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Combined kidney and bone marrow transplantation from the same donor – looking for renal allograft immune tolerance

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Imagine. Just imagine it would be possible to perform organ transplantation in humans without the need for immunosuppression (IMS), so that the transplantation itself would be an instant pathway to a state of complete well-being and perfect health...

This may sound like a mere dream. But many of the most remarkable achievements of mankind started up as dreams. Organ transplantation, though still not as infallible as we would like it to be, is one of them.

The aim in organ transplantation is to achieve tolerance to the allograft. Unfortunately, that is a very rare, and usually an incidental phenomenon in kidney transplantation, discovered after situations in which IMS was reduced – either because of cancer or serious infection episodes or because of patient non-adherence – and good allograft function was maintained or did not deteriorate. Clinical operational tolerance is defined as a situation in which a solid organ transplant recipient maintains good graft

function, without IMS, for at least a year¹. Nowadays, is not yet possible to predict, with a reasonable degree of certainty, in which patients we can, or not, reduce or withdraw IMS.

On the other hand, the available immunosuppressants are far from being “the perfect drug”: apart from inducing a non-specific and generalized state of immunodepression, which can contribute to the occurrence of infection and malignancy, they have a set of adverse effects that can also have other serious impact in patients’ health and survival, like hypertension, diabetes, dyslipidaemia, nephrotoxicity, and so on.

In order to overcome these inconveniences, 3 transplantation units in the United States (US) started a search for clinical tolerance in kidney transplantation and recently reported their results. These three methodologies have in common the administration of blood or bone marrow donor cells with the kidney, and so, until now, these procedures have only been done in living kidney transplantation. Nevertheless,

their effort and results should be highlighted in the transplantation community²⁻⁴.

The fundamentals of this approach came from the attempt to cure patients suffering from multiple myeloma and chronic kidney disease, who were fully matched with their donors, from whom they received both bone marrow and kidney transplants, and it was possible to withdraw IMS without rejection, or kidney disease recurrence⁵.

The foundations behind immune tolerance in those patients are believed to be related with the presence of chimerism in which donor blood cells replace recipients' blood. Chimerism can be 100% or full, in which recipients cells are non-existent, as it happens in bone marrow transplantation for blood malignancies, or partial (mixed), in which donor and recipients' cells coexist, as it happens in the cases described below⁶. A recipient can harbour donor blood cells from all lineages or only T, B or other cell types.

The approach in each of those US transplant units has its own particularities but they all use total body or thymic irradiation in their conditioning regimen.

The Stanford regimen, which has changed over time, according with the results that were obtained, apart from total body irradiation, uses rabbit antithymocyte globulin (thymoglobulin), prednisolone, cyclosporine and mycophenolate mofetil (MMF)². These investigators treated three different groups of patients. The first group included six human leukocyte antigen (HLA) mismatched patients that were transplanted between 2000 and 2003, and did not receive MMF. The criteria to stop IMS in this group were presence of transient chimerism, absence of rejection episodes and absence of graft versus host disease (GVHD). Immunosuppression was stopped in only two patients, that suffered acute rejection a few months later, and IMS was restarted. A second group of 22 fully HLA matched patients was transplanted between 2005 and 2013, the conditioning regimen included MMF for one month. The criteria to withhold IMS in these patients were changed for presence of persistent chimerism for at least 6 months, absence of acute rejection episodes, absence of GVHD and absence of rejection on a protocol biopsy performed 2 weeks before the planned IMS withdrawal. In 17/22 patients, IMS was successfully stopped with an observation period of 2-66 months. One patient restarted IMS

because of a lupus flare. Four patients did not stop IMS because of absence of long-lasting chimerism and/or clinical or subclinical rejection episodes ($n = 3$) and focal segmental glomerulosclerosis recurrence ($n = 1$). In order to achieve a more profound and lasting chimerism that the authors believe to be the basis for allograft acceptance, they transplanted a third group of 10 haplotype matched patients that were subjected to the same conditioning of group 2 but that received increasing numbers of donor CD34+ cells (stem cells) and donor T cells. Indeed, the authors found out, that the highest levels of early chimerism were obtained in the patients that received the highest amounts of both CD 34+ and T cells. Two receptors from this cohort were already tapering IMS at the time of article publication. In the same report, the investigators showed the 100% graft survival at 8 years of 20 fully matched patients subjected to this protocol that was compared with the 86% graft survival of a fully matched group of 49 patients, transplanted between 2001 and 2013 in the same centre, and that had received conventional IMS². They also compared the observed 100% graft survival of 30 patients submitted to the innovative protocol with the 83% expected graft survival at 5 years of matched patients submitted to conventional IMS protocol from the Organ Procurement Transplant Network/Scientific Registry of Transplant Recipients (OPTN/SRTR).

In spite of high levels of initial IMS, the described adverse effects of this approach were relatively mild and easily treatable – several infection episodes in the first year with neutropenic fever occurring in only one patient... The most serious problems were two cases of cancer, breast and thyroid, respectively at 2 and 7 years post-transplant, (that were diagnosed and treated at an early stage and were in remission at the time of the report), and one patient with known coronary artery disease died suddenly after 3 years without IMS. So, to these authors, it is possible, in some patients, under a defined protocol that includes total body irradiation and the administration of donor CD34+ cells and T cells with the kidney allograft, to induce clinical tolerance to the latter, and so maintain its good function without the need for IMS. This was attained in 75% of the recipients and graft survival was 100%.

