Xenotransplantation—current status and future perspectives

Emanuele Cozzi1,2,3,*, Erika Bosio2,3, Michela Seveso2, Marta Vadori2,3 and Ermanno Ancona2,3,4

1Direzione Sanitaria, Padua General Hospital, Via Giustiniani 3, 35128, Padua, Italy, 2CORIT (Consorzio per la Ricerca sul Trapianto d’Organi), Passaggio Gaudenzio 1, 35131, Padua, Italy, 3Department of Medical and Surgical Sciences, University of Padua, Via Ospedale 105, 35128, Padua, Italy and 4Clinica Chirurgica III, Padua General Hospital, Via Giustiniani 2, 35128, Padua, Italy.

Research efforts have shed light on the immunological obstacles to long-term survival of pig organs transplanted into primates and allowed the identification of targets for specific immune intervention. Accordingly, the development of genetically engineered animals has overcome the hyperacute rejection barrier, with acute humoral xenograft rejection (AHXR) currently remaining the most important immunological obstacle. At this stage, a better control of the elicited anti-pig humoral immune response and avoidance of coagulation disorders are the two primary research fronts being pursued in order to overcome AHXR. Nonetheless, it is encouraging that porcine xenografts can sustain the life of non-human primates for several months. Proactive research aimed at the development of a safer organ source is also underway. It is anticipated that ongoing research in several fields, including accommodation, tolerance, immune suppression and genetic engineering, will result in further improvements in non-human primate survival. However, until convincing efficacy data and a more favourable risk/benefit ratio can be established in relevant animal models, progression to the clinic should not be viewed as an option.

**Keywords:** xenotransplantation; pig; primate; humoral rejection; genetic engineering; coagulation; safety

**Introduction**

The requirement for alternative organ and tissue sources to supplement human donation and ensure supply for transplantation is recognized. In this context, xenotransplantation is being explored as a possible avenue to address this issue. Xenotransplantation has had a long and eventful history and has come a long way since the first reported cases of animal bone transplants and blood transfusions to man in the sixteenth and seventeenth centuries [1]. However, its practical application still presents considerable difficulty. Even the process of allotransplantation is associated...
with numerous obstacles. These are partially addressed by careful donor selection, accurate donor–recipient matching and the use of life-long immunosuppression which may often result in undesired side-effects. Allotransplantation also has an associated safety risk. Following the transplantation of cadaveric organs, undiagnosed infections or malignancies derived from the donor organ may be transmitted to recipients, often causing patient death [2, 3]. Nonetheless, xenotransplantation is subject to a more complex immunological scenario than that following allotransplantation, possible physiological discrepancies and reservations regarding its safety.

With the pig as the preferred source species for the potential clinical application of xenotransplantation, genetic modification has enabled the development of more immunologically ‘compatible’ organs which resist hyperacute rejection (HAR), the first immunological insult, by the overexpression of human complement regulatory proteins [4]. Although HAR has largely been overcome, the long-term survival of xenografts is primarily inhibited by the subsequent onset of acute humoral xenograft rejection (AHXR). Accordingly, strategies to avoid AHXR continue to focus on xenoreactive antibody and complement. In addition, novel immunosuppressive approaches designed to induce tolerance or accommodation of xenografts present the latest frontline strategies to control the recipient immune response. Finally, safety concerns regarding the possible transmission of infectious agents following the xenotransplantation of porcine organs have been taken seriously and are the subject of ongoing studies.

This review describes the current status of xenotransplantation with reference to recent preclinical data describing results in pig-to-non-human primate cardiac, renal and islet transplantation studies, with the discussion addressing issues related to immunology, physiology and safety. Where necessary, results generated in other xenotransplantation models will also be reviewed.

**Overcoming the immunological barriers**

Because of the significant antigenic disparities present on tissues derived from different species, the immunological response towards a xenograft is more complex than that towards an allograft. Furthermore, the rejection profiles differ between solid organ and cellular xenotransplantation, and this is discussed herein.

