surg Int.* 2003; 19:340–4. [PubMed: 12845455]
65. Rauch U, Hansgen A, Hagl C, et al. Isolation and cultivation of neuronal precursor cells from the developing human enteric nervous system as a tool for cell therapy in dysganglionosis. *Int J Colorectal Dis.* 2006; 21:554–9. [PubMed: 16267668]
66. Heanue TA, Pachnis V. Prospective identification and isolation of enteric nervous system progenitors using SOX2. *Stem Cells.* 2010; 29:128–40. [PubMed: 21280162]
67. Tsai YH, Murakami N, Garipey CE. Postnatal intestinal engraftment of prospectively selected enteric neural crest stem cells in a rat model of Hirschsprung disease. *Neurogastroenterol Motil.* 2010; 23:362–9. [PubMed: 21199176]
68. Hennig GW, Spencer NJ, Jokela-Willis S, et al. ICC-MY coordinate smooth muscle electrical and mechanical activity in the murine small intestine. *Neurogastroenterol Motil.* 2010; 22:e138–51. [PubMed: 20059699]
69. Lorincz A, Redelman D, Horvath VJ, et al. Progenitors of interstitial cells of cajal in the postnatal murine stomach. *Gastroenterology.* 2008; 134:1083–93. [PubMed: 18395089]
70. Wang XY, Alberti E, White EJ, et al. Igf1r+/CD34+ immature ICC are putative adult progenitor cells, identified ultrastructurally as fibroblast-like ICC in Ws/Ws rat colon. *J Cell Mol Med.* 2009; 13:3528–40. [PubMed: 19220583]
71. Silva AT, Wardhaugh T, Dolatshad NF, et al. Neural progenitors from isolated postnatal rat myenteric ganglia: expansion as neurospheres and differentiation in vitro. *Brain Res.* 2008; 1218:47–53. [PubMed: 18514173]
72. Bondurand N, Natarajan D, Thapar N, et al. Neuron and glia generating progenitors of the mammalian enteric nervous system isolated from foetal and postnatal gut cultures. *Development.* 2003; 130:6387–400. [PubMed: 14623827]
73. Suarez-Rodriguez R, Belkind-Gerson J. Cultured nestin-positive cells from postnatal mouse small bowel differentiate ex vivo into neurons, glia, and smooth muscle. *Stem Cells.* 2004; 22:1373–85. [PubMed: 15579654]
74. Almond S, Lindley RM, Kenny SE, et al. Characterisation and transplantation of enteric nervous system progenitor cells. *Gut.* 2007; 56:489–96. [PubMed: 16973717]
75. Lindley RM, Hawcutt DB, Connell MG, et al. Human and mouse enteric nervous system neurosphere transplants regulate the function of aganglionic embryonic distal colon. *Gastroenterology.* 2008; 135:205–16. e6. [PubMed: 18515088]

76. Natarajan D, Grigoriou M, Marcos-Gutierrez CV, et al. Multipotential progenitors of the mammalian enteric nervous system capable of colonising aganglionic bowel in organ culture. *Development*. 1999; 126:157–68. [PubMed: 9834195]
77. Bondurand N, Natarajan D, Barlow A, et al. Maintenance of mammalian enteric nervous system progenitors by SOX10 and endothelin 3 signalling. *Development*. 2006; 133:2075–86. [PubMed: 16624853]
78. Liu MT, Kuan YH, Wang J, et al. 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. *J Neurosci*. 2009; 29:9683–99. [PubMed: 19657021]
79. Lo L, Anderson DJ. Postmigratory neural crest cells expressing c-RET display restricted developmental and proliferative capacities. *Neuron*. 1995; 15:527–39. [PubMed: 7546733]
80. Schafer KH, Micci MA, Pasricha PJ. Neural stem cell transplantation in the enteric nervous system: roadmaps and roadblocks. *Neurogastroenterol Motil*. 2009; 21:103–12. [PubMed: 19215588]
81. Ourednik V, Ourednik J. Multifaceted dialogue between graft and host in neurotransplantation. *J Neurosci Res*. 2004; 76:193–204. [PubMed: 15048917]
82. Ourednik V, Ourednik J. Graft/host relationships in the developing and regenerating CNS of mammals. *Ann N Y Acad Sci*. 2005; 1049:172–84. [PubMed: 15965116]
83. Martino G, Pluchino S. The therapeutic potential of neural stem cells. *Nat Rev Neurosci*. 2006; 7:395–406. [PubMed: 16760919]
84. Suslov ON, Kukekov VG, Ignatova TN, et al. Neural stem cell heterogeneity demonstrated by molecular phenotyping of clonal neurospheres. *Proc Natl Acad Sci U S A*. 2002; 99:14506–11. [PubMed: 12381788]
85. Ambrosetti DC, Scholer HR, Dailey L, et al. Modulation of the activity of multiple transcriptional activation domains by the DNA binding domains mediates the synergistic action of Sox2 and Oct-3 on the fibroblast growth factor-4 enhancer. *J Biol Chem*. 2000; 275:23387–97. [PubMed: 10801796]
86. Reekmans K, Praet J, Daans J, et al. Current challenges for the advancement of neural stem cell biology and transplantation research. *Stem Cell Rev*. Published Online First: 3 May 2011. 10.1007/s12015-011-9266-2
87. Martucciello G, Brizzolara A, Favre A, et al. Neural crest neuroblasts can colonise aganglionic and ganglionic gut in vivo. *Eur J Pediatr Surg*. 2007; 17:34–40. [PubMed: 17407019]
88. Miyasaka EA, Raghavan S, Gilmont RR, et al. In vivo growth of a bioengineered internal anal sphincter: comparison of growth factors for optimization of growth and survival. *Pediatr Surg Int*. 2011; 27:137–43. [PubMed: 21046117]
89. Chu K, Kim M, Jeong SW, et al. Human neural stem cells can migrate, differentiate, and integrate after intravenous transplantation in adult rats with transient forebrain ischemia. *Neurosci Lett*. 2003; 343:129–33. [PubMed: 12759181]
90. Raghavan S, Gilmont RR, Miyasaka EA, et al. Successful Implantation of Bioengineered, Intrinsicly Innervated, Human Internal Anal Sphincter. *Gastroenterology*. 2011; 141:310–9. [PubMed: 21463628]
91. Amariglio N, Hirshberg A, Scheithauer BW, et al. Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. *PLoS Med*. 2009; 6:e1000029. [PubMed: 19226183]
92. Park H, Conklin JL. Neuromuscular control of esophageal peristalsis. *Curr Gastroenterol Rep*. 1999; 1:186–97. [PubMed: 10980948]
93. Lindley RM, Hawcutt DB, Connell MG, et al. Properties of secondary and tertiary human enteric nervous system neurospheres. *J Pediatr Surg*. 2009; 44:1249–56. [PubMed: 19524749]

