

**PROCEEDS OF THE SIGNATURE SERIES EVENT OF THE INTERNATIONAL
SOCIETY FOR CELLULAR THERAPY ON “ADVANCEMENTS IN CELLULAR
THERAPIES AND REGENERATIVE MEDICINE IN DIGESTIVE DISEASES”**

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Running Title: Proceeds of the First Signature Event on Digestive Diseases

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ABSTRACT

A summary of the First Signature Series Event on “Advancements in Cellular Therapies and Regenerative Medicine for Digestive Diseases” that was held on May 3rd, 2017 in London (United Kingdom) is presented. Twelve speakers from three continents covered major topics in the areas of cellular therapy and regenerative medicine applied to liver and gastrointestinal medicine as well as to diabetes mellitus. Highlights from their presentations together with an overview of the global impact of digestive diseases and a proposal for a shared online collection and data monitoring platform tool are included in this proceedings. Although growing evidences demonstrate the feasibility and the safety of exploiting cell-based technologies for the treatment of digestive diseases, regulatory and methodological obstacles will need to be overcome prior to the successful implementation of these new attractive therapeutic strategies to the clinic.

Key Words. Digestive diseases • Immune tolerance • Mesenchymal stromal cells • Tissue regeneration.

ABBREVIATIONS. CD: Crohn's disease; ESC: embryonic stem cell; iPSC: induced pluripotent stem cells; MSC: mesenchymal stromal cell; RM: Regenerative Medicine.

INTRODUCTION

The Signature Series Event on “Advancements in Cellular Therapies and Regenerative Medicine for Digestive Diseases” was held as a pre-meeting of the 25th International Society for Cellular Therapy annual congress in London, United Kingdom, May 3rd 2017. This was the first workshop organized under the auspices of the Society fully dedicated to the application of stem cell and tissue engineering technologies to digestive diseases. The symposium convened opinion leaders from three continents and seven countries, with a common interest in developing cell therapy platforms and regenerative medicine (RM) technologies for clinical application in liver diseases and diabetes, as well as illnesses affecting the digestive tube. The event represented an opportunity to share knowledge and experience, promote understanding of the supporting technologies and of the potential target populations, with the overarching goal of enabling the clinical implementation of promising cell- and RM-based therapies. This manuscript will succinctly report the topics that were discussed and the debate generated thereof.

BACKGROUND

Prof. Carolina Ciacci: The Global Impact of Digestive Diseases

The Signature Series Event was opened by Professor Ciacci who contextualized the global impact of digestive diseases. According to <http://vizhub.healthdata.org>, in the last 25 years human life expectancy has increased by more than 5 years worldwide, mainly due to a significant decrease of mortality for cardiovascular diseases and cancer (Figure 1, upper panel). By contrast, improvement of management of digestive diseases, including colorectal cancer, accounts for an average increase of life expectancy from 1990 to 2015 of only 0.1 year. In addition, the prevalence of a number of disorders affecting the digestive system, mostly those characterized by a chronic inflammatory process, has undergone a significant increase not only because a growing number of patients are now correctly diagnosed, but also

by virtue of a real spread in the general population [1]. Among the illnesses showing an increased frequency are inflammatory bowel diseases, including Crohn's disease (CD) and ulcerative colitis [2] and other autoimmune conditions affecting the intestine, such as celiac disease [3], the liver, such as autoimmune hepatitis [4], and the pancreas with autoimmune pancreatitis [5] and type I diabetes [6]. These disorders are expected to reach epidemic levels in the near future and, as their peak of incidence is in the young adult age with consequent impairment of patient's work productivity, the consequent social and economic impact may be disastrous. Moreover, recent pooled data demonstrate that the mortality rate of diseases like inflammatory bowel disease is on the rise [7] (Figure 1, lower panel). Specifically, Jess et al. found that from 1982 to 2010, mortality was increased by approximately 10% in patients with ulcerative colitis and by 50% among those suffering from CD compared with the general population [7]. Altogether, this information reveals that currently available therapeutic options are unsatisfactory and that, sadly, a definitive cure is still a too distant horizon. The complex – and in most cases, still unclear – interplay between genetic, epigenetic and environmental factors that underlies and triggers the majority of these illnesses represents the biggest challenge on the way towards the definitive cure [8]. At the same time, *vis a' vis* of inadequate treatment tools, common sense suggests that this apparently inextricable complexity may not be resolved with a “magic bullet” like a pill or an intravenous drug; rather, alternative cellular or RM-based therapeutic tools leveraging the ability of cells to repair and regenerate dysfunctional tissues with the ultimate goal to restore function should be tested. Notwithstanding the therapeutic potential, as of October 31th 2017, among all the clinical trials registered on the National Institutes of Health Clinical Trial Database (<https://clinicaltrials.gov>), only a small proportion was aimed at applying mesenchymal stromal cells (MSCs) and RM in digestive diseases. Specifically, those using MSCs were 55 for liver diseases, 77 for gastrointestinal diseases, 4 for pancreas diseases, and 58 for diabetes, whereas those applying RM were 6 for liver diseases, 11 for gastrointestinal diseases, 1 for

pancreas diseases, and 15 for diabetes. Therefore, the aim of this workshop was to bring together the leading experts in clinical applications of MSCs and RM in digestive diseases in order to overcome this view by sharing of ideas and methodologies and by identifying a number of key challenges, whose solution will accelerate the advent of cellular therapy and tissue engineering as a bedside reality. A synopsis of the presentations followed by a compilation of current key questions and recommendations are listed below. The full program of the meeting and list of participants is available in the online Supplementary Data of this article.

