J. of Cardiovasc. Trans. Res.
DOI 10.1007/s12265-010-9232-4

Stem Cell Update: Highlights from the 2010 Lugano Stem Cell Meeting

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Received: 14 September 2010 / Accepted: 19 October 2010
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Abstract The 2010 edition of the Lugano Stem Cell Meeting, under the auspices of the Swiss center of excellence in cardiovascular diseases "Cardiocentro Ticino" and the Swiss Stem Cell Foundation, offered an update on clinical, translational, and biotechnological advances in regenerative science and medicine pertinent to cardiovascular applications. Highlights from the international forum ranged from innate mechanisms of heart repair, safety, and efficacy of ongoing and completed clinical trials, novel generations of stem cell biologics, bioengineered platforms, and regulatory processes. In the emerging era of regenerative medicine, accelerating the critical path from discovery to product development will require integrated multidisciplinary teams to ensure timely translation of new knowledge into validated algorithms for practice adoption.

Keywords Regenerative medicine · Biotechnology · Heart failure · Disease · Translation · Clinical trials · Mesenchymal stem cells · Induced pluripotent stem cells

The international biomedical community gathered in Lugano, Switzerland on June 22nd–23rd 2010 for the second edition of the Lugano Stem Cell Meeting. The scientific program focused on the translational potential of stem cell platforms moving from discovery science to clinical applications with regulatory authorities highlighting current requirements for clinical trial launch. Organized as an open forum covering topics ranging from stem cell research at the bench to translation at the bedside and applications in patient populations, plenary presentations and keynote lectures are complemented with dedicated poster sessions and awards presented to most promising young investigators in cardiovascular stem cell research. This overview summarizes the proceedings of the 2010 Lugano Stem Cell Meeting. This biennial event was initiated in 2008, and the third edition planned for June 2012.

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Regulatory Stem Cell Science

The first session provided the opportunity for representatives of regulatory agencies in Europe to present the principles and practice of guidelines governing regulatory requirements for stem cell-based clinical trials under the Advanced Therapy Medicinal Products (ATMP) classification act. In the domain of regenerative medicine, the European Commission is committed to monitoring scientific progress and in light of new developments review legislation to ensure patient access to safe, novel treatments. Accordingly, the European Commission institutions agreed on a regulation pertinent to advanced therapies (Regulation EC 1394/2007) designed to facilitate free movement of advanced therapy products within Europe while guaranteeing the highest level of health protection (http://ec.europa.eu/health/human-use/advanced-therapies/index_en.htm). Dr. M.C. Galli, member of the Committee for Advanced Therapy Products at the European Medicinal Agency, stressed the main elements of the regulation, namely the