Figure 2.

Top: β -Tubulin immunoreactivity in rat CNS-derived neural stem cells (CNS-NSC) in co-culture with human colonic smooth muscle cells (visible in the background) indicating that CNS-NSC differentiate into neurons. Bottom: Intracellular calcium measurements in human colonic smooth muscle cells in co-culture with rat CNS-NSC in response to the nicotinic agonist dimethylphenylpiperazinium (DMPP). Cells were loaded with calcium-sensitive fluorescent indicator Fura-2 and changes in intracellular calcium were measured in colonic smooth muscle cells in response to bath application of DMPP (20 μ M, indicated by the white arrows). (A) DMPP induces an increase in intracellular calcium in colonic smooth muscle cells when in co-culture with rat CNS-NSC. (B) This effect is not observed when colonic smooth muscle cells are cultured without rat CNS-NSC. (C) The DMPP-induced response in colonic smooth muscle cells in co-culture with rat CNS-NSC was blocked by the nicotinic receptor antagonist hexamethonium, demonstrating that DMPP is acting on the nicotinic receptor. (D) The response was blocked by the neurokinin A receptor antagonist (CP-099994-01), suggesting that it is mediated by substance P. These data show that rat CNS-NSC stimulated with DMPP induce an increase in smooth muscle calcium, demonstrating that they make functional contact with smooth muscle cells in vitro. Reproduced from Micci and Pasricha¹⁴ with permission from the publisher.

Figure 3.

Transplanted CNS-derived neural stem cells (CNS-NSC) survive in the pyloric wall of the nNOS^{-/-} mice and improve gastric emptying. (A) Representative photographs of CNS-NSC grafts in cross-sections of nNOS^{-/-} mouse pylorus 1 week post-transplantation. Green Fluorescent Protein (GFP)-positive CNS-NSC are shown in green; PGP9.5 immunoreactivity is indicated in red. (B) Representative photographs of CNS-NSC grafts in cross-sections of nNOS^{-/-} mouse pylorus 1 week post-transplantation. GFP-positive CNS-NSC are shown in green; GFAP immunoreactivity is indicated in red. LM, longitudinal muscle; CM, circular muscle. Scale bar 20 μ m. (C) Gastric emptying of liquids measured 20 min after gavage of dye in nNOS^{-/-} mice 1 week after transplantation of CNS-NSC or vehicle. * $p < 0.01$ (two-tailed Student t test). Two-sample equal variance. Data are means $\pm$ SEM. $n = 6$ mice for transplantation of vehicle and $n = 8$ mice for transplantation of CNS-NSC. Reproduced from Micci *et al*⁵⁵ with permission from the publisher.