**Solid organ xenotransplantation**

Distinct types of rejection occur following xenotransplantation of vascularized organs to non-human primates, namely HAR, AHXR,
acute cellular xenograft rejection (ACXR) and chronic rejection. HAR is a very rapid event that results in irreversible graft damage and loss within minutes to hours following graft reperfusion [5]. The process of HAR involves both immunological and non-immunological factors. However, it is triggered by the presence of xenoreactive natural antibodies (XNA) present within the recipient at the time of transplantation [6]. It is widely accepted that natural xenoreactive IgM (and also IgG in the case of rodents [7]) initiates HAR by activating the complement cascade, mediating complement deposition and endothelial cell activation, resulting in platelet activation, coagulation and disruption of vascular endothelial integrity.

Many studies have now conclusively demonstrated that XNA which recognize the Galα1-3 Galβ1-4GlcNAc-R (αGal) epitope are the most important in the onset of HAR. Strategies to remove these XNA and reduce complement-mediated damage have proven efficacious in overcoming the HAR barrier. The removal of XNA has been achieved by ex vivo serum perfusion, plasmapheresis and XNA neutralization, reviewed by Cozzi et al. [8].

Clearly, donor organs not expressing αGal would be a better choice for xenotransplantation. However, the development of genetically modified pigs lacking the galactosyltransferase gene (αGalT−/−), and hence αGal epitopes, has been a technically difficult process. Only recently have such pigs been produced [9], and they are already proving to be an important addition to the xenotransplantation armamentarium. Indeed, following the deletion of this single gene, αGalT−/− hearts and kidneys transplanted to baboons did not undergo HAR [10, 11]. Nonetheless, it is surprising that Sharma et al. [12] could show the presence of residual αGal epitopes in αGalT−/− pigs, possibly added to lipids by iGb3 synthase, albeit at very low levels. Additional studies to further investigate these observations are eagerly awaited.

The problems subsequent to the activation of the complement cascade have been tackled by several different approaches. The administration of cobra venom factor, soluble complement receptor-1, C1-inhibitor, intravenous immunoglobulins or anti-C5 antibody treatment have all demonstrated beneficial effects in preventing the onset of HAR in ex vivo and in vivo pig-to-primate models [13]. However, although effective, systemic complement interference may not only compromise the recipient innate defence system but is associated with the risk of side-effects and is costly. With this in mind, important advancements in complement control were achieved by the development of transgenic pigs expressing human complement regulatory proteins, namely human decay accelerating factor (hDAF; CD55), membrane co-factor protein (MCP; CD46) and CD59. The transplantation of organs from transgenic pigs expressing human complement regulators has resulted in the inhibition of the activation of the
complement cascade and has proven, in the majority of cases, to be effective in overcoming HAR [14–16].

In situations where HAR is avoided, grafts are most often destroyed by a process called AHXR. The pathology of AHXR is not very dissimilar to that observed in HAR, initiating with the deposition of fibrin and the upregulation of tissue factor, ultimately resulting in vascular thrombosis and oedema. Deposits of antibody and complement are hallmark features, causing endothelial cell activation, swelling or disruption. Cellular infiltrates consisting of macrophages, neutrophils, CD8+ T cells and a few NK cells are also observed [5]. AHXR is distinguished from HAR by its delayed kinetics following transplantation (>24 h) combined with the observation of initial graft function before the onset of rejection [14] and is characterized by type II endothelial cell activation [17]. Current widespread opinion concurs that the humoral response, which may involve natural and/or elicited antibodies, is a central event in the establishment of AHXR, with several lines of evidence supporting such thinking. First, anti-pig antibody deposition is considered a hallmark for the diagnosis of AHXR [5]. AHXR may be delayed or prevented by the administration of immunosuppressive agents that also act to inhibit the humoral response [18]. Furthermore, anti-αGal antibody depletion by the systemic administration of αGal-expressing polymers has been shown to reduce the severity or delay AHXR [19]. Finally, xenoreactive antibody removal via immunoapheresis before and after transplantation prevented the onset of AHXR in immunosuppressed primates [20].