Published online: 30 October 2010

technical requirements adapted to the particular characteristics of these innovative products, the benefit from the pooling of expertise at European level, a centralized marketing authorization procedure with direct access to the European Union (EU) market, and the special incentives for small and medium-sized enterprises evolving in this space. This regulation marks the recognition that a number of advanced therapy products combine biological materials, namely tissues or cells, and chemical structures such as metal implants or polymer scaffolds. These combination products are at the interface of the traditional pharmaceutical area and other fields (e.g., medical devices) and cannot be regulated as "conventional" drugs. As further stated by Dr. Galli, efficient translation from discovery into pharmaceuticals is essential for Europe's competitiveness as Advanced Therapy Medicinal Products are among the most innovative medicines and bottlenecks lead to unacceptable delays in the development process. The current EU regulatory framework for medicinal products includes Directive 2001/20/EC, which claims that for all medicines, clinical trial approval is the responsibility of each member state, from first-in-man use through phase III trials. Thus, separate authorizations must be obtained in each member state where the clinical trial is to be carried out, including for multinational clinical trials. As a consequence, conducting cross-border clinical trials and comparing their results might be difficult. Dr. Galli underscored that a harmonized strategy is highly desirable for multinational trials. The European Medicinal Agency contribution to harmonization is represented by the "Guideline on the non-clinical studies required prior to clinical use of gene therapy medicinal products" (CHMP/GTWP/125459/2006). It represents the consensus between member states on the subject and will allow a harmonized approach between Member States in the application of Directive 2001/20/EC at national levels. Referring to national experience, Dr. J. Djonova, head of the Transplant Unit of Swissmedic, reported the requirements for market authorization and clinical studies with transplant products in Switzerland. An important regulatory legislation in Switzerland is represented by Federal law "Transplantation of Organs, Tissues and Cells and its Ordinances" (LTrans) that came into force in 2007. Especially, Art 49 of LTrans defines Standardized Transplants (TrsP), whereby authorization to manufacture a transplant product for the purpose of a clinical trial goes through GMP inspections. In Switzerland, a number of products are currently on the market within this framework, and currently seven submissions with a TrsP are pending for clinical trial authorization, four trials were notified by the Federal Office of Public Health, and three notifications were attributed. Dr. M. Raddrizzani drew the audience's attention on the development of a specific ATMP, i.e., lentiviral vector-transduced hematopoietic stem cells for gene therapy [1]. Dr. Raddrizzani's group is focusing on two gene therapy clinical trials pertinent to the treatment of metachromatic leukodystrophy (MLD) and Wiskott–Aldrich syndrome (WAS). Both trials rely on the infusion of an ATMP, consisting of enriched autologous CD34+ hematopoietic stem cells transduced ex vivo with a lentiviral vector (LV) encoding the therapeutic gene [2,3]. A large-scale process for GMP-grade LV production and purification has been developed, and the process is applicable to vectors carrying different transgenes. The development and validation results were included in the Investigational Medicinal Product Dossiers for MLD and WAS phase I/II clinical trial applications. Both clinical trials have been recently approved by Italian Regulatory Authorities and are currently ongoing. The take-home message for this session is that approval of ATMPs for clinical trial initiation critically depends on joint efforts across the community of practices engendering the continuum of translational science and regulatory affairs. This process is guided by international and national regulations that reflect acquisition of new knowledge in cell therapy platforms.

Stem Cell Sources

Tissue engineering and regenerative medicine have evolved in parallel with recent biotechnological advances and increased understanding of stem cell sources. A case in point, mesenchymal stem cells have an inherent ability for self-renewal, proliferation, and differentiation toward mature tissues depending on the microenvironment by which they are surrounded [4–6]. Such characteristics render this cytotype a prime candidate for use in regenerative medicine. It was originally thought that mesenchymal stem cells were found exclusively in bone marrow, but similar profiles have been more recently reported in diverse adult tissues [7,8]. Adipose tissue has been recognized as particularly attractive, as it is readily accessible following liposuction procedures. Isolating mesenchymal stem cells from adipose tissue is a reproducible process with robust cell yield compared to other sources [9,10]. Dr. P.L. Sánchez, Hospital General Universitario Gregorio Maranon Madrid, Spain, presented and commented on the favorable safety and feasibility data from the APOLLO trial, a randomized clinical study of freshly adipose-derived stem cells in the treatment of patients with ST-elevation myocardial infarction (ClinicalTrials.gov Identifier: NCT00442806). A newly discovered source of mesenchymal stem cells is the human peri-pericardial adipose tissue presented by the stem cell bank and cardiosurgery unit of Cardiocentro Ticino. Patients who underwent cardiac surgery were the donors, with samples collected following thoracotomy or sternotomy. Data indicate that human peri-pericardial adipose tissue contains a subpopulation of multipotent stem cells which are largely comparable—albeit with a higher clonogenic potential—than

