Therapeutic issues in the treatment of vascularized xenotransplants using gal-knockout donors in nonhuman primates

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Purpose of review

Solid organ xenotransplantation could be the future of transplantation, but improved outcomes are required in experimental models before clinical trials are justified. This review summarizes recent advances in solid organ xenotransplantation using organs from α1,3-galactosyltransferase gene-knockout (GTKO) pigs (with or without other genetic modifications) and novel therapeutic approaches.

Recent findings

Work on the development of genetically engineered pigs has been considerable during the past few years, with many research institutes reporting the outcomes of research. Multiple gene modifications on a GTKO background have been reported, and the results of transplantation using organs from these pigs have been published. Progress, however, has been variable, and several obstacles, for example, coagulation dysregulation, have been identified. Heterotopic pig heart xenotransplantation has been associated with graft survival up to 8 months, but kidney graft survival has not improved significantly.

Summary

The availability of GTKO pigs with additional genetic modifications aimed toward expression of multiple complement-regulatory proteins and/or human thromboregulatory genes, combined with novel immunosuppressive regimens, for example, the inclusion of B cell-depleting agents, should improve pig organ survival in the near future.

Keywords

α1,3-galactosyltransferase gene-knockout, complement-regulatory proteins, organs, xenotransplantation

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Introduction

The identification of Galα1,3Gal (Gal) as the major target antigen for human and nonhuman primate (NHP) antipig antibodies and the production of α1,3-galactosyltransferase gene-knockout (GTKO) pigs [1] have helped to resolve the problem of hyperacute rejection. However, acute humoral xenograft rejection, chronic rejection, and other barriers to success, for example, coagulation dysregulation in the form of thrombotic microangiopathy and/or consumptive coagulopathy, need to be addressed if graft survival is to be significantly prolonged.

The initial studies in which kidneys [2] and hearts [3] from GTKO pigs were transplanted into NHPs had encouraging results, but used immunosuppressive regimens that are unlikely to be used clinically. New strategies should be aimed toward extending xenograft survival, minimizing the side-effects of the immunosuppressive agents, and enabling clinical trials to be considered.

Extensive reviews of xenotransplantation in pig-to-NHP models have been reported [4•,5]. The current review summarizes the most recent results of solid organ xenotransplantation using GTKO pig organs (with or without additional genetic modifications) in the pig-to-NHP model in combination with novel therapeutic approaches.

Heart xenotransplantation

Experimental heart xenotransplantation can be performed using three different techniques: heterotopic intraabdominal model, heterotopic intrathoracic model, and orthotopic model (Table 1) [6•,7,8,9•,10,11••,12,13••].

Heterotopic intraabdominal model

Mohiuddin *et al.* [11••] achieved graft survival of up to 8 months using hearts from GTKO pigs expressing a human complement-regulatory protein (CRP), CD46. Median graft survival was increased from 10 to 60 days by the addition of a B cell-depleting agent [anti-CD20

Table 1 Outcomes of heart xenotransplantation in α1,3-galactosyltransferase gene-knockout pig-to-nonhuman primate models between January 2009 and October 2010