Another group of investigators, at the Massachusetts General Hospital, reported their results recently³. They progressively included 10 haplotype matched

patients in a conditioning protocol that includes cyclophosphamide, thymic irradiation, a humanized anti-CD2 monoclonal antibody, which depletes T cells and blocks co-stimulation, and a calcineurin inhibitor (either cyclosporine or tacrolimus). This scheme also changed over time, so that prednisolone and increasing administrations of rituximab were added in order to overcome the production of donor specific antibodies (DSAs) that were documented after IMS taper in some patients. In their protocol, the mixed chimerism was only transient, disappearing in 2-3 weeks after donor bone marrow cells administration in all patients. Almost every patient developed what the investigators called “engraftment syndrome”, composed by a set of symptoms similar to a cytokine release syndrome, alleviated by corticosteroids, except the acute kidney injury (AKI). It occurred in 9/10 patients and was severe (peak creatinine attained 3.5-15.4 mg/dl, between 10-20th day post-transplant). Effectively, this syndrome usually appeared after day 10, at a time when self-blood cells repopulated in the circulation. Another drawback of this protocol, in comparison with the one from Stanford University, is the occurrence of DSAs. There were three allograft losses: one due to AKI that cursed with thrombotic microangiopathy, another because of acute humoral rejection (this patient was discovered to have high level DSA before the kidney transplant) and the last due to acute cellular rejection, after an episode of acute pyelonephritis, that progressed to interstitial fibrosis and tubular atrophy. In two other patients, IMS was restarted, one due to chronic humoral rejection and another because of primary disease recurrence (type I membrano-proliferative glomerulonephritis). At the end, only four out of 10 patients were without IMS by the time of the report. Although the authors state that in those in whom it was possible to withdraw IMS the adverse events were low, we must keep in mind that they were quite severe in the others... As in this protocol only transient chimerism was achieved and, even so, immune tolerance to the kidney was observed in some patients, the investigators hypothesize the occurrence of regulatory cells in the thymus during the chimeric phase that would interact with a specific antigen or cell in the kidney allograft that would perpetuate tolerance. Finally, they conclude that it was possible to achieve immune tolerance to the allograft without IMS (the longest patient was off IMS for more than 10 years) in haplotype-matched recipients with kidney and bone marrow transplantation from the same donor. As stressed above, the

main problems in this protocol were the AKI (which may be severe), and the emergence of anti-donor antibodies (which seemed to be solved by the administration of four rituximab doses in the peri-transplant period).

The Northwestern Memorial Hospital transplant unit is using a manipulated pack of donor bone marrow cells in order to achieve immune tolerance to the transplanted kidney⁴. The conditioning regimen includes the administration of fludarabine, cyclophosphamide, tacrolimus, MMF and total body irradiation. The bioengineered pack of haematopoietic cells also includes tolerogenic CD8+/TCR- graft facilitating cells. This protocol is part of a phase II FDA regulated clinical trial. Another particularity of this unit's study is the inclusion of patients with low HLA-matched kidneys (the lowest, 0 in 6). They transplanted 15 patients and reported their results in 2013⁴. Nine patients developed high levels of full blood and T cell chimerism, which was long-lasting in all except one patient who suffered bone marrow failure at 3 months post-transplant and a viral infection, developed sepsis and lost his allograft. In six out of eight patients with high-level chimerism, the IMS was successfully stopped. Transient donor chimerism was observed in three patients – in two the conditioning protocol had not been done as expected (for technical or clinical reasons) and the third patient was highly sensitized (historical panel reactive antibody (PRA) of 64%; actual PRA of 33%). Failure of donor stem cell engraftment occurred in one HLA completely mismatched patient with a high PRA (52%). He developed early haemolytic uraemic syndrome related to tacrolimus use, and was switched to sirolimus. A later biopsy showed recurrence of his primary kidney disease (IgA nephropathy) and the investigators opted to maintain him under that immunosuppressant drug.

The immune follow-up of all these patients included *in vitro* tests of donor-specific hyporesponsiveness, like the mixed lymphocyte reaction and the cell-mediated lymphocytolysis assays, and the authors initially based their decision to withdraw IMS both in donor-specific hyporesponsiveness and durable chimerism. They found that patients that had persistent chimerism remained tolerant to their donors, but the ones with transient chimerism, even if with donor-specific hyporesponsiveness, could reject their allografts. Therefore, the investigators believe the key

to immune tolerance is durable chimerism, namely of donor T cells.

Concerning adverse effects with the present conditioning protocol, their patients suffered neutropenia for a mean of 10 days, thrombocytopenia for a mean of 12 days, there were several viral infections (herpes zoster, BK viraemia) and a fungal infection (in a patient who lived in an endemic area for histoplasmosis). There were no cases of GVHD, “engraftment syndrome”, or appearance of DSAs.

The studies presented above show us that it is possible to induce immune tolerance to the kidney allograft by using haematopoietic or bone marrow cells from the same kidney donor. Taken together, they seem to be quite instructive in the way to achieve tolerance. It appears that high levels of durable donor chimerism, is essential for such a result, where a good kidney allograft function is maintained without IMS. It looks like that the Stanford and Northwestern protocols are better tolerated. The long term of those patients in which IMS was successfully withdrawn seems to be excellent. The challenges ahead are to extend these procedures to cadaveric kidney transplantation, and eventually, to the transplantation of other organs as well.

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