mesenchymal stem cells isolated from liposuction aspirates [11] and could therefore be potentially exploited for regenerative medicine. Additionally, cultured peri-pericardial samples display very small, round-shaped, colony-forming like cells, spread within the typical fibroblast-like mesenchymal stem cells. These cells are absent in comparable cultured adipose lipoaspirate stromas raising the question on the putative contribution of tissues around the human heart in its repair processes. In the same session dedicated to human tissues, Dr. Giordano's group proposed human cord blood mesenchymal stem cells (hCBMSCs) as a valid source for regenerative medicine. The extensive characterization of human CBMSCs has indicated that they are similar to mesenchymal stem cells of bone marrow origin with respect to morphological characteristics and multipotency [12]. Dr. Giordano's group provided evidence that mesenchymal stem cells derived from CB, intravenously delivered to an experimental model of immunodeficient mice with acute kidney injury (AKI), protect animals from renal function impairment and prolong lifespan [13]. These data indicate that hCBMSCs should be considered as a future option for cell therapy of AKI in humans. Moreover, hCBMSCs have advantages partly related to the immaturity of newborn cells compared with adult stem cells, including possible escape from immune rejection. For these reasons, human umbilical CB have been increasingly banked and used as a source of marrow repopulating stem cells in patients with BM deficit or inborn errors of metabolism [14]. To summarize, both adult and neonatal tissues are being explored as valid sources for clinically applicable protocols. Especially, human adipose tissue has gathered increased scientific attention due to promising properties of derived mesenchymal stem cells with encouraging results in ongoing clinical trials.

Fig. 1 Prof. Piero Anversa (*left*) and Prof. Tiziano Moccetti (*right*). Prof. Anversa presented a lecture entitled "Cardiomyogenesis in the adult human heart" on the first day of the meeting

Resident Progenitor Cells

The adult heart has been traditionally considered a postmitotic organ in which the number of parenchymal cells is established at birth, and cardiomyocytes lost with age or due to disease cannot be replaced by newly formed cells. However, more recently, several studies have challenged this notion as a pool of resident cardiac stem cells (CSCs) has been identified and characterized in the human heart. In fact, significant myocyte renewal, mediated by stem cell activation, has been reported [15–17]. Retrospective ^{14}C birth dating of cells has suggested that roughly half of the human heart can be replaced throughout lifespan. More recent evidence, presented at this meeting in Dr. Anversa's lecture (Fig. 1), indicate that an even larger number of myocytes is regularly formed de novo with myocyte turnover rate in the order of 20% per year and degrees of fibroblast and endothelial cell turnover comparable to that of heart muscle cells [18]. This demonstration is based on cell labeling by a thymidine analog in hearts collected postmortem from cancer patients who received infusion of the radiosensitizer iododeoxyuridine for therapeutic purposes. Importantly, DNA repair, ploidy formation, and cell fusion were not implicated in the assessment of myocyte regeneration. These data suggest that the entire heart, myocyte and non-myocyte compartments, could be replaced several times during the course of life in humans. Collectively, these findings have important clinical implications underscoring the inherent rejuvenating potential of the adult human heart. In describing heart's innate ability for growth and regeneration, Dr. A. Leri's presentation highlighted the role of CSCs in dilated cardiomyopathy. Physiological hypertrophy in the developing heart has been considered the product of an increase in volume of preexisting fetal cardiomyocytes in the absence of myocyte formation [19]. Dr. Leri's group showed an alternative, whereby the mouse heart possesses at birth a pool of c-kit-positive CSCs which are lineage negative, self-renewing, multipotent, and differentiate into myocytes contributing to the expansion of the parenchymal cell compartment postnatally [20]. Dr. Leri stressed the importance of the Notch1 receptor cascade in the regulation of early differentiation of neonatal CSCs and their transition to amplifying myocytes. Alteration of this molecular cascade leads to impaired cardiomyogenesis, prevention of physiological cardiac hypertrophy, and development of life-threatening myopathy. The hypothesis was advanced that Notch1 could be involved in the etiology of idiopathic dilated cardiomyopathy in humans. Dr. Beltrami pointed out that senescent stem cells accumulate in aging leading to premature cardiac senescence and heart failure [21]. Cloning efficiency, population doubling time, and cell expansion