Although anti-αGal antibodies are important in the establishment of AHXR, non-αGal antibodies may also play a role [8]. This was suggested by the study of Kuwaki and colleagues [10] where AHXR was observed in five of eight cases of heterotopic αGalT−/− pig heart transplantation to baboons. In the absence of αGal epitopes, grafts still demonstrated features of AHXR (although reduced) including antibody deposition and consistent thrombotic microangiopathy, inferring that non-αGal antibodies may play a role in its onset. This was also suggested by the data of Chen et al. [21], who demonstrated that AHXR of αGalT−/− kidneys was associated with the presence of circulating elicited anti-non-αGal antibodies. However, in this study, demonstration of a direct role of such antibodies in the pathogenesis of AHXR in these xenografts was not provided. These data in conjunction with those of Lin and colleagues [20] suggest that the avoidance of AHXR will require strategies to control the elicited antibody response against non-αGal antigens and possibly the disorders of the coagulation system.

Although the obstacles presented by the humoral response are clearly the most important with regard to the survival and function of vascularized grafts, the risk of graft damage by cellular mechanisms should not be dismissed. Solid organ xenografts explanted from immunosuppressed
primates have been shown, in some cases, to contain a multifocal lymphocytic infiltrate composed of T and B cells, macrophages and some NK cells in association with the presence of direct tissue damage. This histological picture is defined as ACXR [5]. ACXR is not associated with vascular thrombosis or interstitial haemorrhage nor with significant deposits of fibrin, immunoglobulin or complement components. Notwithstanding strong in vitro demonstrations of cellular xenograft responses (reviewed by Buhler and Cooper [22]), and in contrast to in vivo results in the hamster-to-rat model [23], it is important to underline that ACXR per se does not lead to graft failure following pig-to-primate solid organ xenotransplantation. At this stage, therefore, it would seem that graft damage directly mediated by immune cells can be prevented by the immunosuppressive regimens currently available [22]. On the other hand, a contribution of T cells to the development of the elicited anti-xenograft humoral immune response cannot be ruled out.

With regard to the phenomenon of chronic rejection, very little information is available in the pig-to-primate context. This is most likely because of the fact that long-term survival of xenografts is not routinely obtained because of the problems posed by AHXR. However, very recently, a phenomenon described as chronic xenograft vasculopathy was reported in a pig-to-baboon heterotopic cardiac transplantation model [10]. Despite chronic immunosuppression, in three primate recipients of αGalT−/− hearts which survived 78, 110 and 179 days, respectively, both humoral and cellular features of rejection were associated with the development of chronic xenograft vasculopathy, characterized by intimal thickening, fibrin exudation, complement and immunoglobulin deposition and cellular infiltration. In this context, a further demonstration of chronic xenograft rejection has been achieved in a hamster-to-rat model, where chronic rejection of cardiac xenografts, showing features similar to those observed in allotransplantation, was described. However, differences in the nature of arterial injury, antibody deposition (predominantly IgM rather than IgG as observed in allografts) and the nature of the cellular infiltrates were noted [24]. Most importantly, as observed in both experimental and clinical allotransplantation, immunosuppression with leflunomide and cyclosporine has shown beneficial effects on the chronic rejection process.

**Cellular (islet) xenotransplantation**

The transplantation of non-vascularized tissue, such as islets of Langerhans, is not believed to be subject to classical HAR rejection phenomena [25]. Two principal reasons appear to underlie this observation. First, in contrast to exocrine and endothelial cells, islet endocrine cells appear to express
low to negligible levels of the $\alpha$Gal epitope, making them intrinsically resistant to the binding of the majority of the XNA repertoire [26]. Second, the isolation process results in the removal of most of the islet vasculature, depleting the principal source of $\alpha$Gal target epitopes.

