Stem cell therapy in severe pediatric motility disorders

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Abstract

Pediatric gastrointestinal motility disorders represent a range of severe developmental or acquired conditions that disrupt enteric neuromuscular function. Current medical and surgical therapeutic options are very limited but recent advances have highlighted the possibility of improved or curative stem cell-based treatments. Not only has the ability to harvest, propagate and transplant human-derived enteric neural stem cells (ENSCs) been demonstrated but recent *in vivo* transplantation studies have confirmed that ENSCs are capable of engraftment within recipient intestine of animal models of enteric neuropathy and effecting functional rescue. Pluripotent stem cell-derived cells and pharmacological modulation of both endogenous and transplanted neural stem cells have further enhanced the exciting prospect of clinical application of such stem cell-based therapies in the near future.

Pediatric gastrointestinal motility disorders exist as a wide range of diseases, which can affect nearly every region of the gastrointestinal (GI) tract, including disorders of the esophagus, stomach and intestine such as achalasia,[1,2] gastroparesis,[3,4] pseudo obstruction,[5] slow transit constipation,[6-8] and Hirschsprung disease (HSCR).[9,10] Such conditions can arise from disruption of the neuromuscular syncytium through aberrant development or through acquired processes, which ultimately lead to loss of specific cell populations or disturbances in neuromuscular signaling.

Current therapeutic interventions for pediatric motility disorders are very limited and, apart from those designed to ameliorate immune-mediated or inflammatory aetiologies, can be considered palliative rather than curative. Available interventions comprise medical management such as pharmacotherapy and/or specialized (including parenteral) nutrition and surgery aimed to minimize complications, improve quality of life as well as allow growth and development. Surgical interventions, applied in the majority of severe cases include manipulation (e.g. myotomy) of affected gut segments to facilitate flow of luminal comments and/or decompress the bowel or resection of the affected gut region. Unfortunately, such management strategies are associated with significant morbidity and poor outcomes in the pediatric population, with patients often requiring further surgical management through early childhood and beyond. Hence, there is a real need for alternative approaches to treat these devastating diseases. Recent advances in our understanding of gut development, the identification of gut stem cells and tremendous progress in pluripotent stem cell manipulation have driven investigations into the potential of stem cell-based therapies for the treatment pediatric motility disorders.

Enteric Neural Stem Cell Treatment for Enteric Neuropathies

Whilst pediatric motility disorders may result from disruption or loss any cell type (enteric neurons, interstitial cells of Cajal, PDGFRα^+ cells or smooth muscle cells) involved in gastrointestinal neuromuscular signaling, investigations, to date, have centered on the identification and application of neural stem cells for the treatment of neuropathology within the enteric nervous system (ENS).

The ENS, the largest branch of the peripheral nervous system, is composed primarily of vagal neural crest-derived cells with a smaller contribution of sacral neural crest cells (NCC). During embryonic development NCC delaminate from the dorsal aspect of the neural tube and migrate extensively throughout the embryo to their final anatomical location. Vagally derived enteric neural crest cells (ENCC) enter the foregut at approximately embryonic day 9.5 (E9.5) in mice and in humans at approximately gestational week 4.[11,12] These ENCC proceed to colonize the entire gut in a rostro-caudal fashion by approximately E13.5 in mice[13] or embryonic week 7 in humans[12] with failure of this process leading to severe developmental diseases. Indeed failure of rostro-caudal ENCC colonization of the developing GI tract results in an absence of ENS formation in variable regions of the gut termed “Hirschsprung disease” (HSCR).[9] This incomplete formation of the ENS, in HSCR, leads to severe motility issues with constriction of the aganglionic intestine causing functional blockage of the terminal intestine and subsequent distention of the proximal intestine. HSCR is often diagnosed early in post-natal life with failure to pass meconium within the first 24 hours after birth[14] and the obstruction can be life-threatening without surgical intervention.[10] Given the well-characterized loss of enteric neurons within the aganglionic segment, stem cell-based replacement, of enteric neurons, is an attractive therapy for the treatment of HSCR.