kinetics are significantly impaired by age. Additionally, cardiac pathology significantly reduces the fraction of cardiac progenitor cell (CPCs) able to differentiate toward the cardiomyogenic lineage and the ability of establishing intercellular adhesion sites through Connexin 43 expression [22]. Dr. Beltrami commented that even in circumstances of severe cardiac pathology, primitive cells can be obtained and expanded from failing human hearts. Selection of progenitor cells whose proliferating and differentiating properties are preserved or possibly targeting senescence pathways in CPCs would offer strategies to interfere with progressive degenerative processes. Dr. Gnecchi highlighted mechanisms underlying the "paracrine effect" in adult stem cell signaling and therapy. Secretion of soluble factors that protect the heart, attenuate pathological ventricular remodeling, induce neovascularization, and promote regeneration contributes to functional benefit associated with stem cell transfer in animal models of cardiac injury [23,24]. Conditioned medium from MSCs, particularly from genetically modified MSCs over-expressing Akt-1, is able to recapitulate beneficial effects observed after stem cell therapy and to exert cardiomyocyte protection [25]. In addition, released factors may display autocrine actions on stem cells themselves. Thus, the paracrine/autocrine hypothesis extends the traditional concept of the stem cell niche to include a dynamic influence of stem cell-released factors on the microenvironment modulating stem cell biology and tissue response including survival, repair, and regeneration, involving resident and circulating stem cell populations. Paracrine effects are also involved as major contributors to vascular regeneration as stated in Dr. P. Madeddu's presentation. Recent evidence indicates the presence of progenitor cells in arteries and veins, including human pericyte progenitors from the saphenous vein demonstrated to induce stable and durable neovascularization in mouse models of peripheral ischemia and myocardial infarction [26]. Here, Dr. Madeddu reported the isolation of progenitor cells with proangiogenic capacity in saphenous vein leftovers from elderly cardiovascular patients. This population, dubbed "saphenous vein-derived progenitor cells" (SVPs), establishes interactions with multiple endothelial/endovascular structures, both in coculture systems and in vivo, recapitulating the natural function of perivascular support cells. An ensuing reciprocal paracrine cross-talk results in stimulation of endothelial cell network formation and proliferation and SVP migration. Furthermore, resident $CD34^{+}/CD31^{-}$ cells and their progeny might take part in vein graft remodeling and adaptation by supporting the establishment of vascular connections between vasa vasorum of the graft and the recipient. These data highlight the importance of focusing on more specialized cells to provide the heart with all the elements necessary for its complete repair.

Integrated Stem Cell Science

A magistral lecture by Prof. C. Ricordi, director of the Cell Transplant Center and Diabetes Research Institute in Miami and world expert in pancreatic islet transplantation, drew the audience attention on cellular and regenerative strategies for the treatment of diabetes. As Prof. Ricordi stated, stem cells and regenerative medicine could offer a definitive solution and an alternative to pharmacological treatments [27,28]. Prof. Ricordi underscored that overcoming current challenges of islet transplantation requires emerging multidisciplinary approaches required to advance the promise for β-cell replacement therapies in the years to come. Current approaches include xenotransplantation, cord blood, amniotic, fetal, embryonic, and inducible pluripotent stem cells which expand the potential of transdifferentiation and tissue reprogramming technologies, opening the way to the use of autologous adult tissues as a potential source for insulin-producing cells. In this direction, recent data from his group indicate that adipose-derived stem cells could represent an excellent source obtained from the patient's own subcutaneous tissue following a mini liposuction procedure [29]. Modern methods for processing adipose tissue-derived cells have been described, and initial promising results have been recently published in experimental models where it was possible to transform at least a portion of adipose-derived stem cells into insulin-producing cells. In addition to plastic and reconstructive surgical applications, adipose tissue has become central to an increasing number of translational efforts involving regenerative applications.

A specialized session described technological advances for extraction, storage, and clinical applications of adipose tissue-derived stem cells. Examples included the device STAGRA developed at "Cardiocentro Ticino" in collaboration with the University of Applied Sciences and Arts of Southern Switzerland and Swiss Stem Cell Bank and the Celution system produced and commercialized by General Electrics. Moreover, the Beckman Coulter workshop provided practical insights on the use of flow cytometry. Dr. A. Böhmler and Dr. T. Tallone, after an introductory presentation on stem cells and the classical surface markers used for their characterization, went on to discuss "adult vascular wall resident progenitor cells". Newer markers which could help better characterize adipose tissue-derived multipotent stromal cells were also presented (see short workshop summary in this issue).

