A major advance toward clinical cardiac xenotransplantation

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Central Message

An article in *Nature* reports the survival of orthotopic pig-to-baboon heart xenograft recipients for up to 195 days. If reproducible, these results open a path toward clinical heart xenograft trials.

The Invited Expert Opinion provides a perspective on this topic based on the following paper: *Nature*. 2018; 564(7736):430. <https://doi.org/10.1038/s41586-018-0765-z>.

Consistent achievement of life-supporting cardiac xenograft function in a preclinical model is the benchmark established by an International Society for Heart and Lung Transplantation expert advisory panel in 2000 as a precondition for clinical translation.¹ This long-elusive goal appears to be close at hand based on a recent report by Långin and colleagues,¹ published late last year in *Nature*.² The Munich-based team reported that 4 of 5 consecutive baboon recipients of orthotopically implanted genetically modified pig hearts survived beyond 90 days. Months-long survival is a truly notable achievement in a model in which 30 days has rarely been accomplished previously. In 2 instances, the experiment was terminated by protocol at day 90, when evaluation revealed essentially normal myocardial function and histology. Two subsequent recipients were electively sacrificed after 6 months, with normal systolic and diastolic heart function for as long as the full treatment regimen was continued.

Consistent protection of the pig heart xenograft from immune injury is among the most important of the 4 striking new observations made by the Munich group. The immunosuppressive treatment regimen included either of 2 monoclonal antibodies that block the CD40-CD154 costimulation pathway. Previous work by us and others strongly suggest that targeting this pathway is sufficient and probably necessary to reliably prevent “delayed xenograft rejection” in heart^{3,4} and kidney⁵⁻⁷ models. Although neither antibody has been approved for use in humans, similar antibodies are currently being tested in clinical trials for nontransplant indications.⁸ Many clinician-scientists would consider the Munich team’s pharmacologic regimen “clinically applicable,” because it was well tolerated in this stringent baboon model, was effective to consistently prevent immune injury to the xenograft, and was not associated with clinical evidence of infection. Many of the additional medications used (mycophenolate mofetil, steroids, antithymocyte globulin, anti-CD20 monoclonal antibody, and antibiotic and antiviral drugs) are routinely used

in presensitized kidney and heart recipients, and thus reflect “conventional” immunosuppression and ancillary infection prophylaxis. Additional blockade of interleukin-6, interleukin-1, and tumor necrosis factor were included based on evidence from the authors’ prior experience and reports by others^{9,10} that these proinflammatory cytokines are elaborated by xenograft recipients. Whether cytokine blockade is necessary is a residual question important to clinical trial design, because using 3 cytokine pathway inhibitors indefinitely on top of induction T- and B-lymphocyte depletion followed by costimulation blockade-based (or conventional) immunosuppression would probably leave human xenograft recipients vulnerable to opportunistic infection. Likewise, long-term anticoagulation, with heparin and aspirin in the current report, is associated with well-known (if usually manageable) complications in other contexts and may be unnecessary.

The second remarkable new finding described in this article is that 1 or more mechanisms under the control of the mammalian target of rapamycin (mTOR) controlled physiologically inappropriate hypertrophy of cardiac xenografts, which is often observed in the pig-to-baboon orthotopic heart xenograft model. Prevention of cardiac xenograft hypertrophy apparently required mTOR inhibition, because severe hypertrophy and progressive graft dysfunction were observed in one instance in which a rapamycin pro-drug, temsirolimus, was not used, and in

both 6-month survivors when the drug was discontinued approximately 20 days before elective experimental termination. Whether through direct inhibition of myocyte hypertrophy, as the authors believe, or indirectly as a consequence of suppressing immune response to the graft, addition of mTOR inhibition appeared to be essential to the remarkable results they achieved.

Two other features of the Munich team's protocol deserve notice. The pigs used lacked expression of a carbohydrate antigen, Gal 1-3 α Gal, and contained human genes for membrane cofactor protein (hCD46) and thrombomodulin (hTBM). Gal 1-3 α Gal is recognized by a high proportion of the natural anti-pig antibody normally present in the blood of baboons and humans, and knock-out of the galactosyl transferase gene (GTKO) removes this important antigenic target from the pig's cells. The consequences of residual recipient antibody directed at other "non-Gal" pig antigens was attenuated by expression of a human complement regulatory protein, hCD46 (membrane cofactor protein).¹¹ However, coagulation pathway dysregulation occurs commonly in both the xenograft recipient (consumptive coagulopathy, thrombocytopenia) and xenograft (thrombotic microangiopathy) despite the combined GTKO and CD46 modifications, and even when pharmacologic control of both cellular and humoral rejection is apparently efficient, as reflected by absence of detectable T-cell graft infiltration or elicited anti-pig antibody.¹¹ As first reported by us³ and recently by others,¹² the current report confirmed that expression of hTBM in GTKO.hCD46 hearts coupled with an anti-CD40/CD154-based immunosuppressive regimen and heparin anticoagulation consistently prevented coagulation pathway dysregulation in baboons. Whether this result can be achieved exclusively using clinically approved immunosuppressants¹³ instead of currently unapproved costimulation pathway blockers is an important remaining question of practical importance to obtaining regulatory approval for a proposed pharmacologic "cocktail" to be tested in the first clinical trial. It is also possible that additional genetic modifications to the pig will permit reduced immunosuppression intensity or safely permit anticoagulation minimization or avoidance. However these key questions are answered, it represents a landmark in the xenotransplantation field that a specific pig phenotype (GTKO.hCD46.hTBM) and a specific drug regimen appear to comprise a viable candidate package for consideration by national regulatory authorities, grounding optimism that clinical introduction of safe, effective cardiac xenotransplantation is close at hand.