SESSION I: LIVER DISEASES

In the liver, a number of triggers, such as obesity, alcohol, viruses, drugs and chemicals result in cirrhosis [9], one of the most prevalent conditions worldwide [1]. Currently, liver transplantation is the only therapeutic option for end stage liver disease, although application is limited by inadequate organ supply, high morbidity and costs. As a consequence of the progressively increasing gap between available organs and patients in need for a new liver, the mortality rate for patients on the waiting list has recently achieved a dramatic 20%, which represents a urgent call for action [10]. In this scenario, new treatment strategies aimed at either replacing dysfunctional livers or preventing progression of chronic liver disease towards its end stage are eagerly awaited.

Prof. Michael Ott: Pluripotent stem cells for liver disease

Prof. Ott addressed the role that pluripotent stem cells may have as cell source to treat liver diseases. The need for reliable therapeutic alternatives to orthotopic liver transplantation has driven global efforts to establish cell therapy procedures able to provide stable and reliable sources of functional hepatocytes or of hepatocyte-like cells to be used for repopulation of damaged liver parenchyma. Direct induction of hepatocytes from fibroblasts holds potential

as a strategy for RM. Capitalizing on the original studies from Yamanaka's group on the generation of induced pluripotent stem cells (iPSC) from somatic cells [11], recent studies have shown lineage reprogramming of human fibroblasts into hepatocytes by ectopic expression of transcription factors *FOXA3*, *HNF1A*, and *HNF4A* or the combination of *HNF1A*, *HNF4A*, and *HNF6* together with the maturation factors *ATF5*, *PROX1*, and *CEBPA* [12,13]. In London, Prof. Ott presented landmark results from a series of elegant experiments performed in collaboration with Dr. Sharma showing *in vivo* generation of induced hepatocytes using transcription factor induction and genetic fate tracing in mouse models of chronic liver disease [14]. Transcription factors essential for critical hepatocyte function(s), such as albumin secretion and CYP3A activity, were identified using a loss of function screen in hepatocytes. In response to persistent inflammatory injury large numbers of stellate cells undergo "activation" to pro-fibrogenic myofibroblasts that accumulate in the liver and promote fibrosis. To demonstrate the potential therapeutic effects of direct reprogramming in chronic liver disease, selected transcription factors *FOXA3*, *GATA4*, *HNF1A*, and *HNF4A* required for maintenance of the hepatocyte phenotype were co-overexpressed using a polycistronic lentiviral vector. By day 14, induced hepatocytes had acquired an epithelial-like morphology, a transcriptional profile consistent, albeit not identical, to primary hepatocytes and functional characteristics fundamental to hepatocytes such as cytochrome P450 (*CYP1A2* and *3A*) activity and the ability to store glycogen, to uptake low density lipoprotein, and to secrete albumin. To demonstrate myofibroblasts could be forced to differentiate into induced hepatocytes *in vivo*, the group developed a method of labelling induced hepatocytes derived from non-parenchymal cells by using the *Gt(ROSA)26Sor^{tm4(CTB-tdTomato,-EGFP)Luo}/J* mouse. The *ROSA^{mT/mG}* is a cell membrane-targeted, two-color fluorescent Cre-reporter allele. Prior to Cre-recombination, cell membrane-localized tdTomato fluorescence expression is widespread in cells/tissues. Cre-recombinase expressing cells (and future cell lineages derived from these cells) have cell membrane-localized EGFP fluorescence expression replacing the red one.

Adult mT/mG mice were injected with an adeno-associated virus expressing Cre-recombinase under the transcriptional control of the liver-specific transthyretin promoter. As a result, membranous EGFP fluorescence in hepatocytes and tdTomato membranous fluorescence in non-parenchymal cells of the liver were expressed four weeks after AAV-transthyretin-Cre injection. To demonstrate the potential for *in situ*-hepatocyte regeneration, extensive liver fibrosis was induced by carbon tetrachloride treatment twice a week from day 30 to 90. Forced ectopic expression of the 4 transcription factors to induce hepatocyte generation was performed at day 97. To maximize transduction of the 4 transcription factors essential for induced hepatocyte generation *in vivo*, an adenovirus vector overexpressing the 4 transcription factors of interest with modified tropism was developed. By coupling viral knobs with a peptide fragment of the nerve growth factor, the adenovirus vector was directed with specificity and high-affinity for the p75 neurotrophin receptor present on hepatic stellate cells and myofibroblasts [15]. Co-staining of EGFP with cell-specific markers confirmed preferential transduction of approximately 30% of stellate cells in normal and 20% of myofibroblasts in carbon tetrachloride-induced fibrotic livers of BALB/c mice. The percentage of *in vivo* generated induced hepatocytes among the total hepatocyte population ranged from 0.2% to 1.2% following viral transduction, whereas control mice did not show any reprogrammed cells. Importantly, when the adenovirus vector was injected in uninjured mice, no induced hepatocytes were detected. Induced hepatocytes recovered from livers showed stable reprogramming as determined by absence of exogenous transcription factors, normal proliferative capacity following partial hepatectomy, and chromosomal integrity. Functional improvement was evidenced by the ability of recovered induced hepatocytes to secrete albumin, synthesize urea and store glycogen, as well as by the presence of glycerides and lipids and by cytochrome activity. Moreover, *in vivo* reprogramming of myofibroblasts to induced hepatocytes resulted in significant reduction of collagen and hydroxyproline levels, indicating decreased liver fibrosis. This observation highlights the possible collateral benefit

of using myofibroblasts as target cells for direct reprogramming. Direct reprogramming of hepatic myofibroblasts may not only provide induced hepatocytes to repopulate the damaged liver parenchyma and ultimately restore function but, by reducing the number of myofibroblasts or/and by extinguishing the pro-fibrotic triggers, may also contribute to further reversing fibrosis [16].