Table 1

Summary of studies of the multipotentiality of ENSC in vitro and in vivo

Reference	Functional tests	Transplanted into	Scoring differentiated cells (N, G and/or M)	Cells sorted, unsorted or selected	Source tissue	Age	Species
Kruger <i>et al</i> ⁵⁹	Not performed	Chick embryo in ovo	N, G, M	p75+ and $\alpha 4$ integrin+ cells sorted	Developing whole gut, muscle and plexus layers of normoganglionic postnatal and adult guts	E14.5–P110	Rat
Silva <i>et al</i> ⁷¹	Not performed	Not performed	N, G	Unsorted	Muscle and plexus layers of normoganglionic postnatal guts	P7–P14	Rat
Bondurand <i>et al</i> ⁷²	Not performed	Explants of aganglionic gut from Ret ^{−/−} mouse	N, G	Unsorted	Developing whole gut, muscle and plexus layers of normoganglionic postnatal guts	E11.5–P14	Mouse
Suarez-Rodriguez and Belkind-Gerson ⁷³	Not performed	Not performed	N, G, M	Unsorted	Normoganglionic whole gut of postnatal and adult animals	P3–12 months	Mouse
Bixby <i>et al</i> ⁶⁰	Not performed	Chick embryo in ovo	N, G, M	FACS sorted for p75 and $\alpha 4$ integrin	Embryonic whole gut	Embryonic rats	Rat
Heanue and Pachnis ⁶⁶	Not performed	Explants of mouse ganglionic stomach and aganglionic caecum	N, G	Selected for Sox2	Midgut and hindgut tissue from embryonic and postnatal mice	Embryonic and postnatal mice	Wild type, Wnt1Cre;Rosa26 ^{YFP} , Sox2 ^{fl^{geo}} and B6. Rosa26 ^{YFP} mice
Almond <i>et al</i> ⁷⁴	Not performed	Aganglionic gut explants from E11.5 mouse	N, G	Unsorted	Developing mouse caecum; postnatal normoganglionic human myenteric plexus	E11.5; 3 weeks to 7 months	Mouse, human
Lindley <i>et al</i> ⁷⁵	Bowel contractility and calcium imaging	Aganglionic gut explants from E11.5 mouse	N, G	Unsorted	Developing mouse caecum; postnatal normoganglionic human myenteric plexus	E11.5; neonatal	Mouse, human
Lindley <i>et al</i> ⁷³	Not performed	Aganglionic gut explants from E11.5 mouse	N, G	Unsorted	Postnatal normoganglionic myenteric plexus	Neonatal	Human
Rauch <i>et al</i> ⁶⁵	Not performed	Human aganglionic gut explants	N, G	Unsorted	Normoganglionic myenteric plexus	9 weeks gestation to 5 years	Human
Metzger <i>et al</i> ⁶¹	Not performed	Explants of aganglionic human gut and developing E5 chick gut	N, G	Unsorted	Gut mucosal biopsy tissue	8 weeks gestation to 7 years	Human
Metzger <i>et al</i> ⁶²	Not performed	E18 organotypic in vitro slice culture and aganglionic gut explants from	N, G, M	Unsorted	Longitudinal and circular muscle containing myenteric plexus	26–84 years	Human

Reference	Functional tests	Transplanted into	Scoring differentiated cells (N, G and/or M)	Cells sorted, unsorted or selected	Source tissue	Age	Species
Tsai <i>et al</i> ⁶⁷	Not performed	E11 mouse embryo	Not measured	Sorted for $\alpha 4$ integrin and p75 (high expressors)	Embryonic whole intestine	E14.5	Rat
		E11 mouse embryo					
Natarajan <i>et al</i> ⁷⁶	Not performed	Intraperitoneal cavity of rats with symptoms of Hirschsprung disease	General and specific N; G and M scored post-transplantation	RET+ cells FACS sorted from whole guts of PTY embryonic mice	Embryonic whole guts	E11.5 embryos	Parkes mice (wild type at c-RET locus), Ret.k- mutant mice with and without intercross with Parkes mice and PTY mice
Schäfer <i>et al</i> ⁶⁴	Not performed	Not performed	N, G	Not performed	Embryonic whole guts	E12 embryos	Mouse
Mosher <i>et al</i> ⁶⁸	Not performed	In ovo chick embryos, explants of 5-day-old chick embryonic guts, explants of distal colon of E14.5 rats	N, G, M Specific neuronal subtypes identified post-transplantation	FACS sorted for p75 and $\alpha 4$ integrin	Embryonic fetal whole gut	E14.5 embryos	Wild type rats, hPAP + (human placental alkaline phosphatase) rats, Ednr ^b sl/sl hPAP+ Rats
Bondurand <i>et al</i> ⁷⁷	Not performed	Not performed	N, G	Cultured progenitors from guts transfected with Sox10-GFP whose expression was used to follow cells with different SOX10 expression	Embryonic and postnatal gut	E11.5–E12.5 embryonic and postnatal mice	Wild type, Sox10 ^{+/+} lacZ, ret 51/51, Ednr ^b LacZ/LacZ, CS7Nl/6 carrying Edn3ls allele, Wnt1Cre-YFP mice
Liu <i>et al</i> ⁷⁸	Not performed	Not performed	N (in vitro); N, G (in vivo)	Immunoselected for p75+ cells	Embryonic gut and LM-MP layer from adult male mice	E12 embryonic and adult mice	Mouse
Lo and Anderson ⁷⁹	Not performed	Not performed	N, G	FACS sorted independently for RET+ and p75+ expression	Embryonic whole gut	E14.5 embryonic rats	Rat

G, glial cells; LM-MP, longitudinal muscle containing myenteric plexus; M, myofibroblasts; N, neurons.