Clinical Trials and Next Frontiers

Dr. D.W. Losordo, Northwestern University, described the results of the completed ATC34-CMI Trial (Fig. 2). After

Fig. 2 Prof. Tiziano Moccetti, Medical Director of Cardiocentro Ticino, discussing with Prof. Douglas W. Losordo. Prof. Losordo reported the results of the ATC34-CMI Trial performed in Chicago, IL, USA

ischemic insult, endothelial progenitor cells (EPCs) are believed to home to sites of neovascularization, where they contribute to vascular regeneration by forming a structural component of capillaries and by secreting angiogenic factors [30]. Preclinical data provide evidence of safety and potential bioactivity of autologous CD34+ cells for improving perfusion in ischemic tissue, both in angina and critical limb ischemia [31]. The randomized ATC34-CMI trial provides evidence for feasibility, safety, and bioactivity following intramyocardial injection of autologous mobilized CD34+ cells in patients with intractable angina and permit endpoint selection and power calculations for later phase studies [32]. These insights into the molecular and cellular processes of tissue formation suggest that cardiac function may be preserved after myocardial infarction by transplanting EPCs into ischemic heart tissue, thereby enhancing vascular and myocardial recovery. Dr. R. Ascione at the Bristol Heart Institute addressed neovascularization from a surgical point of view. Circulating EPCs can be characterized by the expression of CD133 (for developmentally immature EPCs), CD34, KDR, and/or VE cadherin. Adult CD133+ cells can be sorted in sufficient number from bone marrow for autologous transplantation and are being used in translational research [33,34]. In two ongoing randomized, double blind, placebo-controlled surgical trials, the safety and efficacy of BM-CD133^{pos} progenitor cells is assessed in patients with acute and chronic myocardial infarction. TransACT-1 and TransACT-2 trials aim to evaluate the efficacy of surgical sub-epicardial injections of autologous BM-CD133^{pos} progenitor cells and surgical intramyocardial (sub-endocardial) injections of autologous BM-CD133^{pos} progenitor cells, respectively. Outcome measures include serial assessment up to 6 months postsurgery of regional and global scar distribution and LV wall function with cardiac MRI, clinical status, quality of life, and safety measures including serial troponin I release and 24 h ECG.

Beyond the strategies of today, Professor A. Terzic, Mayo Clinic, introduced the next frontier for cardiac repair based on advances in nuclear reprogramming and generation of induced pluripotent stem (iPS) cells [35] (Fig. 3). Recently, iPS cell technology has launched a new platform in regenerative medicine aimed at deriving unlimited replacement tissue from autologous sources through somatic cell reprogramming using stemness factor sets [35]. In this way, authentic cardiomyocytes have been obtained from iPS cells and demonstrated in proof-of-principle studies to repair infarcted heart [36]. Within infarcted hearts in the adult, intramyocardial delivery of iPS yielded progeny that properly engrafted without disrupting cytoarchitecture in immunocompetent recipients. Optimizing the cardiogenic potential of iPS progeny would ensure a maximized yield of bioengineered cardiac tissue [37]. In this regard, iPS bioengineered without the oncogene c-MYC achieve highest stringency criteria for bona fide cardiogenesis enabling reprogrammed fibroblasts to yield de novo heart tissue compatible with native counterpart throughout embryological development and into adulthood [38].

Related to Dr. Terzic's main lecture and highlighting the concept of "guiding" stem cell differentiation for enhanced repair, Dr. J. Bartunek presented the study design and initial feasibility and safety results of the C-Cure clinical trial, a prospective, randomized study assessing the therapeutic benefit of a second generation regenerative product. C-Cure is an autologous stem cell-based bio-therapeutic developed for the treatment of chronic heart failure in the setting of ischemic cardiomyopathy. The active ingredients are "cardiopoietic" cells originating from bone marrow-derived mesenchymal stem cells which, when cultured in the presence of cardiotrophic factors, become definitively engaged into the cardiac differentiation program [39,40].