Early studies of ENS development highlighted the critical need of ENCC to proliferate extensively, in embryonic life, to allow for colonization of the expanding gastrointestinal tract and the generation of the approximately 200-600 million enteric neurons and glia which make up the ENS.[15] Such studies have highlighted the role of $SOX10^+$ multi-lineage ENS progenitors (termed here as Enteric Neural Stem Cells - ENSCs) which are maintained as a progenitor pool via endothelin 3 signalling[16] with critical roles of Ret/GDNF signalling[17] in expansion and migration of ENCC along the length of the developing gut.[18] Furthermore, studies of the potential of post migratory ENCC have demonstrated the presence of multipotent $p75^+$ or RET^+ progenitors,[19,20] which can differentiate towards ENS lineages, suggesting the presence of enteric "stem-like" cells within the ENS after colonization. Multipotent ENSCs have subsequently been identified in fetal and postnatal gut tissues from rodent models.[21-23] Moreover, human gut samples, both fetal and post-natal, have been similarly shown to contain ENSC,[24,25] suggesting that a pool of multipotent ENSC are maintained through life, raising the possibility harvesting autologous ENSC for the treatment of ENS disorders. More recently, clinical studies have crucially demonstrated that it may be possible to isolate human ENSC from routine mucosal biopsies, at endoscopy, providing an accessible and routinely practiced method for harvesting autologous ENSC.[24]

Transplantation of Enteric Neural Stem Cells

A number of preliminary and preparatory studies have established the potential for *in vivo* transplantation of ENSC in wild-type colonic segments as a proof-of-principle. *In vivo* transplantation of ENSC (both embryonic and postnatal) sourced from various transgenic reporter models has been shown to lead to the engraftment of donor-derived

cells within recipient colonic *muscularis*.[23,26] Comparative studies have shown that, transplanted ENSC can generate enteric neurons in transplanted colonic tissues at a greater efficiency than CNS derived neural stem cells.[27] These studies additionally demonstrate that ENSC-derived neurons adopt the appropriate localization within the gut *muscularis,* and can give rise to various enteric neurons including the main excitatory (ChAT, VAChT, Calretinin and Calbindin) and relaxatory (nNOS and VIP) neuronal subtypes.[23,26] Immunohistochemical analysis, in the wildtype colon, has also suggested a close anatomical link between transplanted neuronal networks and the endogenous ENS suggesting possible functional integration of donor derived neurons.

Physiological studies of both mouse and human transplanted ENSC-derived neurons have shown the functional integration of individual neurons and/or multiple neurons within the transplanted neural network after *in vivo* transplantation.[22,23,26] These functional studies critically demonstrate that transplanted ENSC-derived neurons integrate with the endogenous circuitry post-transplantation. Furthermore, stimulation of donor ENSC, expressing an optogenetic reporter, has recently been shown to elicit excitatory and inhibitory junction potentials in recipient colonic muscle cells demonstrating the ability of transplanted ENSC-derived neurons to integrate within the gut neuromusculature and mediate motor control.[28] These fundamental preclinical transplantation studies, in wildtype models, provide proof-of-principle data regarding the potential of ENSC transplantation as a possible therapeutic application. However, as the majority of pediatric motility disorders present with neuropathic loss or disruption, further studies were required to demonstrate the potential of stem cell-based strategies to replace lost neurons and rescue functional behavior in models of gut pathophysiology.

Preliminary studies of the effects of ENSC transplantation in "diseased" settings have utilized a range of model systems including aneural or chemically ablated gut segments, and neuropathic animal models. Using these models, murine and human ENSCs have been shown to engraft within aneural chick gut segments or aganglionic gut segments *ex vivo* and within chemically ablated mouse gut after *in vivo* transplantation. [24],[29],[26,30],[31] Similarly, it has been shown that $p75^{+}$ ENSC can be isolated from ganglionated human HSCR colon and that after expansion in culture, these autologous ENSCs could integrate and form neurons in aneuronal sections resected from the same patient.[32] Critically this study demonstrates that an autologous human cell replacement strategy based on ENSC isolation is possible in an *ex vivo* setting.