Dr. Vincenzo Cardinale: Innovative clinical grade cryopreservation and grafting strategies fasten the translation of an effective biliary tree stem cell therapy

Dr. Vincenzo Cardinale presented data from clinical studies that his group at Sapienza University of Rome (Italy) is conducting to prove efficacy of innovative clinical cryopreservation technology and grafting strategies that are expected to fasten the translation of an effective biliary tree stem cell therapy. While several different sources of stem cells including hepatic stem cells, biliary tree stem cells, MSCs, adipose-derived stem cells, umbilical cord cells, amniotic fluid-derived epithelial cells, embryonic stem cells (ESCs) and iPSC have been investigated for their potential as therapy for chronic liver failure, homing of therapeutic cells to the liver remains a challenge [17]. Although the injection into hepatic artery showed the greatest percent of engraftment [18], injection into the liver parenchyma (~10-20%) and into the portal vein (less than 5%) was still consistent with poor engraftment and significant ectopic cell distribution to the vascular beds of other tissues suggesting a high risk of ectopic liver formation [19]. Given that hyaluronic acid is selectively and specifically cleared by the liver and that it has been implicated in various aspects of stem cell therapy optimization [20], Cardinale's team hypothesized that it would not only enhance engraftment but also improve the applicability of human biliary tree stem cells to treat liver cirrhosis [21]. Interestingly, hyaluronic acid coating of human biliary tree stem cells markedly improved viability, colony formation, and population doubling in primary cultures, and resulted in higher expression of integrins that are key players and mediators of cell attachment

to the extracellular matrix. When hyaluronic acid-coated biliary three stem cells were transplanted via the spleen into the liver of immunocompromised mice, the engraftment efficiency increased from 3% of uncoated cells to 11%. Notably, hyaluronic acid-coated human biliary three stem cell transplantation in mice resulted in a 10-fold increase of human albumin gene expression in the liver and in a 2-fold increase of human albumin serum levels with respect to uncoated cells. Moreover, when other organs were sectioned and stained to track the cells in question, only minimal ectopic cell distribution was detected. Furthermore, as one of the major limitations of cellular therapies is the need of their long-term storage, researchers are devising strategies to make this possible. To meet this critical aspect of liver cellular therapies and to optimize their sourcing, Cardinale's group has developed a cryopreservation protocol consisting in the stepwise use of serum-free Kubota's Medium supplemented with 10% dimethyl sulfoxide, 15% human serum albumin and 0.1% hyaluronans [22]. When freshly isolated biliary three stem cells were cultured *in vitro* and compared with their cryopreserved counterparts, no differences were noted in terms of self-replication, stemness traits, and multipotency. Just like freshly isolated cells, cryopreserved cells were able to differentiate into functional hepatocytes, cholangiocytes or pancreatic islets and to yield similar capacity to secrete albumin and glucose-inducible insulin. This technology may be expanded to multiple cell types and promises to facilitate the establishment of cell banks with obvious logistic advantages.

Dr. Debashis Haldar: MSCs for inflammatory liver disease

Dr. Haldar presented a review of the use of MSCs to treat inflammatory liver diseases. Initial observations that bone marrow cells may contribute to hepatic repair and regeneration [23-25] were followed by studies in animal models of chronic liver injury showing variable therapeutic effects [26-28]. While it is well known that MSCs home to areas of acute inflammation, reduce inflammatory damage and oxidative stress, and even contribute to

differentiated epithelium, the impact on prevention of induction and/or progression of fibrosis, however, remains controversial [29]. Along with the anti-inflammatory action of MSCs and various lines of evidence suggesting they may be able to directly exert antifibrotic effects [30], conflicting data paradoxically point to a possible profibrotic role [29]. For instance, MSCs may reduce the *in vitro* production of profibrotic factors and influence macrophages polarization but, in animal models of chronic liver disease, diametrically opposed results have supported both an anti-fibrotic as well as an increase in markers of fibrosis such as collagen deposition. In fact, in an elegant series of gender-mismatched bone marrow reconstitution studies, Russo et al. showed that bone marrow cells contributed to myofibroblasts generation and collagen expression [31]. By using bone marrow cells with a β -gal reporter under the control of a $\alpha 2(I)$ collagen enhancer, this group demonstrated the presence of β -gal-expressing cells around areas of scarring in the liver after 12 weeks of carbon tetrachloride injury and *in situ*-hybridization confirmed collagen expression in bone marrow-derived cells. To determine if the bone marrow-derived cells functionally contributed to increased fibrosis in this model, irradiated mice received bone marrow-derived cells from Col-1a1^{rr} mice, which have a mutated collagenase-resistant collagen, and upon injury wild-type mice developed extensive pericellular fibrosis similar to that seen in Col-1a1^{rr} mice. To determine which cells within the bone marrow were responsible, this group performed new gender-mismatched reconstitution experiments showing that marrow stromal cells, not hematopoietic stem cells, were responsible for bone marrow-derived myofibroblasts in the liver. The same group also published a case series of male patients who had undergone liver transplantations from female donors and who had gone on to require another transplant for cirrhosis [32]. Explanted liver tissues were analyzed for Y chromosome by *in situ* hybridization together with markers for hepatic stellate cells and myofibroblasts, revealing that 14–45% of myofibroblast were indeed of recipient origin. In contrast to these reports implicating bone marrow stromal cells in fibrosis, Miyata et al. provides evidence of a