Author [ref]	Type	Pigs	Recipients	*N*	Survival (days)	Mean (median) Survival (days)	Immunosuppression	Anticoagulant	Outcome
Azimzadeh *et al.* [6•]	HIA	GTKO	Baboon	14	n.a.	n.a.	None ($n=4$), ATG+anti-CD154+CVF+MMF+Cs (±CTLA4-Ig)	Heparin, aspirin	Early graft failure, complement deposition
	HIA	GTKO/CRP	Baboon	9	n.a.	n.a.	None ($n=1$), ATG+anti-CD154+CVF+MMF+Cs (±CTLA4-Ig)	Heparin, aspirin	CRP (either CD46 or CD55). Reduced incidence of early graft failure, minimal complement deposition
Burdorf *et al.* [7]	HIA	GTKO	Baboon	3	<1,<1, 6	3 (1)	ATG+anti-CD154+CVF+MMF+Cs ($n=2$)+CTLA4-Ig ($n=1$)	n.a.	n.a.
	HIA	GTKO/CD46	Baboon	6	2, 8, 10, 10, 12, 28	12 (10)	ATG+anti-CD154+CVF+MMF+Cs ($n=4$)+CTLA4-Ig ($n=2$)	n.a.	n.a.
Byrne *et al.* [8]	HIA	GTKO	Baboon	6	n.a.	n.a. (21)	Splenectomy+rituximab+ATG+TAC+RAPA	n.a.	HAR in 90 min ($n=1$), AHXR, chronic vascular antibody deposition, complement deposition
	HIA	GTKO/CD55	Baboon	5	n.a.	n.a. (28)	Splenectomy+rituximab+ATG+TAC+RAPA	n.a.	No complement deposition, chronic vascular antibody deposition
Ezzelarab *et al.* [9•]	HIA	GTKO	Baboon	9	2.5 h,1, 6, 6, 7, 12, 12, 35, 56	15 (7)	None ($n=2$), CVF only ($n=1$), CVF+ATG+leflunomide ($n=1$), low-dose anti-CD154+CTLA4-Ig+MMF ($n=1$), ATG+CVF+anti-CD154+MMF+Cs ($n=4$)	Heparin, ketorolac, PGI2	No accommodation, AHXR, CC, TM, stenosis at anastomoses, ischemic myopathy
Brenner *et al.* [10]	HIA	GTKO	Baboon	2	8, 8	8 (8)	Rituximab+TAC+RAPA+Cs+FcγRIIb	n.a.	No rejection. Died due to ileus
	HIA	GTKO/CD46	Baboon	3	7, 17, 29	18 (17)	Rituximab+TAC+RAPA+Cs+FcγRIIb+ATG±immunoadsorption	n.a.	Mild-moderate rejection, sepsis, left ventricular thrombus
	HIA	GTKO/HO-1	Baboon	2	8, 13	11 (11)	Rituximab+TAC+RAPA+Cs+FcγRIIb±ATG	n.a.	Humoral rejection, cellular rejection, graft failure
Mohiuddin *et al.* [11••]	HIA	GTKO/CD46	Baboon	22	n.a. 179, >195 ongoing	n.a −(10, $n=6$) (60, $n=14$)	None ($n=2$), ATG+MMF+CVF+Cs+anti-CD154 ($n=6$) ATG+MMF+CVF+Cs+anti-CD154+Rituximab ($n=14$)	Heparin, aspirin, ketorolac	HAR when no IS. Several focal complications related to heterotopic xenografting (abdominal adhesions, bleeding, intestinal obstruction)
Bauer *et al.* [12]	HIT	GTKO/CD46	Baboon	2	<1, 50	26 (26)	Immunoadsorption pretransplantation, rituximab+TAC+RAPA+MMF+Cs	n.a.	Died from cerebral air-embolism (10 h). Poor myocardial perfusion due to ventricular fibrillation
McGregor *et al.* [13••]	OHT	GTKO/CD55	Baboon	6	2, 14, 23, 34, 40, 57	28 (29)	ATG+TAC+RAPA+Cs	n.a.	Rejection (extensive data are n.a.)

AHXR, acute humoral xenograft rejection; ATG, antithymocyte globulin; CC, consumptive coagulopathy; CRP, complement-regulatory protein; Cs, corticosteroids; CVF, cobra venom factor; HAR, hyperacute rejection; HIA, heterotopic intraabdominal; HIT, heterotopic intrathoracic; IS, immunosuppression; MMF, mycophenolate mofetil; n.a., not available; OHT, orthotopic; PGI2, prostacyclin; RAPA, rapamycin; TAC, tacrolimus; TM, thrombotic microangiopathy.

monoclonal antibody (mAb)]. However, their study was not without significant complications associated with surgical technique, immunosuppressive regimen, and other aspects of recipient management [14,15].

Byrne *et al.* [8] performed 11 transplants using GTKO or GTKO/CD55 pig hearts. Despite T- and B-cell depletion and splenectomy, median survivals were not prolonged in comparison to other reports in the literature. However, expression of CD55 reduced complement deposition in the graft. Similarly, Azimzadeh *et al.* [6•] transplanted GTKO or GTKO/CRP (CD46 or CD55) pig hearts into baboons and demonstrated that complement deposition and early graft failure were significantly reduced by CRP expression.