Moreover, as islet revascularization results from the in-growth of recipient capillaries [25, 27], the endothelium, which is of recipient origin, is not a target for circulating anti-donor xenoreactive antibodies, avoiding subsequent antibody-mediated damage. Non-$\alpha$Gal antibodies have been shown to be able to mediate islet damage in vitro. However, observations following in vivo transplantation studies currently point to a very small contribution of the natural and elicited humoral responses in islet rejection.

Nonetheless, a phenomenon described as the instant blood-mediated inflammatory reaction (IBMIR) may be just as damaging to islets immediately following their exposure to primate blood as HAR has proven to be in the case of solid organ xenografts. Indeed, the exposure of porcine islets to whole blood results in an immediate inflammatory reaction, characterized by macroscopic coagulation, the activation of complement, consumption of platelets and infiltration of leukocytes [28]. Although the reaction occurs with a kinetics similar to HAR, failure to observe antibody deposition on the graft is a distinguishing feature. Nevertheless, in the context of intraportal islet infusion, the IBMIR may account for the premature loss of grafted islets and the consequent large tissue volume required to achieve a functional islet mass following transplantation via this route. In this regard, the use of novel approaches such as dextran sulfate [29] may prove to be beneficial in abrogating this response.

Islets which survive the IBMIR subsequently succumb to cell-mediated rejection phenomena. Studies assessing pig islet fate following transplantation to non-human primates in the absence of immunosuppression predominantly demonstrate engrafted islet destruction via the infiltration of immune cells (largely T cells) at the graft site, resulting in localized graft destruction [30, 31]. Porcine islet survival following transplantation to non-human primates varies greatly, depending on the immunosuppression regimen employed and the site of islet implantation, and is also likely influenced by the expertise of the transplantation team involved. It is of interest that using an immunosuppression regimen consisting of anti-IL2R and anti-CD40L monoclonal antibodies, FTY720, everolimus and leflunomide, Hering and colleagues [32] were able to obtain survival of pancreatectomized non-human primates xenografted with porcine islets for greater than 180 days. Nonetheless, islets showing histological signs of rejection were characterized by CD4$^+$ and CD8$^+$ T-cell and macrophage infiltrates, in the absence of antibody deposition, underlining the central role of the cell-mediated immune response in porcine islet rejection, as previously reported by others [31, 33, 34].
Novel immunosuppressive strategies directed towards the specific abro-gation of the cellular response, such as novel molecules which target T-cell co-stimulation pathways, are expected to result in prolonged islet survival. Alternatively, immunoprotection via encapsulation has already demonstrated beneficial effects in primates [35]. Finally, some are trying to counteract the anti-pig T-cell-mediated immune response by transgenic manipulation. Specifically, transgenic pigs expressing the NK- and T-cell apoptotic inducer TRAIL have been generated [36]. The in vivo advantage for xenograft survival conferred by such transgene expression has yet to be determined.

**Accommodation and tolerance**

Achieving long-term xenograft survival in primates in the absence of continued immunosuppression may, at some stage, become possible by the establishment of accommodation or tolerance. In the case of accommodation, grafts survive long-term notwithstanding the continuing presence of xenoreactive antibodies and complement. Tolerance, on the other hand, is the result of recipient non-responsiveness towards a graft. By inducing tolerance, the immune system of the recipient accepts donor antigens as self.

With regard to accommodation, it has been suggested that changes in both the graft itself and the host immune response contribute to its establishment [37]. Changes in the graft include alterations that primarily affect the antigenic profile of the cells within the graft, such as the reduced expression of antigenic determinants or increased expression of molecules such as complement regulators [38, 39]. Alternatively, accommodation of the graft could occur as a consequence of the regeneration of protective substances such as heparan sulfate in the graft or following the acquired resistance of endothelial cells to environmental insults. In this case, the upregulation of protective genes such as heme oxygenase-1, A20, Bcl-2 and Bcl-xl is believed to be a central event [40], although this idea has recently been challenged [41].