dominant role for hematopoietic stem cells [33], and, although other groups have not been able to replicate a role for bone marrow-derived stem/stromal cells in fibrosis [34], an important contribution of hepatic stellate cells to fibrosis [35] has emerged. Significant heterogeneity in experimental designs, i.e., approaches to inducing chronic liver disease, timing, route of administration and animal models have precluded an in-depth understanding of potential MSC-mediated mechanisms of action in this condition. Despite mixed results, over ten clinical trials between 2007 and 2014 using either autologous [36] or allogenic [37] MSCs suggest that they are at least safe in chronic liver disease [38,39]. The endpoints of the studies were to evaluate the safety and efficacy of bone marrow and umbilical cord MSC transplantation. The cells were mostly infused intravenously even if two studies reported infusions via the hepatic artery [39,40], and one in the spleen [41]. Moreover, there was a great variation in both the number of cells infused per patient and the frequency of injections among the trials. The results of the studies seemed promising in terms of improvement of liver function and model for end stage liver disease score. This score is based on objective variables (international normalized ratio and serum creatinine, bilirubin, and sodium concentration) and has been validated as a predictor of survival among patients with advanced liver disease [42]. However, for most of the studies discussed by Dr. Halder, data regarding evaluation of liver histology after cell transplantation is lacking, most studies were underpowered to detect significant differences, controls were either lacking or inadequate and the follow-up period was inadequately short. One of the primary challenges remains the inability to track and monitor the transplanted cells and the absence of standardized transplantation protocols. Thus, standardizing and harmonizing protocols that fully agree on the timing of cell injection following the stage of liver fibrosis, number (perhaps type) of cells and administration route would significantly allow for meaningful advances in the field to be achieved. Overall, a large body of literature suggests that MSCs may exert a potent anti-inflammatory effect in the setting of liver diseases, that when exploited in a timely fashion

following acute liver inflammation, may reduce progression to fibrosis. The need for well-controlled randomized clinical trials in this area is paramount to address key questions regarding the potential applicability of this technology as a wide-accepted strategy for the management of acute liver disease.

Dr. Basak Uygun: Bioengineering the liver: future prospects

Dr. Uygun presented the state of the art of liver bioengineering starting by emphasizing that a major issue in manufacturing hepatic grafts for transplantation purpose is the difficulty of producing a three dimensional architecture that mimics the *in vivo* environment. At Harvard Medical School her group [43-45] is using native extracellular matrix-based scaffolds to bioengineering liver grafts. In fact, native extracellular matrix represents a biochemically, geometrically and spatially ideal platform for organ bioengineering and regeneration, because it is biocompatible, has both basic components (proteins and polysaccharides) [46], retains an intact and patent vasculature which – when implanted *in vivo* – sustains physiologic blood pressure, and is able to drive differentiation of progenitor cells into an organ-specific phenotype [47]. When cells are seeded within an intact extracellular matrix scaffold and are allowed to mature into bioreactors, cells attach on, proliferate and show signs of active metabolism and effective function [46]. For these reasons, organ-derived scaffolds have become the scaffolding material of choice in the bioengineering of complex organs [48]. By adapting the perfusion decellularization technique to liver and introducing perfusion recellularization to innovate the process of cell seeding of the extracellular matrix graft, Uygun and coworkers were able to preserve both the liver-specific extracellular matrix and three-dimensional architecture [43]. Most importantly, the decellularized liver matrix retained the underlying matrix of the vascular network, which can be readily connected to the circulation, facilitating rapid oxygen and nutrient delivery after transplantation. Retention of vascular structures allowed for transplantation of partially recellularized liver graft in rats, and

post-transplantation analysis demonstrated preservation of hepatocyte structure and function with minimal signs of ischemic damage. Preliminary recellularization with endothelial cells also indicated attachment and viability within the recellularized matrix *in vitro*. For translation, the major challenge besides the regeneration of the parenchymal compartment – that, as of now, not only has never been reported but its feasibility has been critically questioned [49] – is the scaling up to human compatible models. In that respect, Mazza et al. recently reported on the complete decellularization of whole human liver and lobes to form an extracellular matrix scaffold with a preserved architecture [50]. Decellularized human liver cubic scaffolds were repopulated for up to 21 days using different human cell lines of hepatic stellate cells, hepatocellular carcinoma and hepatoblastoma, with excellent viability, motility and proliferation, and remodeling of the extracellular matrix. The key contribution of Mazza and coworkers is the development of a novel decellularization protocol based on high g-force oscillation leading to the successful removal of immunogenic cellular materials, while maintaining the extracellular matrix protein composition and 3D architecture. This allowed the entire human liver to be decellularized instead of the wedge sections of normal human liver tissue obtained by surgical resections for the development of biological human liver extracellular matrix scaffolds.

SESSION II: INTESTINAL DISEASES

Regarding the intestine, almost all the information available on cell therapies refers to CD, given that ulcerative colitis has a rescue and definitive therapeutic option with proctocolectomy for refractory cases. The goal in the therapeutic management of CD patients relies in the induction and maintenance of remission while achieving mucosal healing, with the ultimate effect of preserving intestinal function [51]. Currently accepted therapies are based on the use of immunomodulators or cytokine-directed immune-neutralizing antibodies in order to modify the natural history of the illness and therefore ameliorating the overall

outcome [52]. Nevertheless, a high proportion of treated patients either does not respond or tolerate these molecules fostering interest towards alternative treatment approaches, in particular cellular therapies based on the use of MSCs.