Ezzelarab *et al.* [9•] demonstrated that costimulation blockade with an anti-CD154 mAb prolonged graft survival, but early or late xenograft failure was associated with activation of the innate immune system. Consumptive coagulopathy occurred in six of nine recipients. Using the same model and immunosuppression as Ezzelarab *et al.* [9•], Burdorf *et al.* [7] concluded that a high pretransplantation level of antinonGal antibodies limited the efficacy of costimulation blockade. There is clearly a need for alternative or adjunct therapies to control the humoral response.

Brenner *et al.* [10] transplanted hearts from GTKO, GTKO/CD46, or GTKO/HO-1 (heme oxygenase-1) pigs. There was no difference in median survival in relation to the various genotypes of the organ-source pig, but extended survival was achieved when immunoadsorption was carried out.

The identification of major antinonGal antibodies and development of strategies to reduce the corresponding antigens on the pig [16] may lead to prolonged survival.

Heterotopic intrathoracic model

Bauer *et al.* [12] employed the original Losman and Barnard technique of intrathoracic heterotopic heart transplantation [17]. This would be a clinically applicable approach because, in view of the continuing presence of the recipient's native heart, graft failure would not necessarily result in death of the patient [12]. They reported two cases using GTKO/CD46 pig hearts in baboons and incorporated pretransplantation immunoadsorption of antipig antibody in the recipient. The first recipient died within hours from air embolism and brain damage, whereas the second graft functioned for 50 days.

Orthotopic model

McGregor *et al.* [13••,18] reported orthotopic heart transplantation of GTKO/CD55 pig hearts into baboons using a clinically applicable immunosuppressive regimen (Table 1). Longest survival was 57 days (with good cardiac function), which is the longest reported to date, and median survival was 28.5 days.

Kidney xenotransplantation

In a nonimmunosuppressed pig-to-monkey model, an Australian group showed that the combination of GTKO with expression of CD55/CD59/HT (H-transferase) improved renal xenograft survival and delayed or prevented the development of consumptive coagulopathy (Table 2) [6•,7,9•,19•,20–23,24••,25].

Under the Xenome Project of the European Union, pigs with multiple genetic modifications (GTKO/CD55/CD39/HT) were transplanted into NHPs. Cozzi *et al.* in cynomolgus monkeys [19•] and Le Bas-Bernardet *et al.* in baboons [21] reported similar outcomes using different clinically applicable immunosuppressive regimens. In the experience of Cozzi *et al.* [19•], multiple genetic modifications were not associated with prolonged recipient survival, though preliminary coagulation data were consistent with a lower extent of coagulopathy when compared with previous studies using pigs expressing CD55 on a wild-type background. They drew attention on the presence of $CD20^+$ infiltrating cells in the majority of the grafts, which correlates with Mohiuddin's experience that the addition of a B cell-depleting agent to the immunosuppressive regimen is beneficial.

The longest survivals of baboons with life-saving pig kidney grafts were reported by the Boston group in their thymokidney transplantation models [20,25]. Griesemer *et al.* [20] performed seven GTKO pig-to-baboon thymokidney transplants. Median survival was 49 days, with the longest survival being 83 days. This baboon was the only one that received whole body radiation and appears to have been reported previously [2]. Several immunosuppressive regimens, including different T cell-depleting (antithymocyte globulin or monoclonal mouse antihuman CD2b antibody) and B cell-depleting (anti-CD20 mAb) agents were tested. The kidney grafts showed no signs of cellular infiltration or deposition of IgG, and no grafts were lost from rejection. In the same thymokidney model, the same group reported that high levels of preformed cytotoxic antinonGal antibody did not induce hyperacute rejection, but were associated with early graft loss [25], correlating with the observations of Burdorf *et al.* [7].

With the goal of inducing transplantation tolerance, the Boston group performed GTKO pig-to-baboon renal xenotransplants using an extensive immunosuppressive regimen combined with GTKO pig bone marrow administration [23]. Two baboons received kidney transplants 2 or 17 days after bone marrow administration. One baboon

Table 2 Outcomes of kidney xenotransplantation in α1,3-galactosyltransferase gene-knockout pig-to-nonhuman primate models between January 2009 and October 2010