Accommodation could also ensue as a consequence of changes in the host immune response. These include a shift of the elicited antibody repertoire towards a class of antibodies with a relatively limited capacity to activate complement, the shift from a Th1 to a Th2 response or a situation in which T-cell help is compromised. In any case, it is clear that the process of accommodation is still incompletely understood and further insight into its onset is necessary to develop the appropriate immunomodulatory strategy to reproducibly obtain establishment of this phenomenon in the primate.

Many approaches have been applied in efforts to induce tolerance at both the central and peripheral levels. Although tolerance has been reproducibly achieved in rodent xenotransplantation models by several
strategies, achieving tolerance in large animal xenotransplantation models is still elusive despite the application of strategies including mixed haematopoietic chimerism, thymic transplantation, the induction of regulatory T-cell populations and anergy by costimulation blockade [42, 43]. In contrast to allotransplantation, it is likely that a successful tolerance protocol for xenotransplantation will require the induction of both T- and B-cell tolerance, given the significant role of the humoral response in all phases of xenograft rejection [44]. However, it is encouraging that such tolerance has been achieved in rodent models [45] and that prolonged survival has been reported following co-transplantation of porcine kidneys with thymic tissue. Indeed, Yamada et al. [11] could demonstrate that the application of a tolerance-inducing protocol enables normal graft function in the absence of rejection for up to 83 days. This approach seems promising also in the light of demonstrations of pig-specific unresponsiveness via mixed lymphocyte reactions in two long-term surviving recipients and the absence of a pig-specific cytotoxic T lymphocyte response in one case. On the other hand, the role of an immune-mediated mechanism behind the presence of a mild and focal thrombotic microangiopathy in the grafts cannot as yet be ruled out. Similarly, the requirement for continuous immunosuppression throughout the study, including the administration of a human anti-CD154 monoclonal antibody occasionally associated with thrombotic complications [46, 47], suggests that further refinement will be necessary before complete tolerance can be achieved. Nonetheless, these results provide optimism for the successful induction of this process in the primate.

**Physiology**

The existence of potential physiological incompatibilities between donor and recipient in the context of xenotransplantation is an important consideration. Currently, because of their comparable organ size, rapid growth rate, large litters, the possibility of genetic manipulation and a more manageable ethical profile in comparison with other species, pigs are considered to be the most appropriate organ source [48]. Although physiological incompatibilities between pigs and primates are recognized, to date these do not appear to represent an insurmountable challenge to the long-term survival of porcine renal or cardiac xenografts [11, 49–51]. Such incompatibilities include molecular differences between the complement [13] and coagulation systems [52] of the pig and primate. Some have also hypothesized that anaemia, often observed in renal pig-to-primate xenotransplantation, could be related to the inability of porcine erythropoietin to adequately stimulate primate haematopoietic precursors [53], although conclusive evidence is still lacking.
As far as coagulation is concerned, it is of note that porcine von Willebrand factor interacts with human platelet receptors with high affinity, possibly resulting in elevated procoagulant activity. Porcine tissue factor pathway inhibitor (TFPI) is not able to neutralize human factor Xa and is therefore unable to inhibit the direct activation of human prothrombin to thrombin. In addition, although porcine thrombomodulin has been shown to bind human thrombin and Protein C, the human thrombin–porcine thrombomodulin complex is a poor activator of Protein C. The insufficient production of activated Protein C contributes to enhanced levels of thrombin favouring the initiation of clotting. Approaches such as the use of platelet fibrinogen receptor antagonist (GPIIbIIIIa), P-selectin inhibitor and soluble ATP diphosphohydrolase (ATPDase/CD39, the major vascular nucleoside triphosphate diphosphohydrolase, whose activity generates the anti-thrombotic and anti-inflammatory mediator adenosine) may provide some benefit in prolonging the survival of xenografted organs in primates but have yet to be tested (as reviewed by Robson et al. [52]).