Dr. Stefania Vetrano: MSCs: the potential to regenerate and restore tolerance in immune-mediated intestinal diseases

Dr. Vetrano provided evidence of the potential of MSCs in inducing regeneration of the intestinal epithelium and restoring self-tolerance in immune-mediated intestinal diseases (Figure 2). After successful preliminary experiments in mouse models of colitis showing the striking ability of MSCs to prevent and improve tissue damage [53,54], a number of open-label phase 1-2 studies were conducted to test the use of autologous or allogeneic systemic infusions of bone marrow- and placenta-derived MSCs for the treatment-resistant CD [55-58]. The results showed this therapeutic approach to be feasible and safe, as well as significantly effective since disease remission was achieved in half of the patients with a follow-up ranging from 6 weeks to 24 months. Notably, the best outcome was obtained when serial infusions were performed [58], thus opening up the question of the half-life or at least the duration of the therapeutic effects of MSCs in this clinical setting, a crucial point in establishing the right schedule of infusions. Importantly, although initially it was believed that MSCs would exert their effects locally after engraftment in the damaged dysfunctional tissue [59], it has now become clear that homing in the gastrointestinal district is not *a sine qua non* condition, because MSCs actually exert their function through the release of paracrine factors [60] in consistence with data from other fields of health sciences [61-64]. Vetrano's group has recently observed that although an intraperitoneal injection of bone marrow-derived MSCs ameliorated the mucosal features and overall outcome in an experimental model of colitis [65], only less than 1% of injected MSCs reached the inflamed colon. Indeed, most of the MSCs remained in the site of injection forming aggregates together with immune cells (i.e.,

macrophages, T- and B-lymphocytes), thus promoting the secretion of immunoregulatory molecules. Interestingly, neither therapeutic effect nor MSC-aggregates were observed when the cells were administered intravenously, thus raising the importance of the route of administration of MSCs in order to exert their beneficial effects. The tumor necrosis factor- α -stimulated gene-6, an anti-inflammatory protein, was found highly increased in sera of mice treated with MSCs within 72 hours from the intraperitoneal injection compared to placebo controls, which correlated with the amelioration of colitis. Moreover, similarly to MSCs, recombinant tumor necrosis factor- α -stimulated gene-6 treatment improved survival rate by reducing both systemic and mucosal levels of inflammatory mediators, neutrophil infiltration, metalloproteinase activity, and enhancing expansion of macrophages and T-cells with a regulatory phenotype. These findings strongly correlate with other reports demonstrating that MSCs modulate inflammatory response both promoting a switch from a T-helper 1 cytotoxic response to a less detrimental T-helper 2 one [66] and recruiting transforming growth factor- β and interleukin-10 producing regulatory T cells in inflamed gut [67], while promoting mucosal healing by stimulating angiogenesis [68]. The evidence that MSCs lacking of tumor necrosis factor- α -stimulated gene-6 fail to exert any therapeutic effect in experimental colitis further corroborates the hypothesis that this molecule is crucial for MSC activities and can be considered a predictive biomarker to assess the response to MSC-based therapy in the treatment of CD. It has also emerged that the paracrine functions of MSCs could be largely mediated by their extracellular vesicles. These are membrane bodies that comprise microvesicles and exosomes [69] containing full bioactive molecules, namely proteins, lipids, mRNA, microRNAs, and nuclei acids [70]. Following receptor-mediated attachment or internalization, target cells eventually undergo a wide variety of epigenetic reprogramming and phenotypic changes [71], which may be exploited for therapeutic purposes. In this regard, the role of extracellular vesicles as conveyors of immune and regenerative capabilities has already been demonstrated in several *in vitro* [72], and *in vivo* experimental models [73-75].

Extracellular vesicles have a series of advantages over their cellular counterpart since they are more stable, induce stronger and reproducible signaling, they are easier to sterilize, store and deliver, and carry no risk of aneuploidy or immune rejection upon allogeneic administration. MSC-derived extracellular vesicles, therefore, may be regarded as an alternative, cell-free therapy for chronic inflammatory conditions [71,76].

Prof. Daniel C. Baumgart: Fistulizing Crohn's disease: can stem cells successfully take on the challenge?

Prof. Baumgart reported the results of a randomized, double-blind, parallel-group, placebo-controlled study conducted in 49 hospitals of seven European countries and Israel from July 6th, 2012, to July 27th, 2015, whereby expanded allogeneic adipose-derived MSCs (Cx601®) were used to treat perianal fistulas complicating CD [77]. These lesions affect up to 28% of patients during the first two decades after diagnosis – particularly those with colonic disease and rectal involvement [78] – and, as they are frequently complex and penetrate into neighboring organs [79], they tend to cause severe morbidity [80] thus dramatically impairing patients' quality of life. Unfortunately, treatment armamentarium remains inadequate. In fact, conventional medical treatment strategies (namely, antibiotics and immunomodulators) are poorly effective, while lesions tend to recur in more than two thirds of patients [78,80]. Overall, only a few patients achieve long-term remission [80] and debilitating surgeries with diverting stomas or proctocolectomy are often the last resort after failure of or intolerance to medical treatment. In this scenario, the need of alternative effective and non-invasive therapies is an urgent matter. Following promising phase 1 and 2 studies (see Table 1), 212 patients suffering from CD and draining complex perianal fistulas who did not respond to conventional or biological treatments, or both, were recruited and randomly assigned to receive either a single local injection of placebo or of an industrial preparation of adipose-derived MSCs (Cx601®) [77]. Cell therapy resulted more effective

than placebo in achieving combined remission (defined as closure of all treated draining external openings, and absence of collections >2 cm of the treated perianal fistulas confirmed by Magnetic Resonance Imaging), since 50% of the MSC-treated patients achieved remission versus 34% in the placebo group at week 24, within a shorter period of time (6.7 instead of 14 weeks). Moreover, MSC-treated patients experienced a lower complication rate as demonstrated by the fact that only 17% of them versus 29% of those enrolled in the placebo group reported treatment-related adverse events (namely, anal abscess and proctalgia). Other than safety and efficacy, it is worth emphasizing that the cell preparation studied offers a ready-to-use cellular suspension that may overcome one of the major hurdles commonly encountered in cellular therapy, namely the lack of standardization in the manufacturing of the cells of interest and the need for a Good Manufacturing Protocol-certified cell factory. However, although the sustainability of the MSC effect at 6-12 months (see Table 1) appears higher than that observed with biological agents that represent the first line treatment for this condition [90], if follow-up is extended to two or five years [91,92], the rate of fistula recurrence increases progressively, thus highlighting the need to repeat the treatment over time.