Author [ref]	Pigs	Recipients	*N*	Survival (days)	Mean (median) Survival (days)	Immunosuppression	Anticoagulant	Outcome
Azimzadeh *et al.* [6•]	GTKO	Baboon	7	n.a.	n.a.	None ($n=1$), ATG+anti-CD154+CVF+MMF+Cs (±CTLA4-Ig)	Heparin, aspirin	Early graft failure, complement deposition
	GTKO/CRP	Baboon	5	n.a.	n.a.	ATG+anti-CD154+CVF+MMF+Cs (±CTLA4-Ig)	Heparin, Aspirin	CRP (either CD46 or CD55), reduced early graft failure incidence, minimal complement deposition
Burdorf *et al.* [7]	GTKO	Baboon	3	<1, 5, 7	4 (5)	ATG+anti-CD154+CVF+MMF+Cs (±CTLA4-Ig)	n.a.	n.a.
	GTKO/CD55	Baboon	3	<1, 12, 12	8 (12)	ATG+anti-CD154+CVF+MMF+Cs	n.a.	n.a.
	GTKO/CD46	Baboon	2	5, 12	9 (9)	ATG+anti-CD154+CVF+MMF+Cs (±CTLA4-Ig)	n.a.	n.a.
Ezzelarab *et al.* [9•]	GTKO	Baboon	3	2, 3, 5	3 (3)	CVF only ($n=1$), low-dose anti-CD154+CTLA4-Ig+MMF ($n=1$), ATG+CVF+anti-CD154+MMF+Cs ($n=1$)	Heparin, ketorolac, PGI2	AHXR, CC, renal artery thrombosis
Cozzi *et al.* [19•]	GTKO/CD55/CD59/CD39/HT	Cyno	6	8–22	16 (16)	CyP+CSA+MMF+Cs	n.a.	Kidney failure, abdominal bleeding ($n=1$), AHXR
Griesemer *et al.* [20]	GTKO	Baboon	7	18, 28, 40, 49, 57, 81, 83	51 (49)	(a) Thymokidney+rituximab+ATG+LoCD2b + anti-CD154+TAC+MMF ($n=4$), (a) – LoCD2b ($n=2$), (a) – TAC + WBI ($n=1$)	n.a.	Died from drug reaction, invasive CMV infection, AMI, pleural effusion from proteinuria, ARDS,
Le Bas-Bernardet *et al.* [21]	GTKO/CD55/CD59/CD39/HT	Baboon	5	4, 4, 12, 13, 14	13 (13) (ISed)	None ($n=2$) or CyP+TAC+MMF+C1 inhibitor	n.a.	AHXR
Salvaris *et al.* [22]	GTKO/CD55/CD59/HT	Baboon	6	<2 h, 2, 3, 3, 4, 5	3 (3)	None	None	HAR, CC
Griesemer *et al.* [23]	GTKO	Baboon	2	8, 24	16 (16)	Splenectomy+WBI+Thymus Irradiation+ATG+LoCD2b+TAC+GTKO BM (+CVF in one case)	Heparin, PGI2	Renal failure, pulmonary edema, gross enlargement of the kidney, hemorrhage and thrombi
Lin *et al.* [24••]	GTKO	Baboon	1	7	7	ATG+anti-CD154+CVF+MMF+Cs	Heparin, ketorolac, PGI2	CC
	GTKO/CD46	Baboon	6	2, 4, 9, 10, 10, 16	9 (10)	None ($n=1$), ATG+anti-CD154+MMF ($n=4$), ATG+anti-CD154+MMF+Cs ($n=1$)	Heparin, ketorolac, PGI2	Fluid overload, CC
Nishimura *et al.* [25]	GTKO	Baboon	4	16, 16, 17, 50	25 (17)	Thymokidney+ATG+rituximab+anti-CD154 +low dose TAC+MMF	n.a.	Sudden death (thrombi in vessels), AHXR

AMI, acute myocardial infarction; ARDS, acute respiratory distress syndrome; BM, bone marrow; CMV, cytomegalovirus; CSA, cyclosporine; cyno, cynomolgus monkey; CyP, cyclophosphamide; ISed, immunosuppressed; LoCD2b, mouse antihuman CD2b antibody; WBI, whole body irradiation. Other abbreviations as for Table 1.

died from renal failure (day 8) and the other developed thrombocytopenia and consumptive coagulopathy and expired with pulmonary edema after 24 days.