In vivo transplantation of murine derived ENSCs within aganglionic models has been shown to lead to successful integration and appropriate differentiation to ENS lineages providing further evidence that donor ENSCs survive within aganglionic gut segments. [33],[34] Furthermore, unsorted ENSCs harvested from ganglionated human HSCR bowel has been shown to colonize aganglionic ($Ednrb^{-/-}$) colonic segments after *in vivo* transplantation giving rise to both neurons and glia.[29]

Unfortunately, such *in vivo* transplantation studies have, to date, been hampered by short survival times of aganglionic transgenic mouse lines, which has essentially precluded in-depth studies to determine the degree of functional rescue, which is imparted by the development of donor ENSC-derived neurons. Recent *in vivo* transplantation studies in less severe phenotypes such as the neuronal nitric oxide knockout ($nNOS^{-/-}$) mouse model, which displays slow colonic transit,[35] have shown that transplantation of ENSCs is able to lead to the development of $nNOS^{+}$ neurons and the restoration of nitrergic responses in the distal bowel.[36] Moreover, ENSC

transplantation within this model led to non cell-autonomous effects increases in interstitial cells of Cajal (ICC) numbers and rescue of colonic motility, providing the first direct evidence that *in vivo* ENSC transplantation can restore function, in a pathophysiological disease model.

Transplantation of Pluripotent Stem Cell Derived Cells

While these studies demonstrate the potential of an autologous ENSC-based therapy for the treatment of neuropathic motility disorders, recent advances in the manipulation of pluripotent cell sources have led to the exciting prospect of pluripotent cells for the treatment motility disorders.

Similarly, due to the well characterized neuropathic models of dysmotility, significant early investigations have focused on the derivation of nervous system cell types from pluripotent sources as a treatment strategy. *In vivo* transplantation of neural stem cells derived from amniotic fluid has been shown to lead to increased improvement in colonic transit as assessed by bead expulsion *ex vivo*.[37] Moreover, transplantation of ENCC from human pluripotent stem cells, including both human ES and iPSCs has been recently shown rescue a Hirschsprung phenotype with 100% survival of *Ednrb*$^{s-l/s-l}$ (SSL/LEJ) mice demonstrating the potential use of pluripotent stem cell derived neurons in the treatment of neuropathic motility disorders.[38] However, as several questions remain as to the safety of pluripotent cell sources for therapeutic treatment it will be necessary to fully characterize the fate of transplanted pluripotent derived-donor cells and the mechanisms by which they appear to rescue diseased gut.

Pharmacological modulation of stem cells for the treatment of severe motility disorders

Interestingly, studies targeting pluripotent stem cells as potential therapeutic tools have provided key insights as to the ability to pharmacologically modulate cell fate and behavior in order to maximize therapeutic impact. Thorough molecular characterization studies of ENCC-derived from human ES and iPSC sources have demonstrated the ability to drive pluripotent stem cells towards an ENCC fate using various pharmacological protocols.[38,39] Further pharmacological targeting has been shown to promote derivation of terminal neuronal subtypes from pluripotent sources including nNOS, ChAT, calretinin, tyrosine hydroxylase VIP and 5-HT neurons both *in vitro* and in *ex vivo* culture conditions.[38,40-42] Furthermore, the vast numbers of ENCC which can be derived from pluripotent stem cell sources provide an excellent model for drug discovery. Using a high throughput approach, pluripotent stem cells have been used to model HSCR to serve as a screening platform for molecules which may modulate the migratory behavior of ENCC, *in vitro*. Interestingly, pretreatment with validated compounds, such as Pepstatin A, Endothelin 3 or EDNRB inhibitor (BQ-778) where found to modify migratory behavior.[38] Similarly, the behavior of autologously derived ENSC, including migratory and neurogenic potential, has been found to be enhanced via exposure to GDNF both *in vitro* and after *in vivo* transplantation, providing further evidence that pre-transplantation pharmaceutical modulation of ENSC or pluripotent stem cell-derived ENS cells may be possible as a therapeutic strategy.[43],[44]

Conclusions

Recent preclinical investigations have provided significant steps towards the application of a stem cell-based therapy for the treatment of severe pediatric motility disorders. Such studies have critically shown significant progress in the ability to isolate and manipulate stem cells for the treatment of enteric

neuropathies. However, in order to transition to first-in-human studies an improved understanding and enhanced diagnostics of gut motility disorders is required, in terms of cellular or functional pathology, before application of stem cell treatment can be applied. For example, in disease states where enteric neuropathy is immune or virus mediated a stem cell-based transplantation strategy may not be beneficial as any transplanted cells may be themselves targeted by the underlying disease process. Furthermore, where enteric neuropathy is driven by genetic mutation autologous transplantation without genetic manipulation would result in the transplantation of "defective" cells which may not provide a therapeutic benefit. In such cases enhanced understanding of the causative features of individual disease mechanisms combined with advances in gene therapy may provide avenues to provide a "personalized" stem cell transplantation strategy to overcome such complications.

