Cobi J. Heijnen, PhD, Andreas E. Kulozik, MD, PhD, Olaf Witt, MD, PhD, and Nico Wulffraat, MD, PhD, are the Guest Editors for this special all-review issue on cell-based and cancer stem cell–targeted therapies in pediatric medicine. Dr Heijnen is professor of neuroimmunology and head of the Laboratory of Neuroimmunology and Developmental Origins of Disease at the University Medical Center Utrecht in Utrecht, The Netherlands. Dr Kulozik is professor of pediatric oncology, hematology, and immunology at Heidelberg University, Germany. Dr Witt is professor of pediatric oncology and hematology and head of CCU Pediatric Oncology at the German Cancer Research Center and University Medical Center Heidelberg, Germany. Dr Wulffraat is head of the Department of Pediatric Immunology at the University Medical Center Utrecht, The Netherlands.

Stem cells in pediatrics: state of the art and future perspectives

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Both the general public and a major proportion of clinicians are excited by the idea that stem cells can be used to repair or replace damaged tissues. In this special issue, a unique, cutting-edge overview is presented of the therapeutic potential and risks of stem cell–based and cancer stem cell–targeted therapies in pediatric medicine. The reviews highlight the already existing clinical applications as well as novel possibilities for the near and not-so-near future. It is clear that the major reasons for the thus-far rather limited use of stem cell therapies are the known and feared risks of cell-based therapies involving hematopoietic stem cells, mesenchymal stem cells (MSCs), and induced pluripotent stem cells to fight pediatric disorders such as chronic inflammatory diseases, leukemias and solid tumors, primary immunodeficiencies, brain damage, and cardiac disease. The anticipated risk is also a major hurdle for treatments targeting cancer stem cells that represent the other side of the coin. The need to eliminate cancer stem cells in order to prevent recurrence of malignancies is now considered a major target in cancer treatment and a serious scientific challenge.

The first section of this review issue was edited by Andreas Kulozik and contains three articles that focus on the use of hematopoietic stem cells for the treatment of pediatric diseases. The review by Domen *et al.* (1) provides a broad overview of the current successful clinical use of hematopoietic stem cells for treatment of diverse disorders, including autoimmunity, and an inherited metabolic disease (Hurler syndrome). It also provides in-depth information on the emerging use of hematopoietic stem cells to cure HIV infection as well as a new approach to tolerance induction in organ transplants. In line with the majority of the articles in this review issue, there are still major safety issues, even in cases where stem cell therapy is already widely used, and there is clearly an urgent need to alleviate this pressure in order to increase the applicability of stem cells in pediatrics specifically, and in medicine in general.

The clinical application of hematopoietic stem cells has long been limited to patients for whom human leukocyte antigen–matched donor bone marrow was available (2). Oevermann and Handgretinger (2) summarize the existing evidence that employment of haploidentical donors is finally developing into a feasible alternative. This is especially important as it expands the possibility of using stem cell therapy for the non-Caucasian population for which the availability of human leukocyte antigen–identical cells is currently a major limiting factor.

Severe combine immunodeficiencies, which are usually caused by mutations in a single gene, have been treated via the use of autologous hematopoietic stem cells in which the genetic defect has been corrected by viral vectors. Mikkers *et al.* (3) have concluded that a novel approach is needed to circumvent the risk of malignant transformation. They propose that it would be much safer to apply homologous recombination to repair the genetic defect. Unfortunately, hematopoietic stem cells are not the best target for homologous recombination because of technical limitations. Autologous induced pluripotent stem cells, which can now be well

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targeted with homologous recombination for gene repair followed by *in vitro* differentiation into hematopoietic-like stem cells, could provide an interesting alternative, albeit with its own specific limitations regarding safety.

The focus of the second section, stem cell therapy and chronic inflammatory diseases, edited by Nico Wulffraat, is the clinical outcome of stem cell transplantation for the treatment of severe autoimmune diseases. Tyndall (4) performed a retrospective analysis of several clinical trials in which mostly autologous hematopoietic stem cells have been applied. He discusses the crucial problem of treatment-related mortality vs. the potential long-term benefits and makes clear that many more randomized trials should be performed to determine whether the ultimate effects of these interventions do indeed outweigh the risks. He also discusses the use of multipotent mesenchymal stromal cells, or mesenchymal stem cells (MSCs), which could represent a safer alternative for the use of hematopoietic stem cells. The major known advantages of MSCs over hematopoietic stem cells are the apparent lower acute toxicity and the finding that MSCs confer immune modulation, which could be helpful in dampening a chronic autoimmune process.