Burdorf *et al.* [7] reported kidney transplants in baboons from GTKO, GTKO/CD55, and GTKO/CD46 pigs. Various immunosuppressive regimens included T-cell depletion and costimulation blockade. Prolonged survival was achieved with GTKO/CD55 and GTKO/CD46 pigs when compared to GTKO pigs. They observed the same outcomes as in their heart xenotransplantation model in that a high pretransplant level of antinonGal antibodies was associated with a substantial incidence of early graft failure. This antibody response has been reviewed by others [16,26].

Ezzelarab *et al.* [9•] reported that the GTKO genetic modification alone with depletion of complement activity in the recipient by cobra venom factor was not enough to prevent acute humoral xenograft rejection and/or consumptive coagulopathy. Lin *et al.* [24••] studied the role of tissue factor expression on circulating platelets and peripheral blood mononuclear cells (PBMCs) following either GTKO or GTKO/CD46 pig kidney transplantation in baboons. Tissue factor was detected on platelets on posttransplant day 1, but was not detected on PBMCs until consumptive coagulopathy was beginning to develop. Graft histopathology showed fibrin deposition and platelet aggregation ($n = 6$), but with only minor features indicating a humoral immune response, and no macrophage, B- or T-cell infiltration. Prevention of recipient platelet activation will be critical for successful pig kidney transplantation.

Liver xenotransplantation

The first and only xenotransplantations using GTKO pig livers came from the Pittsburgh group [27••], who reported 10 liver xenotransplants from GTKO or GTKO/CD46 pigs using a clinically applicable immunosuppressive regimen. The importance of size matching between pig liver and recipient baboon was emphasized. The narrow abdomen of baboons necessitated the use of livers from pigs that were almost 60% smaller in weight than the recipient baboon. Six of 10 baboons survived for 4–7 days. No obvious difference was observed between GTKO and GTKO/CD46 pig livers. In all cases, liver function was adequate, as evidenced by tests of detoxification, complement activity, coagulation parameters, and production of pig proteins, including coagulation factors [28•]. Pig coagulation factors and proteins appeared to function adequately in baboons, although interspecies compatibility of such proteins remains to be confirmed (Table 3).

Survival beyond 7 days was prevented by a profound thrombocytopenia that developed within 1 h after reperfusion of the graft, ultimately resulting in spontaneous hemorrhage at various sites [27••]. The authors postulated that the thrombocytopenia was associated with the expression of tissue factor on platelets after contact with the pig endothelium, resulting in platelet and platelet-PBMC aggregation and deposition of aggregates in the liver graft, although phagocytosis of the platelets by pig Kupffer cells or hepatocytes could not be ruled out.

The Pittsburgh study was primarily undertaken to determine whether the pig liver could be used as a bridge to allotransplantation in patients with fulminant liver failure. Bridging options, and inclusion and exclusion criteria have been discussed [29•]. The immediate development of thrombocytopenia needs to be prevented before a clinical trial would be justified.

Coagulation dysregulation

Three review papers discussed this topic [30•,31••,32] and are reviewed elsewhere in this issue by Cowan *et al.* [33]. The need for pigs expressing one or more human 'thromboregulatory' genes, such as tissue factor pathway inhibitor (TFPI) or human thrombomodulin, is crucial. Two attempts using CD39 pig kidneys have been reported (Table 2), but adequate expression of CD39 was doubtful [19•,21]. The effect of expression of a thomboregulatory gene or genes, therefore, remains untested. There are several pharmacologic agents that would likely prove beneficial, for example, heparin, aspirin, ketorolac, and prostacyclin (Tables 1–3), but which interventions will be beneficial, yet effective and safe, also remains uncertain [30•,32].

