# *Introduction to Themed Series on Intestinal Stem Cells and the NIDDK Intestinal Stem Cell Consortium*

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This short piece serves to introduce our themed series entitled, "The Engineered Gut: Use of Stem Cells and Tissue Engineering to Study Physiology and Disease." Given the remarkable progress in our understanding of intestinal stem cells (ISCs) over the last decade, it seems timely to review the topic of ISC in some depth. Indeed, it has been approximately ten years since the first report of Lgr5 as an ISC marker, and the creation of the Intestinal Stem Cell Consortium by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The ISC field and the Intestinal Stem Cell Consortium are moving into the next phase of discovery; consequently it is reasonable to take stock of where we are and touch on the many challenges that await us.

Theories of the origins of intestinal epithelial cells date back to the 1950's and 1960's, with the discovery of DNA and DNA labeling studies in rodents using $^{3}$H-thymidine. Proliferation was found not to be evenly distributed throughout the intestinal epithelium, but largely restricted to the lower three-fourths of the crypt, with a general flow upwards. The initial concept was that if dedicated ISC exist, they would reside at the base of the proliferative column, immediately above the Paneth cells[3]. Detailed studies using autoradiography and electronic microscopy by Cheng and Leblond enumerated the various progenitors, and these investigators eventually came up with the "Unitarian Theory", suggesting that all four main epithelial cell types (columnar, enteroendocrine, goblet and Paneth) are derived from the same precursor[5]. Importantly, they identified undifferentiated (granule free) potential progenitors located in the lower intestinal crypts (at cell positions +1 to +9) that they believed represented stem cells. Interestingly, some of these Crypt Base Columnar (CBC) cells were located below Paneth cells at the +1 position. Around the same time, Potten and Cairns were working on radiation studies in the small intestine, studying the segregation of DNA, and found that radiation induced apoptosis in a number of progenitors, but led to retention of the $^{3}$H-thymidine DNA label for more than 30 days by a subset of cells[11]. They went on to show that these label-retaining cells are rare cells that reside between positions +2 to +10, with a peak at +4 - +5; thus the concept of the label-retaining cell or + 4 cell was developed[12]. As a result, for decades the general thinking was that the stem cell compartment of the intestinal epithelium was achieved by a hierarchical age structure, with rare, slowly dividing radioresistant cells at the top of the hierarchy.

However, our notions were dramatically changed with the discovery by Clevers and Barker in 2007 of the Lgr5 cells[2], abundant in the lower crypts, with more rapid division, consistent with many of the features of the Crypt Base Columnar (CBC) cells first described by Leblond. The Lgr5-CreERT knock-in allele was able to lineage trace all intestinal epithelial lineages in a mosaic fashion over a fairly short time course, consistent with an active stem cell. However, in 2008 Sangiorgi and Capecchi reported the existence of a Bmi1+ population located at the +4 position in the intestinal crypts, that appeared to divide slowly, self-renew, and give rise to all of the differentiated lineages[13]. This reignited the debate regarding CBC versus the +4 ISC, which was later resolved somewhat with the notion of a 2 stem cell model[9].

At this critical launching point for the ISC field, the Intestinal Stem Cell Consortium or ISCC came together. The notion of such a consortium was formulated by the NIDDK during their workshop on Local Influences on Health and Repair of Intestinal Epithelium (March 25-26, 2008). Formally established in 2009, from the beginning the ISCC has functioned as a highly collaborative, interactive team-science initiative, funded by the NIDDK and the National Institute of Allergy and Infectious Diseases (NIAID). The primary initial focus of the ISCC was to advance the understanding of intestinal epithelial stem cell biology during development, homeostasis, regeneration and disease.

In the first phase of the ISCC ('Gen 1', 2009-2014), there were 8 intestinal stem cell research centers (**Fig. 1A**), geographically well distributed, and coordinated by the ISCC Coordinating Center at the City of Hope. A major goal of the consortium has been to accelerate ISC research through collaborations and rapid exchange of information, and to expand the tools available to the research community as a whole. The ISCC initially focused on major questions in stem cell biology, including the role of the CBC versus the +4 ISC, and the possible utility of other stem cell markers, with a major focus on the mouse intestine. The group collaborated on an RNAseq project for different cell subsets, established common FACS methods for sorting intestinal epithelial cells, and developed standardized nomenclature for intestinal in vitro cultures.