To this end, researchers in the field have recently compiled a key white paper summarizing the key methodologies and strategies as well as the obstacles that must be overcome in order to progress from successful preclinical studies in animal models to ENS stem cell therapies in the clinic.[45]

Moreover, despite considerable strides in the application of a stem cell treatment for neuropathic motility disorders, little work has been provided to investigate applications for myopathic or combined neuropathic/myopathic disorders. Hence, significant further work will be required in order to demonstrate the ability to treat these challenging diseases with stem cell therapeutics.

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1. Csendes A, Smok G, Braghetto I, Gonzalez P, Henriquez A, Csendes P, Pizurno D: **Histological studies of auerbach's plexuses of the oesophagus, stomach, jejunum, and colon in patients with achalasia of the oesophagus: Correlation with gastric acid secretion, presence of parietal cells and gastric emptying of solids.** *Gut* (1992) **33**(2):150-154.

2. Goldblum JR, Rice TW, Richter JE: **Histopathologic features in esophagomyotomy specimens from patients with achalasia.** *Gastroenterology* (1996) **111**(3):648-654.

3. Harberson J, Thomas RM, Harbison SP, Parkman HP: **Gastric neuromuscular pathology in gastroparesis: Analysis of full-thickness antral biopsies.** *Dig Dis Sci* (2010) **55**(2):359-370.

4. Hasler WL: **Gastroparesis: Pathogenesis, diagnosis and management.** *Nat Rev Gastroenterol Hepatol* (2011) **8**(8):438-453.

5. Thapar N, Saliakellis E, Benninga MA, Borrelli O, Curry J, Faure C, De Giorgio R, Gupte G, Knowles CH, Staiano A, Vandenplas Y *et al*: **Paediatric intestinal pseudo-obstruction: Evidence and consensus-based recommendations from an espghan-led expert group.** *J Pediatr Gastroenterol Nutr* (2018) **66**(6):991-1019.

6. Bassotti G, Villanacci V, Cathomas G, Maurer CA, Fisogni S, Cadei M, Baron L, Morelli A, Valloncini E, Salerni B: **Enteric neuropathology of the terminal ileum in patients with intractable slow-transit constipation.** *Hum Pathol* (2006) **37**(10):1252-1258.

7. Giorgio V, Borrelli O, Smith VV, Rampling D, Koglmeier J, Shah N, Thapar N, Curry J, Lindley KJ: **High-resolution colonic manometry accurately predicts colonic neuromuscular pathological phenotype in pediatric slow transit constipation.** *Neurogastroenterol Motil* (2013) **25**(1):70-78

8. Rao SS, Rattanakovit K, Patcharatrakul T: **Diagnosis and management of chronic constipation in adults.** *Nat Rev Gastroenterol Hepatol* (2016) **13**(5):295-305.

9. Heanue TA, Pachnis V: **Enteric nervous system development and hirschsprung's disease: Advances in genetic and stem cell studies.** *Nat Rev Neurosci* (2007) **8**(6):466-479.

10. Amiel J, Sproat-Emison E, Garcia-Barcelo M, Lantieri F, Burzynski G, Borrego S, Pelet A, Arnold S, Miao X, Griseri P, Brooks AS *et al*: **Hirschsprung disease, associated syndromes and genetics: A review.** *J Med Genet* (2008) **45**(1):1-14.

11. Anderson RB, Newgreen DF, Young HM: **Neural crest and the development of the enteric nervous system.** *Adv Exp Med Biol* (2006) **589**:181-196.

12. Wallace AS, Burns AJ: **Development of the enteric nervous system, smooth muscle and interstitial cells of cajal in the human gastrointestinal tract.** *Cell Tissue Res* (2005) **319**(3):367-382.

13. Young HM, Hearn CJ, Ciampoli D, Southwell BR, Brunet JF, Newgreen DF: **A single rostrocaudal colonization of the rodent intestine by enteric neuron precursors is revealed by the expression of phox2b, ret, and p75 and by explants grown under the kidney capsule or in organ culture.** *Dev Biol* (1998) **202**(1):67-84.

14. Bradnock TJ, Knight M, Kenny S, Nair M, Walker GM, British Association of Paediatric Surgeons Congenital Anomalies Surveillance S: **Hirschsprung's disease in the uk and ireland: Incidence and anomalies.** *Arch Dis Child* (2017) **102**(8):722-727.