Prof. Paolo De Coppi: Bioengineering the gut: future prospects of regenerative medicine

Prof. De Coppi updated us on the work done by his team in the field of upper gastrointestinal bioengineering and whose overarching goal is to provide alternative therapies for esophageal atresia. This is a relatively rare congenital defect that affects 1 out of 2,500-4,000 births [93] and comprises a variety of congenital anatomic defects that are caused by an abnormal embryological development. Anatomically, the most frequent defect is a congenital obstruction of the esophagus with interruption of the continuity of the esophageal wall, with or without a concomitant tracheoesophageal fistula. The treatment depends on the severity of the condition. In about 90% of cases, primary anastomosis of the esophagus can be done but

in the most severe cases, where an extremely long gap exists, treatment consists in esophageal replacement by gastric pull-up or transposition of a colonic or jejunum segment.

Unfortunately, these procedures are burdened by high morbidity and both the prognosis and quality of life of affected patients remain largely inferior to the general population. In this setting, an *ad hoc* bioengineered esophageal segment manufactured from patient's own cells may potentially offer a valuable therapeutic alternative. Notably, previous experience with upper airway bioengineering [94,95] has shown feasibility of such an approach, even if safety and long term efficacy still remain to be proven [96]. Work from his team at University College of London (United Kingdom) has shown that hollow organs like the gastrointestinal tract can be successfully decellularized to obtain acellular extracellular matrix scaffolds that may be used as template for the regeneration and bioengineering of the organ of interest [97]. Capitalizing on this successful approach and understanding of the molecular embryology underlying foregut development and the defects associated with esophageal atresia, his group is using a combinational approach whereby acellular extracellular matrix scaffolds obtained from the pig are seeded with patient's derived cells. Investigations have progressed quite successfully to the point that the team has announced that the first clinical trial may start in 2018 following approval from the Medicines and Healthcare Products Regulatory Agency (<http://www.gosh.nhs.uk/research-and-innovation/nih-great-ormond-street-brc/brc-news/researchers-announce-oesophagus-regeneration-trial>).

SESSION III: ENDOCRINE PANCREAS AND TYPE 1 DIABETES

Type 1 diabetes represents a formidable platform for the application of cellular therapies aiming at either replenishing the β -cell compartment or modulating the immune system in order to either arrest the disease or prevent its recurrence after whole pancreas or islet transplantation. The symposium focused on the first topic. In general, the management of diabetes mellitus consist in the combination of a medical – based on oral antidiabetic agents

and insulin injections – and behavioral – dietary restrictions and regular physical activity – approaches. However, although exogenous insulin therapy is effective at preventing acute metabolic decompensation and is life-saving, less than 40% of type 1 diabetic patients achieve recommended therapeutic goals and therefore a large number of diabetic patients –especially those who do not use insulin-pump devices [98] – are inadequately controlled and destined to eventually develop one or more end stage organ complications during their lifetime. In this scenario, β -cell replacement is the only therapy that, at the moment, reliably establishes a long-term stable euglycemic state, however its applicability in the form of either whole pancreas or islet transplantation, has been limited by critical hurdles like shortage of usable pancreases, need for anti-rejection therapy, costs and associated morbidity [99,100]. Moreover, while islet transplantation may appear more appealing due to its lower costs and morbidity when compared with whole pancreas transplantation, so far it has not been able to replicate the same outstanding outcome results of whole pancreas transplantation [101-104]. Because the superior results of the latter, this currently represents the standard of care in β -cell replacement and should be offered to patients with a low surgical risk, while type 1 diabetic patients with a high surgical risk should undergo islet transplantation [104]. Yet, at a recent IPITA-TTS Opinion Leaders Meeting on the Future of β -Cell Replacement, it was felt that islet transplantation will become the preferred therapeutic procedure for β -cell replacement, because of the metabolic efficiency and the superior safety profile of the islet in relation to whole pancreas transplantation [105]. For islet transplantation to replace pancreas transplantation, the most important future improvements need to be made in isolation techniques so that single infusions with consistently high islet yields become the norm, as well as in delivery methods so that islets can engraft and be viable long term in friendly and supportive niches, as well in the identification of potentially inexhaustible sources of islets across species and in camouflaging techniques to prevent allorecognition.

Prof. Lorenzo Piemonti: State of the art of cell therapy for type 1 diabetes

Prof. Piemonti gave the introductory lecture with which the state of the art of cellular therapies as they are applied to type 1 diabetes [106] was illustrated starting with the revival of xenotransplantation [107]. The use of xeno islets represents an old alternative that has become newly of age with novel recent perspectives [107]. Porcine islets are a valuable source of transplantable islets due to their physiological affinity to their human counterparts and their theoretical abundance and, very importantly, because pigs can be genetically modified for making their islets more suitable for the transplantation in humans [108]. In fact, the deletion of the galactose- α 1,3-galactose gene, a saccharide expressed on cells of lower mammals but not on cells of humans or monkeys and against which humans have natural preformed antibodies, as well as the (recently reported) inactivation of porcine endogenous retroviruses enabled by CRISPR-Cas9 technology [109], bear potential to rule out the two major hurdles of islet xenotransplantation, namely the risks of an hyperacute immunologic rejection and zoonosis that so far have been limited to non-human species. Moreover, neonatal pig islets are also being microencapsulated to prevent allorecognition and escape the host immune surveillance, and translation into humans have already occurred, although with unclear results (reviewed in[106]). Currently, however, the most significant advances and the greatest promise come from the stem cell field, which is offering opportunities for the cell therapy of single-cell disorders like type 1 diabetes. In fact, various groups have shown that human ESC and iPSC are able to generate pancreatic progenitors and/or functional β -cells *in vitro* (reviewed in[106]). In particular, the optimization of *in vitro* strategies to differentiate human ESC into mature insulin-secreting cells has made considerable progress and recently led to the first clinical trial of stem cell treatment for this condition. As well, the discovery that it is possible to derive human iPSC from somatic cells has shown that it may be possible to derive a sufficient amount of patient-specific β -cells through the reprogramming and differentiation of patients' own cells suggesting that in the