During this first Gen1 version of the ISCC, there were several other major advances in the field. First, the technique of growing intestinal organoids from intestinal crypts and even single Lgr5+ cells using 3D-cultures in Matrigel with ENR media were reported by Sato et al[14]. At around the same time, Ootani et al described a technique for long-term culture of both intestinal epithelial and mesenchymal structures using air-liquid interface[10]. Shortly thereafter, the group led by Jim Wells from Cincinnati reported the ability to grow human intestinal organoids (HIOs) from endodermal tissue derived from pluripotent iPS or ES cells. They developed a robust methodology to direct differentiation of human pluripotent stem cells into intestinal tissue in vitro through sequential growth factors that mimic embryonic intestinal development[15]. Further refinements resulted in structures with both crypt-villus epithelium and mesenchyme, able to be engrafted in vivo[18]. Finally, the scientific basis for the observation that R-spondin was a critical factor in the growth of intestinal organoids was clarified with the report by several groups that Lgr5 was a receptor for R-spondin, and also associated with Wnt receptors[7]. Thus, while Wnt molecules possibly derived from Paneth cells were initially considered to be a major niche factor, greater attention turned to R-spondins and their role in regulating ISCs.

Studies by the ISCC and other labs during this first five-year period laid the groundwork for the second phase of the ISCC, so-called 'Gen 2' (2014-2019) (**Fig. 1B**). A number of new centers were added to the ISCC, resulting in a greater focus on human tissues and human intestinal organoids (HIOs), as well as on mitigation of

radiation injury to the intestine. With extensive research and validation of Lgr5 as an active stem cell marker, the initial two-stem cell model evolved into the concept of "plasticity" by reserve stem cells in the intestinal crypts, and multiple populations including enteroendocrine cells were shown to lineage trace in the setting of Lgr5 cell loss or injury [21, 17, 16, 4]. The role of reserve populations was shown to be particularly important and/or clinically relevant under conditions of radiation injury or infection. Increased focus on human tissues resulted in widespread use of the HIO technology by the group, with the remarkable observations that engineered HIOs derived from pluripotent stem cells are able to develop a functional enteric nervous system[19]. An additional major accomplishment by the group was the use of novel gain-of-function and loss-of-function technologies to make the remarkable insight that R-spondin and Wnt ligands are not identical in their activity as previously assumed, but rather that R-spondin is in fact the major driver of stem cell self-renewal[22]. As a next step, given the growing data that cell types beyond the Paneth cell can contribute to ISC function and behavior, the group has begun additional collaborative studies to define the role of stromal cells that constitute the ISC niche, with a particular focus on mesenchymal cells expressing Foxl1[1], Grem1[20] and PDGFRα[8]. The group also has shifted into studying the importance of matrix contributions to stem cell behavior[6].

As the ISCC looks to 2019 and beyond, it is clear that the ultimate vision is *to develop novel therapies targeting intestinal stem cells and their supportive niche to regenerate and rebuild the human intestine.* Thus, the consortium is establishing new priorities, and increasingly seeking new ways to translate discoveries in the stem cell field to the bedside. Many of these new priorities are highlighted in this "Themed Series" on intestinal stem cells. This series consists of 9 mini-reviews (**Table 1**) that cover many active and emerging areas of research on ISCs, most of which involve ISCC researchers and authors.

The work by the ISCC continues to be based on a solid foundation of intestinal stem cell biology. In the first several themed reviews, our authors discuss a "Comparison of Mouse and Fly ISCs", highlighting the tremendous insights in ISC biology that derive from research in a simpler invertebrate species, *Drosophila melanogaster*. The second themed review discusses the diversity of ISCs, which includes more active ISCs (e.g. Lgr5+ cells) as well as the more quiescent and/or reserve population. Much of the past work on ISC function and lineage relationships comes from genetic lineage tracing using inducible Cre-drivers; however investigators have increasingly recognized the limitation of models employing tamoxifen-induced Cre. Further, bulk RNAseq analysis often fails to take into account the complexity and heterogeneity of intestinal tissue. Thus, in our third themed review, our authors explore the utility and contribution of single cell approaches (i.e. single cell RNAseq) to our understanding of the intestinal epithelium and lineage relationships. In our fourth article, we move from a solitary focus on the epithelium to the surrounding mesenchyme, and our authors discuss the concept of the stem cell niche as it applies to the mammalian intestine.

In our fifth themed review, our authors discuss the role of epigenetics in the regulation of intestinal stem cells. Insights into pluripotent stem cells and genetic reprogramming have clarified the tremendous importance of epigenetic regulation in maintaining the stem cell state, while also providing for the potential for plasticity by intestinal crypt cells. In our sixth themed review, we describe the development of HIOs from pluripotent stem cells by many of the original investigators in this field, and their potential for use in translational research. In our seventh review, investigators discuss how intestinal organoids can be used in vitro to study the regenerative responses to diverse forms of intestinal damage, thus providing insights into newer approaches for treating or mitigating such injury. In our eighth themed review, our teams of biologists and bio-engineers comment on the latest approaches and strategies for regenerative medicine, and how they could be applied to the intestinal epithelium. Finally, in our last review, our authors discuss a possible roadmap for bringing ISC technology to the bedside, and how bioengineered organs or cells can potentially achieve FDA approval for the treatment of human disease.