Furthermore, transgenic modulation of the clotting cascade by de novo expression or induction of anticoagulants, or elimination of procoagulant molecules on xenogenic vascular endothelium, may represent an additional potential therapeutic strategy. In this context, several target gene candidates for transgenic expression (e.g. CD39, TFPI, thrombomodulin, hirudin and CD73) or knockout (e.g. Tissue Factor, PAR3, PAR4 and Fgl-2) in pig tissues have been identified. Encouraging results, although only obtained in vitro [54, 55] and in small animal models [56–60], have provided a basis for the future genetic manipulation of porcine organs, able to overcome thrombotic events that compromise xenograft survival.

Notwithstanding the physiological differences reported and the advantages that transgenesis may provide, studies in non-human primates suggest that porcine heart and kidney are able to work in primates and sustain their life for up to several months [10, 51, 61]. During such a time, the organs support normal levels of activity, with the recipients exhibiting normal social behaviour. Together, these observations suggest that an adequate control of the immune response such as that achieved by Lin et al. [20] could mitigate the functional significance of the physiological differences reported, further extending the survival of transplanted pig organs in the primate.

Safety

Ensuring a high safety profile for xenotransplantation is of primary importance in view of its possible clinical application. Indeed, there is
the need to minimize the risk of potential transmission of infectious agents through this approach. Accordingly, research has been undertaken by many groups in this area. Currently, using specific pathogen-free colonies and specialized animal husbandry, it is possible to exclude the vast majority of known bacterial, viral and parasitic pathogens from pig herds. Furthermore, research conducted during the past 10 years and retrospective studies in humans exposed to live porcine cells and tissues have not shown transmission through these procedures of any potential infectious agents to human, including viruses [62].

Nevertheless, studies assessing the safety of xenotransplantation are ongoing and are primarily focused on viruses, especially porcine endogenous retrovirus (PERV) which are encoded in the germ line DNA. In this respect, extensive investigations have demonstrated that multiple copies of PERV are integrated in the genome of all pig strains and that viral particles are produced by normal pig cells (reviewed by Fishman and Patience [62]). Three classes of infectious PERV have been identified (PERV-A, PERV-B and PERV-C) and classified based on differences in receptor recognition, which are responsible for differences in host range [63]. PERV have been isolated from both porcine cell lines and primary cells, although viral particles derived from primary pig cells are generally present in low titres and show limited replication competence, a favourable aspect with respect to the xenotransplantation of pig organs. Although PERV-A and PERV-B can infect human cells in vitro, the PERV-C subgroup lacks this capacity [63]. However, it has recently been observed that in vivo recombination between PERV-A and PERV-C is possible and can produce a human-tropic recombinant virus [64, 65]. Nevertheless, recombinant PERV-A/C proviruses have not been identified in the germ line DNA of pigs capable of transmitting PERV [66]. Most importantly, no evidence of human infection with any PERV, even with the recombinant PERV-A/C virus, has been reported.

As far as exogenous viruses are concerned, particular interest has been paid to the four families of herpesviruses identified in pigs: porcine cytomegalovirus (PCMV) and porcine lymphotropic virus 1, -2 and -3 (PLHV-1, PLHV-2 and PLHV-3). Although PCMV activation has been documented in pig-to-primate xenografts, causing clinical disease in the xenotransplanted organ and the detection of viral DNA in primate tissues, it does not appear to cause invasive disease in transplanted primates [67]. Moreover, it has been demonstrated that PCMV can be effectively excluded from source pigs by early weaning [68]. Of the three PLHV viruses identified, only PLHV-1 is associated with a lymphoproliferative syndrome similar to post-transplantation lymphoproliferative disease following allogeneic bone marrow transplantation in swine, but such a disorder has not been observed in pig-to-primate xenotransplantation [67].
It has been pointed out that some of the strategies aimed at minimizing xenograft rejection may increase the risk of zoonoses. First, the use of immunosuppressive regimens and tolerance induction protocols may exacerbate the risk of infection from otherwise non-infectious or latent animal pathogens. Second, viruses released by cells that do not express αGal, such as those of αGalT−/− pigs, lack αGal epitopes on their envelope and cannot be recognized by anti αGal antibodies, therefore becoming less sensitive to complement-mediated inactivation [69, 70]. Third, the presence of human complement regulators on transgenic pig cells may reduce complement-mediated defence mechanisms against infections. Last, some human complement regulatory proteins are receptors for human viruses. Therefore, in genetically engineered pig lines expressing such human complement regulatory proteins, pig viruses may adapt to infect humans once porcine organs have been transplanted [71]. However, it is reassuring that, despite all these considerations, to date, no experimental or clinical findings have supported these concerns.