15. Furness JB, Callaghan BP, Rivera LR, Cho HJ: **The enteric nervous system and gastrointestinal innervation: Integrated local and central control.** *Adv Exp Med Biol* (2014) **817**: 39-71.

16. Bondurand N, Natarajan D, Barlow A, Thapar N, Pachnis V: **Maintenance of mammalian enteric nervous system progenitors by sox10 and endothelin 3 signalling.** *Development* (2006) **133**(10):2075-2086.

17. Gianino S, Grider JR, Cresswell J, Enomoto H, Heuckeroth RO: **Gdnf availability determines enteric neuron number by controlling precursor proliferation.** *Development* (2003) **130**(10):2187-2198.

18. Young HM, Turner KN, Bergner AJ: **The location and phenotype of proliferating neural-crest-derived cells in the developing mouse gut.** *Cell Tissue Res* (2005) **320**(1):1-9.

19. Kruger GM, Mosher JT, Bixby S, Joseph N, Iwashita T, Morrison SJ: **Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness.** *Neuron* (2002) **35**(4):657-669.

20. Natarajan D, Grigoriou M, Marcos-Gutierrez CV, Atkins C, Pachnis V: **Multipotential progenitors of the mammalian enteric nervous system capable of colonising aganglionic bowel in organ culture.** *Development* (1999) **126**(1):157-168.

21. Bondurand N, Natarajan D, Thapar N, Atkins C, Pachnis V: **Neuron and glia generating progenitors of the mammalian enteric nervous system isolated from foetal and postnatal gut cultures.** *Development* (2003) **130**(25):6387-6400.

22. Cooper JE, Natarajan D, McCann CJ, Choudhury S, Godwin H, Burns AJ, Thapar N: **In vivo transplantation of fetal human gut-derived enteric neural crest cells.** *Neurogastroenterol Motil* (2017) **29**(1):e12900

23. Hotta R, Stamp LA, Foong JP, McConnell SN, Bergner AJ, Anderson RB, Enomoto H, Newgreen DF, Obermayr F, Furness JB, Young HM: **Transplanted progenitors generate functional enteric neurons in the postnatal colon.** *J Clin Invest* (2013) **123**(3):1182-1191.

24. Metzger M, Caldwell C, Barlow AJ, Burns AJ, Thapar N: **Enteric nervous system stem cells derived from human gut mucosa for the treatment of aganglionic gut disorders.** *Gastroenterology* (2009) **136**(7):2214-2225.

25. Almond S, Lindley RM, Kenny SE, Connell MG, Edgar DH: **Characterisation and transplantation of enteric nervous system progenitor cells.** *Gut* (2007) **56**(4):489-496.

26. Cooper JE, McCann CJ, Natarajan D, Choudhury S, Boesmans W, Delalande JM, Vanden Berghe P, Burns AJ, Thapar N: **In vivo transplantation of enteric neural crest cells into mouse gut; engraftment, functional integration and long-term safety.** *PLoS One* (2016) **11**(1):e0147989.

27. Findlay Q, Yap KK, Bergner AJ, Young HM, Stamp LA: **Enteric neural progenitors are more efficient than brain-derived progenitors at generating neurons in the colon.** *Am J Physiol Gastrointest Liver Physiol* (2014) **307**(7):G741-748.

28. Stamp LA, Gwynne RM, Foong JPP, Lomax AE, Hao MM, Kaplan DI, Reid CA, Petrou S, Allen AM, Bornstein JC, Young HM: **Optogenetic demonstration of functional innervation of mouse colon by neurons derived from transplanted neural cells.** *Gastroenterology* (2017) **152**(6):1407-1418.

29. Cheng LS, Hotta R, Graham HK, Belkind-Gerson J, Nagy N, Goldstein AM: **Postnatal human enteric neuronal progenitors can migrate, differentiate, and proliferate in embryonic and postnatal aganglionic gut environments.** *Pediatr Res* (2017) **81**(5):838-846.

30. Hotta R, Cheng LS, Graham HK, Pan W, Nagy N, Belkind-Gerson J, Goldstein AM: **Isogenic enteric neural progenitor cells can replace missing neurons and glia in mice with hirschsprung disease.** *Neurogastroenterol Motil* (2016) **28**(4):498-512.