## Table 1:

## Articles in the Themed Series on Intestinal Stem Cells

| Topic |
|---|
| Introduction to ISCs and the Consortium |
| Comparison of Mouse and Fly ISCs |
| Diversity of Stem Cells: Active and Quiescent Populations |
| Single-cell Approaches to Studying the Intestinal Epithelium and Lineage Relationships |
| Stem Cell Niche is Defined by the Signals that Maintain and Control the Activity of the Stem Cells |
| Epigenetic Regulation of ISCs |
| Use of Organoids to Study Regenerative Responses to Intestinal Damage |
| Development of HIOs |
| Bioengineering Strategies for Regenerative Medicine |
| Translational/Clinical: Working towards FDA Approval |

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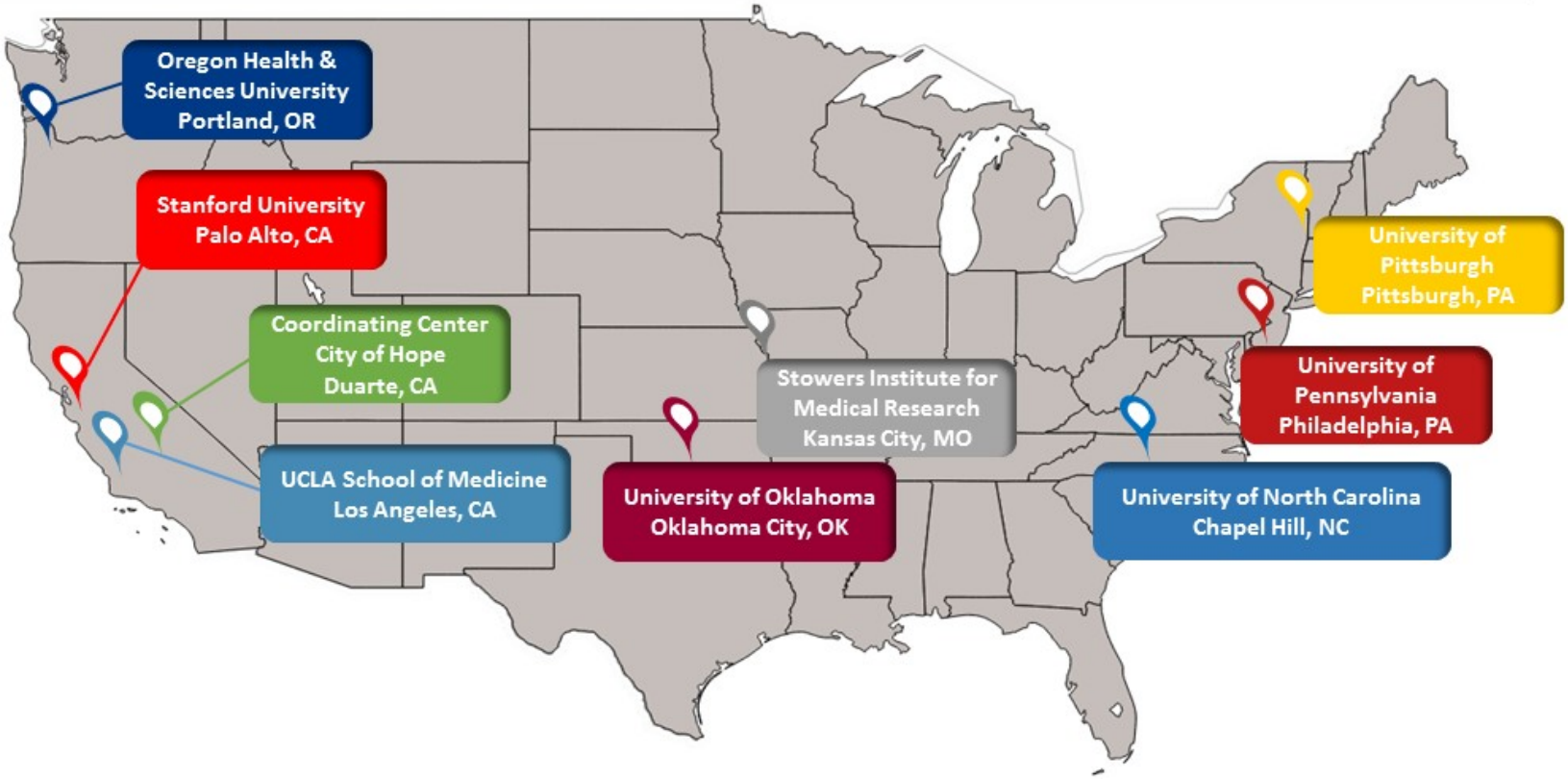
**Figure Legend**

Figure 1: Figures 1A and 1B: ISCC Institutions during “Gen 1” and “Gen 2

# MAP of ISCC INSTITUTIONS
# 'GEN 1' 2009-2014

1A



# MAP of ISCC INSTITUTIONS
# 'GEN 2' 2009-2014

1B