Next, Daikeler *et al.* (5) concentrate on the potential complications of hematopoietic stem cell treatment and of the conditioning regimen required for lymphodepletion. The authors underline the importance of patient selection, especially in view of potential preexisting organ damage, which is a serious drawback due to the increased risk of therapy-related complications.

Dalal *et al.* (6) report on the promising approach of using MSCs for the treatment of Crohn's disease, particularly for patients who fail to respond to corticosteroids, anti–tumor necrosis factor drugs, and immunosuppressants such as methotrexate. They first explain the mechanism of action of MSCs on immune function *in vitro* and continue by discussing the phase I trials in which MSC treatment was the primary intervention. MSCs seem to be nontoxic, and no treatment-related serious events have been reported so far. However, although systemic treatment with MSCs has shown promising first results in patients with Crohn's disease, much more research is needed to define timing of MSC treatment, dose, and route of administration. An interesting aspect of this review is the notion that local administration of MSCs may hold promise as an effective therapy for Crohn's disease.

Norambuena *et al.* (7) describe the use of MSCs for osteoarticular diseases, which was initiated for the most part because MSCs have been shown to be capable of differentiating into bone and cartilage. Although only a few studies have supported a benefit of MSCs for pediatric osteoarticular disorders, studies in adults have reported beneficial effects on bone healing and early osteonecrosis. Again, no toxicity has been reported in these studies. There is also no evidence for development of malignancies in the long term, although the latter issue definitely remains the major concern for the use of stem cells, including MSCs, in general. The authors conclude by saying, "MSCs are no longer second-class citizens but first-line players." This statement might be a little overenthusiastic, but it makes us realize that the therapeutic possibilities of MSCs should be taken seriously for the treatment of disorders for which conventional treatments fail.

In the third section of this special issue, edited by Cobi Heijnen, attention is focused on the use of human cord blood and MSCs as stem cell–based therapies for the treatment of brain damage, e.g., after hypoxic–ischemic events, and for cardiac disorders.

Carroll (8) presents an interesting overview of preclinical studies as well as clinical trials using cord blood in children with brain damage. No definitive reports on the efficacy of the trials are available yet. In addition, he points out that only two trials have included patients with acute neonatal hypoxic–ischemic injury—most such trials test the effect of cord blood on more chronic types of brain injury, e.g., established cerebral palsy. The author concludes by proposing a randomized multicenter trial in children born at term who have developed encephalopathy in the aftermath of a hypoxic–ischemic event are treated by applying cord blood cells as early as possible after the insult.

The question of which cell type(s) in cord blood—e.g., $CD34^+$ hematopoietic stem cells, endothelial precursors, lymphocytes, monocytes, or MSCs—contributes to the repair of brain damage is discussed by Pimentel-Coelho *et al.* (9). Apart from the immune-modulating effect of lymphocytes and monocytes, hematopoietic stem cells and endothelial precursors in the cord blood may be the cells that are actively involved in repair, whereas the number of MSCs present in cord blood may be too low to contribute. However, *in vitro* experiments have shown that the various cell types may interact and/or share activities involved in the restoration of tissue. The authors also highlight the possible mechanism of action of the cord blood cells, such as inhibition of neuronal apoptosis, prevention of the suppression of axonal growth *in vitro*, and secretion of neurotrophic and angiogenic factors. Furthermore, they provide a detailed description of imaging methods to trace transplanted cells in the brain.

van Velthoven *et al.* (10) describe the beneficial effects of MSCs on hypoxic–ischemic brain damage in the neonatal mouse. The authors underline the notion that the strong effects of MSCs on repair in the neonatal brain as compared with the adult brain might be due to the inherent plasticity of the developing brain. They summarize evidence that MSCs adapt to the local milieu in the brain and change the neurogenic niche via the secretion of several growth factors and differentiation factors. It is proposed that local parenchymal cells respond to the secretome of MSCs, leading in turn to reduced lesion size and amelioration of sensory motoric behavior of the mouse in the long term.