In addition, it is noteworthy that PERVs and other viruses are susceptible to some of the currently available anti-viral agents [72]. Furthermore, pig lines have recently been identified, which are incapable of transmitting PERV to human cells in vitro [64, 73]. Finally, genetic manipulation of the porcine genome may provide an additional strategy to remove the viral risk. Indeed, both specific knockouts of endogenous retroviruses [74] and short interfering RNAs specific for PERV sequences [75] have been proposed.

Moreover, the development of microarray-based technology capable of rapidly identifying known and as yet unidentified potential infectious agents [76] may allow their timely identification and control in the xenotransplantation setting.

In conclusion, the described genetic procedures, in combination with controlled breeding conditions and lifelong source animal monitoring will ultimately result in the availability of source animals with a high safety profile and minimized risk of zoonoses.

**Concluding remarks**

The xenotransplantation field is moving ahead rapidly. Many carefully designed studies have been instrumental in the elucidation of the fundamental mechanisms involved in xenograft rejection and the enhanced comprehension of physiological and safety issues, resulting in the development of appropriate intervention strategies. In this light, the versatile technology of genetic engineering has proven a powerful tool enabling multiple transgenic modifications, designed to overcome the hurdles still associated with pig-to-primate xenotransplantation. This has made
possible the elimination of HAR by the use of transgenic pig organs expressing human complement regulatory molecules or indeed lacking the αGal epitope. Ongoing refinements are aimed at the mitigation of coagulopathy and the improvement of the safety profile of pig-to-primate xenotransplantation. These approaches, in combination with the development of novel immunosuppression and tolerance-inducing protocols, provide hope that a strategy enabling safe, long-term xenograft survival will be available in the not too distant future.

Nonetheless, progress in this field must meet the highest ethical and safety requirements. In addition, the risk/benefit ratio of a potential clinical application of xenotransplantation must be carefully and stringently evaluated. In this context, the ethical principles outlined in the position paper of the Ethics Committee of the International Xenotransplantation Association [77] remains the cornerstone document governing the conduct necessary for clinical trials. In addition, it is noteworthy that the development of internationally accepted guidelines, with the aims of promoting cooperation and harmonizing global practices related to xenotransplantation procedures, is a key objective of a resolution recently adopted by the World Health Organization [78]. In all cases, convincing efficacy data in non-human primate preclinical models is an indispensable requirement for progression to the clinic. In this light, the recent publication by Valdes et al. [79], in the absence of documented proof-of-concept data in non-human primates, raises the concern that the study may not have been conducted in keeping with the abovementioned position paper recommendations. Indeed, it is the authors’ opinion, and that of many, that only timely, well-planned, scientifically rigorous and ethically acceptable clinical studies will provide the confidence that will be needed by society at large to allow this emerging field to meet its clinical potential.

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**References**


12 Sharma A, Naziruddin B, Cui C et al. Pig cells that lack the gene for alpha1-3 galactosyltransferase express low levels of the gal antigen. Transplantation 2003;75:430–6.


42 Tseng YL, Dor FJ, Kuwaki K et al. Bone marrow transplantation from alpha1,3-galactosyltransferase gene-knockout pigs in baboons. Xenotransplantation 2004;11:361–70.