The discrepancies between the results reported by various groups with regard to the development of coagulopathy cannot be fully explained, though a number of factors may be playing roles, for example, differences in the source of the pigs, the immunosuppressive regimen, and the organ transplanted. The literature suggests that the development of consumptive coagulopathy is less common or delayed when intensive immunosuppressive therapy is administered [34], though the exact mechanism of this effect remains uncertain. It is well known that the costimulation blockade agent, anti-CD154 mAb, is associated with thromboembolic events [35–37], and it is possible that this may be a factor in some of the reported studies, though thrombotic microangiopathy develops in NHPs receiving conventional immunosuppressive therapy [34]. Our experience of transplanting GTKO pig organs does not indicate to us that the GTKO genetic modification increases the 'thrombogenicity' of the organ graft. However, Knosalla *et al.* [38•] have reported that there is a clear heterogeneity between renal and cardiac xenograft endothelium, which may account for the observed increased incidence

Table 3 Outcomes of liver xenotransplantation in α1,3-galactosyltransferase gene-knockout pig-to-nonhuman primate models between January 2009 and October 2010

Author [ref]	Type	Pigs	Recipients	*N*	Survival (days)	Mean (median) Survival (days)	Immunosuppression	Anticoagulant	Outcome
Ekser *et al.* [27••]	Orthotopic	GTKO	Baboon	2	<1, 6	4 (4)	ATG+TAC+MMF+Cs	PGI2	Donor versus recipient size-mismatch ($n = 1$), death or euthanasia due to hemorrhage in abdomen and lungs
	Orthotopic	GTKO/CD46	Baboon	8	<1, <1, 1, 4, 5, 6, 6, 7	4 (5)	ATG or CyP+TAC+ MMF+Cs, CL (to two donors), CVF (to one recipient)	Heparin (in two cases), ketorolac (in four cases), PGI2	PNF on CL treatment. Donor versus recipient size-mismatch ($n = 1$), very high TAC levels, death or euthanasia due to hemorrhage in abdomen and lungs

CL, clodronate liposomes; PNF, primary nonfunction. Other abbreviations as for Tables 1 and 2.

of consumptive coagulopathy after pig kidney xenotransplantation.

Complement regulation

Miyagawa *et al.* [39••] published a valuable review on complement regulation in the GTKO era. After GTKO pig organ xenotransplantation, complement is activated by the classical pathway, by the interaction of antinonGal antibodies and non-Gal antigens, and by ischemic injury; the alternative pathway, especially after pig islet transplantation; and the lectin pathway. The complement system represents an important recognition and effector mechanism of acute humoral xenograft rejection. The various CRPs, for example, CD35, CD46, CD55, CD59, C1-inhibitor, regulate complement activation at different points in the complement cascade. Therefore, it will be preferable to express multiple human CRP genes in GTKO pigs rather than rely on a single gene.

Cowan and d'Apice [32] emphasized the importance of antinonGal antibodies in the humoral response to a GTKO pig organ. Without complete suppression of production of these antibodies, there is progressive activation and injury of the graft endothelium, resulting in thrombotic microangiopathy, with subsequent graft loss [31••].

The potential role of cytotherapy

Cytotherapy [e.g., the infusion of bone marrow, T-regulatory cells, or mesenchymal stem cells (MSCs)] to the recipient of a solid organ xenograft could be beneficial [23]. In their quest for microchimerism, Griesemer *et al.* [23] administered GTKO bone marrow to baboons. Of the four baboons, two received pig kidney transplants, with no significant prolongation of graft survival compared to that in baboons that did not receive bone marrow (summarized above).

Although there are several different subtypes of T-regulatory cells, $CD4^+CD25^+Foxp3^+$ T cells are the most characterized and have been suggested as an immunosuppressive strategy in xenotransplantation [40•,41•,42]. Although in-vitro studies show expanded T-regulatory cells inhibit the baboon cellular response to pig PBMCs, the effect on prolongation of xenograft survival needs confirmation in NHP studies [43]. In contrast to cadaveric allotransplantation, the organ donor is known prior to xenotransplantation, which should facilitate therapy with T-regulatory cells.

The report of successful treatment of severe graft-versus-host disease after clinical bone marrow transplantation by MSCs created substantial interest [44]. MSCs prohibit the proliferation of T cells, inhibit

dendritic cell maturation, and induce T-regulatory cells, and hence may prove a potential therapy for solid organ transplantation. Ezzelarab *et al.* [45] discussed the potential of (organ) donor-specific genetically modified pig MSCs.

Future directions

Five years after the first results of GTKO pig-to-NHP organ transplantation were reported, the addition to GTKO of one or more human CRPs has been associated with better outcomes. The expression of multiple CRPs is probably preferable. Antithrombotic gene expression is required to reduce coagulation dysregulation. Table 4 [46•,47,48•,49•,50,51,52•,53,54] summarizes recently generated genetically engineered pigs. In this regard, human thrombomodulin-expressing pigs have been produced [55], but not yet tested *in vivo* [52•].