future this technology may allow immunosuppression-free transplantation. In both cases of ESC- and iPSC-derived β -cells, cells are phenotypically and physiologically similar to β -cells and can treat diabetic mice after implantation. Moreover, the stem cell approach may synergize well with other developing innovations such as the generation of immune isolating and retrievable devices, fundamental to allow cell therapy without immunosuppression and to overcome the safety concerns.

Dr. M. Cristina Nostro: Generation of pancreatic progenitors from human pluripotent stem cells

Dr. Cristina Nostro addressed a major hurdle that the field is encountering. In fact, despite it is possible to engineer human pluripotent stem cells in order to generate pancreatic progenitor cells that may be used as source of functional β -cells (and other mature pancreatic cells) when transplanted into mice, the efficiency of pancreatic progenitor cell generation *in vitro* is highly variable (ranging between 6% and 80%) and cell-dependent, thus negatively impacting reproducibility and validation of *in vitro* and *in vivo* studies, and consequently, translation to the clinic. Therefore, increasing purity of pancreatic progenitor cells is crucial towards producing mature cell types for clinical applications. Her group in Toronto recently described the use of a proteomics approach to phenotypically characterize pancreatic progenitor cells obtained from human pluripotent stem cells and thus allow sorting of these cells from non-pancreatic progenitor cell populations during differentiation [110]. Specifically, the pancreatic secretory granule membrane major glycoprotein2 – which is co-expressed with NKX6-1 and PTF1A in human developing pancreata – was identified as critical pancreatic progenitor cell-specific surface marker. Strikingly, these glycoprotein2⁺ cells obtained from human pluripotent stem cells were able to generate β -like cells (C-PEPTIDE⁺/NKX6-1⁺) more efficiently compared to glycoprotein2⁻ and unsorted populations, highlighting the potential therapeutic applications of this marker.

Dr. Alice Tomei: Conformal coating to improve islet encapsulation technology

Islet encapsulation has been designed to immunoisolate transplanted cells and prevent direct immune attack against alloantigens thus allowing immunosuppression-free islet transplantation. By this technique, islets are incorporated within biocompatible capsules that allow nutrient diffusion and glucose-stimulated insulin secretion while providing immunoisolation from allo- and auto-reactive cells. Clinical trials have proven the safety but not the long-term efficacy of islet encapsulation [111]. However, the majority of trials have been conducted with capsules of uniform 500-1000 μm diameter – despite islets having a variable size between 50 and 350 μm , and with the peritoneal cavity as the transplant site. Therefore, the reasons for clinical failure of those traditional encapsulation protocols can be associated with the large capsule size which impairs diffusion of nutrients to the encapsulated islets, causes delays in glucose-stimulated insulin secretion and limits the choice of the transplant site. In fact, it is known that cells can only survive within a 300 μm -distance from the source of nutrients [111]. If we consider that islet size ranges between 50 and 350 μm , and that capsules may measure up to 1,000 μm , larger islets are prone to central ischemia and delayed glucose-stimulated insulin secretion, with the larger the capsules the worse these effects will be. To minimize the distance between the islets and the source of nutrients – namely, the host capillary bed – naked isolated islets can be transplanted in confined and vascularized sites, where they can be rapidly revascularized reaching the optimal configuration that eliminates the risk of central hypoxia [112]. Conversely, due to their large volume islets encapsulated in traditional large capsules cannot be transplanted in confined sites at curative doses [113]. In this regard, Tomei's group has recently developed an encapsulation technology that allows 'wrapping' each individual islet with a uniformly thin (≈ 15 μm) layer of biomaterial, generating capsules that 'conform' to the size and shape of the islet [114]. By reducing the diffusion distance of 10-fold, this conformal coating allows

increased nutrient transport. By reducing the overall graft volume more than 100-fold, conformal coating also makes possible islet transplantation in well-vascularized confined sites without the need to use the intraperitoneal cavity as site of injection, further maximizing nutrient transport. Importantly, contrary to islets in traditional microcapsules, conformal coated islets display no delay in glucose-stimulated insulin secretion [114,115]. Tomei's group demonstrated long-term euglycemia after transplantation of fully MHC-mismatched conformal coated grafts in diabetic mice without immunosuppression using polyethylene glycol hydrogels [115] and they were able to show that their conformal coating platform is suitable for use with essentially unlimited insulin-secreting cell sources derived from stem cells (unpublished results). Moreover, as macrophage activation and suboptimal oxygen tension at the graft site right after transplantation are responsible for loss of a portion conformal coated islets in the first few days after transplantation, nanomedicine approaches that target innate immune cell activation [116] and increase local oxygen concentrations were successfully integrated within the conformal coating system (unpublished results).

CONCLUSIONS

Dr. Catherine Klersy: Proposal of an online platform for collection and monitoring of data from clinical trials exploring the use of MSCs in digestive diseases.