The fourth notable Munich protocol feature was use of continuous ex vivo organ perfusion rather than cold storage of the heart xenografts. Before this technique was adopted, these investigators consistently encountered perioperative cardiac xenograft dysfunction (PCXD).¹⁴ Occasional examples of survival of pig-to-baboon heart xenograft recipients

to 39 days (in 2000)¹⁵ or even 57 days¹⁶ (reported in 2015) clearly demonstrate that pig hearts can provide physiologically adequate circulation for a primate. However operative survival remained limited by PCXD to approximately 50% even in the hands of expert surgeons supported by experienced clinical anesthesia, perfusion, and veterinary intensive care teams.^{8,16} What causes PCXD? Pig hearts might be intrinsically intolerant of ischemia-reperfusion injury or particularly vulnerable to innate immune injury by natural killer cells, anti-non-Gal antibody, or other mechanisms. The much improved initial performance of perfusion-stored GTKO.hCD46.hTBM hearts reported by the Munich group for the first time enabled consistent operative survival in this previously daunting model. Whether by inducing cytoprotective pathways in endothelium or myocardium, by somehow altering expression of pig antigens or adhesion molecules or simply by minimizing pig heart xenograft ischemia, the durable graft performance they consistently accomplished places cardiac xenotransplantation on a viable path toward clinical application. Additional genetic modifications to the pig, to remove additional carbohydrate targets of anti-non-Gal antibody or improve thromboregulatory and anti-inflammatory defenses in the xenograft, may render machine perfusion unnecessary.^{7,17} Meanwhile, the authors have shown us a simple solution to a previously vexing (and still incompletely understood) phenomenon. Consequently, the field is no longer hobbled by inability to consistently "get out of the gate" with a well-functioning heart xenograft.

What are the current obstacles to clinical cardiac xenotransplantation? Internationally agreed standards propose that pigs intended for use in humans must be reared under conditions that minimize the risk of transmitting a known infectious agent to the recipient. At present, facilities designed around these standards are uncommon and not yet populated with the particular lines of pigs tested by the Munich team. Guidance for investigators, host institutions, corporate sponsors, institutional review boards, and national regulatory authorities regarding the design and conduct of xenotransplantation clinical trials have been developed and published under the auspices of the World Health Organization.¹⁸⁻²⁰ These recommendations have been revisited because of the perception that the risk/benefit ratio for xenotransplantation trials has shifted favorably in light of recent progress in the field.^{21,22} Specifically, it appears unlikely that porcine endogenous retrovirus will pose a significant risk to a xenograft recipient. Pig husbandry methods and clinical trial protocols have been developed that should detect and contain the spread of porcine endogenous retrovirus or any other currently known infectious organism or even any unsuspected pathogen that may arise. Thus the risk side of the risk-benefit equation has shifted in favor of the xenotransplantation field.

Meanwhile, encouraging results now reported by the Munich group for heart xenografts and xeno-kidneys by multiple other groups⁵⁻⁷ demonstrate that the probable benefit side of the risk-benefit equation has also improved. As noted earlier, some additional work is justified to demonstrate reproducibility of the specific regimen intended for use in the initial clinical trial and to learn whether particular components of the regimen are needed that might be expected to increase the risk of predictable complications (prolonged anticoagulation or multi-cytokine blockade). For example, if a CD40/CD154 costimulation pathway inhibitor were approved for clinical use for another indication or can be replaced with agents already in clinical use for allotransplantation, the regulatory approval path is likely to be less complicated.

Multiple groups have described patient populations with end-stage heart failure who might be among the first offered a cardiac xenograft.^{8,16,23} The US Food and Drug Administration has recommended that xenotransplantation initially should be limited to “patients with serious or life-threatening diseases for whom adequately safe and effective alternative therapies are not available... [and] who have potential for a clinically significant improvement with increased quality of life following the procedure.”²⁴ Patients with heart failure with limited timely access to allografts, relatively poor outcomes after transplantation, and few or no good alternative treatment options include highly sensitized patients after prior transplant or left ventricular assist device, and patients requiring biventricular mechanical support who have relative or absolute contraindications to allotransplantation. Likewise, patients with contraindications to systemic anticoagulation or to left ventricular assist device implantation (severe aortic insufficiency, a ventricular septal defect, refractory ventricular arrhythmias, or a prior mechanical valve prosthesis) might be offered a heart xenograft as a bridge to allotransplantation or a destination therapy.

If the Munich group's results are reproducible and can at least occasionally be extended to 1 year in the rigorous orthotopic pig-to-baboon heart xenograft preclinical model, it is likely that regulatory authorities will choose to permit carefully conducted clinical trials using a regimen directly informed by the current report. In my estimation, Längin and colleagues² have opened up a clear path for themselves and others to advance cardiac xenotransplantation toward the clinic. Successful clinical translation of xenotransplantation would finally realize the many potential favorable impacts on human health and welfare that would attend timely availability of an unlimited supply of healthy xenogeneic cells, tissues and organs.

Conflict of Interest Statement

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