Dalous *et al.* (11) highlight current pediatric and adult trials using human MSCs derived from cord blood. They conclude that MSCs could have great potential in treating several neurologic disorders, including autism, hereditary ataxia, and multiple sclerosis. Specific attention should be given to further optimize the treatment, such as standardization of MSC preparations, route and timing of administration, and, most interestingly, the manipulation of MSCs before transfusion to enhance their therapeutic potential.

Bernstein and Srivastava (12) conclude this section of the issue by describing the possibilities of stem cell therapies for cardiac diseases. In contrast to some other organs, cardiac tissue has a very limited intrinsic capacity to repair after injury. To compensate, myocardial engineering, including stem cell therapy, is of

utmost importance. The authors describe the beneficial effects of MSC trials in patients with acute myocardial infarction, in whom positive functional effects on cardiac function were observed. From a practical point of view, it is interesting that the trial also included the use of non–human leukocyte antigen–matched MSCs, which proved to be safe in patients with no ectopic tissue formation. This opens the possibility of applying "off the shelf" MSCs, which could ensure early treatment. The authors also describe the potential of resident cardiac progenitors and of cardiospheres, derived from cultured explants of human atrial and ventricular biopsies, which are currently being tested in a phase I trial in patients with myocardial infarction. In addition, they report on the use of embryonic stem cells, although their clinical applicability is not evident at present owing to only short-term effects and the possibility of immune rejection by the host. The latter problem may be solved by using induced pluripotent stem cells, which are extensively described in this article in the context of repair of cardiac tissue.

The last section of this issue, edited by Olaf Witt, shifts gears to focus on targeting and eliminating cancer stem cells in pediatric oncology. The cancer stem cell hypothesis implies that a fraction of tumor cells is responsible for self-renewal and therapy resistance of neoplastic disease. Therefore, successful treatment of cancer is thought to be dependent to a large extent on the capacity to eliminate these cancer stem cells, which have become key targets for intervention. Friedman *et al.* (13) describe the application of oncolytic viruses to target and specifically kill cancer stem cells and tumor cells. This therapy may evolve as a realistic approach to treat children with chemo- and radioresistant malignancies in the future, e.g., through the use of genetically engineered viruses expressing gene products engaged in cell death. This review gives a detailed description of viruses already used clinically and also focuses on next-generation viruses that are engineered to specifically target transformed cancer stem cells without interacting with normal stem cells. Obviously, the problem of specific targeting to the cancer stem cells must be solved before oncolytic therapy can be applied in the developing child.

In the next review, Kamijo (14) focuses on (cancer) stem cell molecules, such as BMI1 in neuroblastoma, as putative targets to kill cancer stem cells and tumor cells. Knockdown or transfection of stem cell–related molecules in neuroblastoma cells reveals the mechanism of action of these molecules and how they regulate tumor growth and differentiation. Knowledge of the expression and function of these cancer stem cell–related molecules can now be used for the future development of molecular targeted therapy for refractory neuroblastoma in children.

Manoranjan *et al.* (15) report on novel cellular target molecules on cancer stem cells in the most frequently malignant brain tumor diagnosed in children: medulloblastoma. The authors focus on the signaling pathways of Sonic hedgehog and Wingless and the novel BTIC self-renewal pathway. Regulatory mechanisms and how these molecules initiate and support tumor growth are discussed in the context of targeted molecular interventions.

Castelo-Branco and Tabori (16) conclude this special issue by discussing the clinical possibility of targeting cancer stem cells in pediatric oncology. In many pediatric cancers, the initial response to therapy is relatively high but tumors may recur. The authors explain this phenomenon by noting that, in general, cancer stem cells are resistant to chemotherapy and radiation, facilitating tumor recurrence. Molecular targeting to exhaust cancer stem cells might become one of the most effective ways to fight cancer, especially as an add-on therapy after the bulk of the tumor load has been eradicated.

In conclusion, the excellent reviews in this issue highlight the potential benefits of stem cell–based therapies as well as of targeting aberrant stem cells in pediatrics. The major hurdle that still prevents widespread application of the promising preclinical findings is the very important issue of safety. The successful clinical application of hematopoietic and MSC treatment in immune-related pediatric disorders holds promise for the future and should encourage translational research in this rapidly developing field.

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