The final presentation from Dr. Klersy is a call for an online-platform for collection and monitoring of data from clinical trials exploring the use of MSCs in digestive diseases. The expectation is that developing of a shared platform for collection and monitoring cell therapy related trial data shall facilitate the identification of the right design and outcome measures needed to speed up the translation of this enormous potential to patients' care. Therefore, she proposed to set up a new online Registry by using RedCap© (Research Electronic Data Capture), a novel workflow methodology and software tool that expedites the electronic collection of research data from a single-site or multi-site clinical research study that was

developed at the Vanderbilt University (Nashville, Tennessee, USA) [117]. The software supports a secure web-based application for developing fully functional case report forms and surveys. In particular, through RedCap© it is possible to implement: (a) full user authentication (log-on/password) to restrict users to study functions; (b) an intuitive interface for validated data entry; (c) real-time data validation, integrity checks for insuring data quality; (d) de-identification options to be applied to data exports to remove fields that contain notes and other information that could identify patient; (e) centralized, secure storage of research data with back-ups (f) automated export procedures for seamless data downloads to common statistical packages and (g) procedures for importing data from external sources [117]. All data collected will be entered into a local regulation compliant Data Management System provided by the Service of Biometry & Clinical Trial Center of the I.R.C.C.S. Policlinico San Matteo Foundation (Pavia, Italy).

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FIGURE LEGENDS

Figure 1. Mortality rate in the United Kingdom over time. During the last 45 years, we witnessed a significant decrease of mortality as showed by the data from both female and male adult populations in the United Kingdom (upper panel). By contrast, in the last 30 years, the mortality rate in inflammatory bowel disease (IBD) has risen (lower panel). Data are from the <http://www.healthdata.org/gbd> website.

Figure 2. Role of mesenchymal stem/stromal cells in mucosal regeneration and immune-modulation. Once injected *in vivo*, mesenchymal stem/stromal cells are able to rescue enterocytes from apoptosis by blocking both cytolytic mechanisms, that is, Fas-Fas Ligand cognate interaction and perforin-granzyme secretion, inhibit IL-15 secretion and function, preserve the epithelial barrier by reassembling claudins, the apical-most proteins of the tight junctions, and protect crypt stem cells (left side). Moreover, mesenchymal stem/stromal cells form aggregates with lymphocytes and release TSG-6, a potent anti-inflammatory soluble factor, that reduces the levels of proinflammatory mediators, such as TNF- α , IFN- γ , IL-6, IL-17A and IL-21, inhibit the recruitment of neutrophils, block the MMP activity and immunoglobulin secretion, while promoting the expansion of T-cells (mainly the FoxP3⁺) and macrophages with immunosuppressive and regulatory activities. In addition, they cause an abortive maturation of dendritic cells, thus preventing efficient antigen presentation to T lymphocytes (right side). Red lines indicate an inhibitory effect; green lines indicate a stimulatory effect.

Abbreviations: AJ, adherens junctions; B, B-cell; DC, dendritic cell; FasL, Fas Ligand; Fox, transcription factor Forkhead box; HGF, hepatocyte growth factor; IDO, indoleamine 2,3-dioxygenase; IEL, intraepithelial lymphocyte; IFN, interferon; Ig, immunoglobulin; IL, interleukin; M-CSF, macrophage-colony stimulating factor; M reg, regulatory macrophage; MMPs, matrix metalloproteinases; MSC, mesenchymal stem/stromal cell; NK, natural killer cell; PGE2, prostaglandin E2; PMN, PMN: polymorphonuclear leukocytes; Th, T helper; Treg, regulatory T cell; TGF, transforming growth factor; TNF, tumor necrotizing factor; TJ, tight junctions; VEGF, vascular endothelial growth factor.

Table I. Mesenchymal stromal cell therapy in fistulizing Crohn's disease

Study (year)	Number of patients (F/M) Age (years)	Disease duration (years)	Study Phase	Source	Number of injections	Fistula closure total/partial	Follow-up	Relapse
Garcia-Olmo (2009) ⁸¹	14 (NA) NA (range 33-62)	NA	Phase 2	Autologous adipose-tissue	2	Yes	12 months	Yes
Ciccocioppo (2011) ⁸²	10 (3/7) Median 33 (range 16-59)	Median 9,5 (range 2-21)	Phase 1-2	Autologous bone marrow	4	Yes	12 months	Not
de la Portilla (2013) ⁸³	24 (13/11) Mean 36.0 (SD 9.0)	>12 months	Phase 1-2	Allogeneic adipose-tissue	2	Yes	6 months	Yes
Lee (2013) ⁸⁴	43 (13/30) Median 26 (range 21-32)	Median 5,5 (range 1-8)	Phase 1	Autologous adipose-tissue	2	Yes	12 months	Not
Cho (2013) ⁸⁵	10 (6/4) Mean 26.5 (SD 6.0)	NA	Phase 1	Autologous adipose-tissue	1	Yes	8 months	Not
Molendijck (2015) ⁸⁶	21 (9/12) Median 29 (range 24-41)	Median 5 (range 0,5-10)	Phase 2	Allogeneic bone marrow	1	Yes	6 months	Not
Park (2015) ⁸⁷	6 (2/4) Mean 32	Range 1-12	Phase 1	Allogeneic adipose-tissue	1	Yes 3/3	8 months	Yes
Garcia-Arranz (2016) ⁸⁸	10 (10) Mean 35 (range 31-55)	Mean 11.6 (range 1-23)	Phase 1-2a	Allogeneic adipose-tissue	1-2	Yes (Recto-vaginal)	12 months	Yes
Dietz (2017) ⁸⁹	12 (6/6) Mean 54 (range 18-58)	Mean 6.5 (Range 2-17)	Phase 1	Autologous adipose-tissue +Bioabsorbable matrix	1	Yes 10/2	6 months	Not
Abbreviations: NA: Not Available; F/M: Female/Male.								