Fig. 3 Prof. Andre Terzic, eminent course director of this second edition of the Lugano Stem Cell Meeting, chairing the session on "Cell Therapy in Chronic Ischemic Diseases"

Fig. 4 Dr. Gianni Soldati (*right*), Scientific Director of Swiss Stem Cell Bank, Prof. T. Moccetti (*center*), and the winner of the Best Poster Award 2010 (*left*)

Based on preclinical studies [41], the pharmacodynamic profile of "cardiopoietic" guided mesenchymal stem cells is superior to unguided counterparts and involves promotion of myocardial repair in the setting of myocardial infarction following direct delivery into host heart parenchyma. This therapeutic impact is achieved as transplanted "cardiopoietic" cells have the propensity to engraft and give rise to new myocardium within the diseased heart microenvironment. The feasibility results were excellent opening the way to a further and larger trial design to assess efficacy of this newest stem cell product for cardiac regeneration.

Dr. Houtgraaf described in detail the first-in-man APOLLO Trial. The objective of the study was to determine safety and feasibility of adipose-derived stem cells delivered via intracoronary route [42]. Although the number of patients was limited (N=14), the study shows indications of efficacy, consistent, and concordant with the preclinical experience in large animal studies [43]: reduction of infarct size, improvement in myocardial perfusion by SPECT, and indication on improvement of global LVEF with SPECT and cMRI. Additionally, Dr. Houtgraaf announced the imminent start of the related ALPHA trial, which will involve 200 patients and 20 centers in Europe.

A very interesting talk by Dr. A. Zeiher introduced a hot issue of discussion: Are unselected mononuclear cells still the first choice after myocardial infarction? Even though there is no definitive answer, the current experience in terms of safety, efficacy, and clinical outcome in patients is the largest with unselected bone marrow cells (BMCs). According to results obtained by meta-analysis, available evidence suggests that BMC transplantation is associated with modest improvements in physiologic and anatomic parameters in patients with both acute myocardial infarction and chronic ischemic heart disease, above and beyond conventional therapy [44]. Therapy with BMCs seems safe. These results support conducting large randomized trials to evaluate the impact of BMC therapy versus the standard of care on patient-important outcomes. One key question concerns the putative mechanisms of action of these cells. Vasculogenesis certainly plays a crucial role in functional cardiac regeneration, and intracoronary BMC administration is proven to normalize coronary flow reserve. Repeated intracoronary BMC treatment is associated with lower mortality than Seattle Heart Failure Model predicted mortality [45]. Importantly, application of functionally competent BMCs is essential to maximize outcome.

New Generation of Investigators: Poster Award

Among outstanding young candidates, the scientific committee using rigorous criteria that included clinical applicability selected the abstract entitled "Mesodermal progenitor cells isolated from human bone marrow can differentiate towards endothelial lineage" from the Department of Oncology of the University of Pisa for oral presentation and the Swiss Stem Cell Bank Prize [46] (Fig. 4). Given the very high level of received abstracts, it is anticipated that the highest quality applications will be received for the next edition of the Lugano Stem Cell Meeting in 2012.

Conclusion

The 2010 Lugano Stem Cell Meeting has provided a forum to highlight regenerative medicine as a new perspective of future clinical practice. Patients and society increasingly expect that regenerative medicine will lead to repair of diseased organs, injured tissues, or congenital anomalies. Aimed toward functional restoration, not a mere moderation of symptoms, regenerative medicine offers a therapeutic strategy uniquely poised to transform cardiovascular

Fig. 5 Landscape view of Lugano from the meeting venue

health care by providing tailored, curative solutions for the unmet needs of our patients [47]. Building on breakthroughs in stem cell biology [48], maximizing potential return mandates an integrated roadmap across the translational continuum of discovery–development–regulation–use to ensure optimal application of regenerative medicine in practice. In a wonderful environment of southern Switzerland (Fig. 5), we look forward to hosting you all during our next planned Lugano Stem Cell Meeting in Summer 2012.

Acknowledgments The authors wish to thank the Cardiocentro Ticino Congress Committee, namely Mrs. Annapaola Sürder-Boschet, Mrs. Rosi Parillo, and Mr. Alessandro Tomei, for their excellent professional support and their warm and collaborative attitude in the challenging organization of the meeting.

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