Pigs with potential resistance to the human cellular response are also becoming available. Stimulatory and inhibitory receptor interactions in xenotransplantation have been reviewed elsewhere [56]. The production of human Fas ligand-expressing pigs, which may prevent human $CD8^+$ and natural killer cell cytotoxicity, has recently been reported [48•]. With the aim of protecting against injury by natural killer cells, Weiss *et al.* [47] produced HLA-E/human beta2-microglobulin-expressing pigs. Pigs expressing CTLA4-Ig have been produced so successfully that the level of CTLA4-Ig in the blood was several times higher than the therapeutic level [46•]; these pigs demonstrated features of immunoincompetence, for example, infections, and required euthanasia. Major histocompatibility complex (MHC) class II transactivator (CIITA) gene knock-down pigs have been generated (*Dai Y et al.*, unpublished). In-vitro testing of the human $CD4^+$ T-cell response to cells from CIITA pigs indicated a much diminished human T-cell response, suggesting that organs and cells from these pigs should be significantly protected against the human/NHP cellular immune response [49•].

Other pigs that have become available include human HO-1-expressing pigs [51] and human A20-expressing pigs [50]. These will hopefully provide some antiapoptotic, anti-inflammatory, and cell protective effects. Porcine endogenous retrovirus siRNA-expressing transgenic pigs, aimed at preventing the activation and propagation of porcine endogenous retrovirus, have been produced [53,54].

Newer immunosuppressive and adjunctive agents, for example, anticoagulants, may also be beneficial. Alemtuzumab (anti-CD52 mAb) combined with mycophenolate mofetil depleted and maintained the number of $CD4^+$ cells at less than 25% for at least 1 year in cynomolgus

Table 4 Recent generation of genetically engineered pigs

Author [ref]	Genetically engineered pigs	Site of expression	Function	Mechanism of action
For prevention of immune injury				
Phelps *et al.* [46•]	Porcine CTLA4-Ig	High, ubiquitous	Costimulation blockade and decreased T-cell response	Porcine CTLA4-Ig binds to pig CD80 or CD86 and prevents direct response (pigs susceptible to opportunistic infection)
Weiss *et al.* [47]	HLA-E/human β2-microglobulin	Endothelium	Protection against human antipig NK cells	Interacts with inhibitory receptor CD94/NKG2A on human NK cells
Choi *et al.* [48•]	Human FAS ligand	Membrane bound - metalloproteinase resistant	Prevents human $CD8^+$ and NK cell cytotoxicity	FAS–FAS ligand-induced apoptosis
Hara *et al.* [49•]	CIITA-DN	Not available	Immunoprotection	Inhibition of SLA class II expression
Oropeza *et al.* [50]	Human A20	Skeletal muscles, heart and PAECs	Anti-inflammatory and antiapoptotic	Protects against TNFα-mediated apoptosis, blocks NF-κB and caspases
Petersen *et al.* [51]	Human hemeoxygenase-1	Not available	Cytoprotective	Antiapoptotic and cell protective
For prevention of coagulopathy				
Peterson *et al.* [52•]	Human thrombomodulin (hTM) in hCD59/CD55 background	Kidney>heart>> lungs> liver	Anticoagulant/anti-inflammatory activity	Binds human thrombin and activates protein C (anticoagulation)
For prevention of PERV				
Dieckhoff *et al.* [53] and Ramsoondar *et al.* [54]	Transgenic PERV siRNA expression	Ubiquitous	Prevention of propagation of PERV	RNA interference of *gag* and *pol* PERV genes

CIITA-DN, major histocompatability complex class II transactivator gene knockdown; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cell; NK, natural killer; PAEC, porcine aortic endothelial cell; PERV, porcine endogenous retrovirus; SLA, swine leukocyte antigen; TNF, tumor necrosis factor.

monkeys [57•] and, therefore, may have a role in xenotransplantation.

Conclusion

In summary, the increasing number of genetic modifications being made to GTKO pigs [58] and more effective immunosuppressive, anticoagulant, and anti-inflammatory regimens provide the expectation of improved experimental results that may eventually lead to the initiation of clinical trials.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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