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INVITED COMMENTARY

Maximising impact of small cohort studies

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The current practice of post-transplant *immunosuppression* is based on the use of relatively unspecific small molecule agents and targeted antibodies. This methodology attempts to balance the risk of rejection against health risks associated with immunosuppression. Overall, this approach has led to the great success of organ transplantation with a very reasonable patient and graft survival for most organs. However, pharmacological immunosuppression is also associated with a variety of unwanted side effects. The alternative strategy of inducing specific graft acceptance would avoid the risks associated with traditional immunosuppressives and, in addition, may favorably contribute to long-term organ survival by reducing chronic rejection.

Infusion of immune-modulating cells as a means of achieving tolerance is not new, being first observed many years ago following donor-specific blood transfusions. Many protocols and cell types have been evaluated in a variety of preclinical models: from splenocytes via whole bone marrow, T-regulatory cells, regulatory myeloid cells, dendritic cells to mesenchymal- and even embryonic-stem cells. Throughout these developments, the game-changing potential of these therapeutic concepts has always been evident to the knowledgeable observer. However, the great success of immunosuppressive pharmacotherapy has hampered the development and adoption of novel, cellular-based therapies.

So, how should one study new immunosuppressive strategies when unclear risks are associated and a viable clinical alternative is existent? Randomized controlled studies have the greatest regulatory impact and are generally oriented toward avoiding false positive study results (type I error rate). However, such trials are not necessarily designed to detect signals that infringe patient safety and the risk of failure is high – especially in relationship with the costs. These considerations are of particularly important for new biological agents, such as cell therapy, where despite encouraging initial results, uncertainty continues to exist regarding dose-ranging and safety (e.g. tumorigenicity, modulation of immunity, and differentiation). Thus, new study concepts are needed for this clinical field; consequently there is great interest in the possibility that clinical development can include adaptive features, such as on-study protocol changes or analyses guided by examination of the accumulated data at interim points.

Giuseppe Remuzzi's laboratory in Bergamo is using such an adaptive approach to address the issue whether administration of mesenchymal stromal cells (MSC) can be used to induce tolerance. They have promoted a "tailor-made stepwise" approach, opting to treat only two patients per cohort, but to subsequently study these subjects extensively with best available immunological methods. Findings are then applied to the next cohort and the protocol is modified appropriately. In the first study, two living-related

donor kidney recipients were given *ex-vivo* expanded, autologous, bone marrow-derived MSC at day 7 post-transplant, after induction therapy with basiliximab and low-dose thymoglobulin [1]. In that study, MSC infusion did induce a pro-tolerogenic environment characterized by lower numbers of memory/effector CD8+ T-cells, expansion of CD4+Tregs and reduction in donor-specific CD8+ T-cell cytotoxicity (compared to control kidney transplant recipients given the same induction therapy but not MSC). However, shortly after cell infusion, both MSC-treated patients developed acute renal insufficiency. Histologic and immunohistochemic analysis of graft infiltrating cells did exclude an acute cellular or humoral rejection, but intragraft recruitment of neutrophils together with MSC, as well as complement-C3 deposition was observed.

In this issue of *Transplant International*, Perico *et al.* report the results from the second cohort of two patients [2] treated under this "tailor-made step-wise" protocol. As additional murine studies had outlined that timing of the MSC infusion is critical and that MSC localize to lymphoid organs where they promote early expansion of Tregs, the clinical protocol was modified accordingly [3]. And indeed, in these next patients, the engraftment reaction seen with early post-transplant administration of MSC was absent. In particular, the clinical course of patient 1 further suggests that MSC are a clinically feasible form of alternative induction therapy, provided they are given prior to the transplantation. However, the second patient illustrates that acute rejection could not be prevented despite induction of an enriched Treg cell population. Higher HLA haplotype mismatches in this patient can possibly explain the occurrence of this rejection episode. Importantly, this patient did receive adequate depletion therapy. This observation perhaps illustrates the limited potential of MSCs under those circumstances.

Taken together, we thus believe that Remuzzi and co-workers are correct to study few patients very extensively before going forward with larger clinical endeavors. Their series of patients highlights the importance of an adaptive approach to identify an optimal dosing strategy for MSC infusions to induce a tolerogenic signature while reducing side effects. Nevertheless, the clinical benefit of the investigational treatment protocol over standard-of-care immunosuppression is entirely open and the protocol should be continued to finally prove efficacy compared to conventional immunosuppressive therapy. Clearly, adaptations during a development program may also result in a biased assessment of potential treatment or side effects. This stands against early large clinical studies that have been carried out in other regions of the world [4].

Overall, we thus support the concept of developing immunomodulative cell therapy in the field of solid organ transplantation in the hands of few well-organized laboratories *on small cohorts of patients* [5,6]. Additionally, the installment of independent monitoring committees with well-described roles for adapting methodology and international exchange among clinical programs [7] will be the key to ongoing successful development of cell therapies – not only for the field of solid organ transplantation.

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References

1. Perico N, Casiraghi F, Introna M, *et al.* Autologous mesenchymal stromal cells and kidney transplantation: a pilot study of safety and clinical feasibility. *Clin J Am Soc Nephrol* 2011; **6**: 412.
2. Perico N. MSC and kidney transplantation: pre-transplant infusion protects from graft dysfunction while fostering immunoregulation *Transplant Int* 2013. [Epub ahead of print].
3. Casiraghi F, Azzollini N, Todeschini M, *et al.* Localization of mesenchymal stromal cells dictates their immune or proinflammatory effects in kidney transplantation. *Am J Transplant* 2012; **12**: 2373.
4. Tan J, Wu W, Xu X, *et al.* Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. *JAMA* 2012; **307**: 1169.
5. Reinders ME, de Fijter JW, Roelofs H, *et al.* Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation: results of a phase I study. *Stem Cells Transl Med* 2013; **2**: 107.
6. Popp FC, Fillenberg B, Eggenhofer E, *et al.* Safety and feasibility of third-party multipotent adult progenitor cells for immunomodulation therapy after liver transplantation – a phase I study (MISOT-I). *J Transl Med* 2011; **9**: 124.
7. Franquesa M, Hoogduijn MJ, Reinders ME, *et al.* Mesenchymal stem cells in solid organ transplantation (MiSOT) fourth meeting: lessons learned from first clinical trials. *Transplantation* 2013; PMID: 23759879. [Epub ahead of print].