31. Hetz S, Acikgoez A, Voss U, Nieber K, Holland H, Hegewald C, Till H, Metzger R, Metzger M: **In vivo transplantation of neurosphere-like bodies derived from the human postnatal and adult enteric nervous system: A pilot study.** *PLoS One* (2014) **9**(4):e93605.

32. Rollo BN, Zhang D, Stamp LA, Menheniott TR, Stathopoulos L, Denham M, Dottori M, King SK, Hutson JM, Newgreen DF: **Enteric neural cells from**

hirschsprung disease patients form ganglia in autologous aneuronal colon. *Cell Mol Gastroenterol Hepatol* (2016) **2**(1):92-109.

33. Tsai YH, Murakami N, Gariepy CE: **Postnatal intestinal engraftment of prospectively selected enteric neural crest stem cells in a rat model of hirschsprung disease.** *Neurogastroenterol Motil* (2011) **23**(4):362-369.

34. Cheng LS, Hotta R, Graham HK, Nagy N, Goldstein AM, Belkind-Gerson J: **Endoscopic delivery of enteric neural stem cells to treat hirschsprung disease.** *Neurogastroenterol Motil* (2015) **27**(10):1509-1514.

35. Dickson EJ, Heredia DJ, McCann CJ, Hennig GW, Smith TK: **The mechanisms underlying the generation of the colonic migrating motor complex in both wild-type and nnos knockout mice.** *Am J Physiol Gastrointest Liver Physiol* (2010) **298**(2):G222-232.

36. McCann CJ, Cooper JE, Natarajan D, Jevans B, Burnett LE, Burns AJ, Thapar N: **Transplantation of enteric nervous system stem cells rescues nitric oxide synthase deficient mouse colon.** *Nat Commun* (2017) **8**: 15937.

37. Zhou Y, Besner G: **Transplantation of amniotic fluid-derived neural stem cells as a potential novel therapy for hirschsprung's disease.** *J Pediatr Surg* (2016) **51**(1):87-91.

38. Fattahi F, Steinbeck JA, Kriks S, Tchieu J, Zimmer B, Kishinevsky S, Zeltner N, Mica Y, El-Nachef W, Zhao H, de Stanchina E *et al*: **Deriving human ens lineages for cell therapy and drug discovery in hirschsprung disease.** *Nature* (2016) **531**(7592):105-109.

39. Hackland JOS, Frith TJR, Thompson O, Marin Navarro A, Garcia-Castro MI, Unger C, Andrews PW: **Top-down inhibition of bmp signaling enables robust induction of hpscs into neural crest in fully defined, xeno-free conditions.** *Stem Cell Reports* (2017) **9**(4):1043-1052.

40. Li W, Huang L, Zeng J, Lin W, Li K, Sun J, Huang W, Chen J, Wang G, Ke Q, Duan J *et al*: **Characterization and transplantation of enteric neural crest cells from human induced pluripotent stem cells.** *Mol Psychiatry* (2016).

41. Schlieve CR, Fowler KL, Thornton M, Huang S, Hajjali I, Hou X, Grubbs B, Spence JR, Grikscheit TC: **Neural crest cell implantation restores enteric nervous system function and alters the gastrointestinal transcriptome in human tissue-engineered small intestine.** *Stem Cell Reports* (2017) **9**(3):883-896.

42. Workman MJ, Mahe MM, Trisno S, Poling HM, Watson CL, Sundaram N, Chang CF, Schiesser J, Aubert P, Stanley EG, Elefanty AG *et al*: **Engineered**

human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. *Nat Med* (2017) **23**(1):49-59.

43. McKeown SJ, Mohsenipour M, Bergner AJ, Young HM, Stamp LA: **Exposure to gdnf enhances the ability of enteric neural progenitors to generate an enteric nervous system.** *Stem Cell Reports* (2017) **8**(2):476-488.

44. Cheng LS, Graham HK, Pan WH, Nagy N, Carreon-Rodriguez A, Goldstein AM, Hotta R: **Optimizing neurogenic potential of enteric neurospheres for treatment of neurointestinal diseases.** *J Surg Res* (2016) **206**(2):451-459.

45. Burns AJ, Goldstein AM, Newgreen DF, Stamp L, Schafer KH, Metzger M, Hotta R, Young HM, Andrews PW, Thapar N, Belkind-Gerson J *et al*: **White paper on guidelines concerning enteric nervous system stem cell therapy for enteric neuropathies.** *Dev Biol* (2016) **417**(2):229-251.