nature biotechnology
VOLUME 20 • NO 3 • MARCH 2002

Will these pigs ever fly?

For those readers who feel a sense of déjà vu, you have seen the pig on the cover before. She is one of five piglets—named Noel, Angel, Star, Joy, and Mary in keeping with their December 25 birthday—that occupied news pages in early January. After delivering the pigs, PPL Therapeutics' subsidiary in Blacksburg, Virginia, also delivered a premature announcement, claiming the world's first report of cloned knockout pigs. In fact, another group of researchers collaborating with Immerge BioTherapeutics had produced a litter of four cloned knockout piglets months before, the results of which appeared in Science (295, 1089–1092, 2002) a couple of days after PPL's release.

Competition was fierce because these clones are the latest step in the race to turn pigs into organ factories for humans. The gene (GGTA1) that was deleted in these animals encodes α1,3-galactosyltransferase, which synthesizes one of the most important antigens in eliciting hyperacute rejection and (to a lesser extent) acute vascular rejection to xenografts. Importantly, because of the differences in immune responses to organs and cells, the lack of GGTA1 is likely to be particularly important for transplants of whole pig organs.

Essentially, two advances were required to produce the knockout piglets: adaptation of nuclear transfer technology to pigs (no mean feat considering the notorious fragility of pig embryos and the idiosyncrasies of pig reproduction); and the refinement of homologous recombination technology to enable specific targeting of genes implicated in immune rejection. Both groups went about creating their knockouts using a similar approach (despite differences in vectors, pig strain, and means of preparing sows for artificial impregnation). Gene targeting was used to inactivate GGTA1 in pig fetal fibroblast cultures, cells containing the deletion were then selected, and nuclear transfer was carried out to generate embryos that lacked one copy of the gene. The PPL paper, presented on page 251, provides independent confirmation of the results obtained by Prather and colleagues in Science, relates previously unreleased data confirming targeting of GGTA1 in pigs, and confirms deletion of the gene via Southern blots.

The next task for researchers is to produce—by either breeding or further rounds of targeting/cloning—pigs lacking both copies of GGTA1. When they've done that, they need to engineer pigs to carry five or six more genes that inhibit human complement activation and clotting around the xenograft, and then target pig adhesion molecules that could recruit human inflammatory cells. Put simply, we are still a very long way from ever turning this research into a clinical reality. What's more, the inability of current detection technologies to verify that transplants are free of viral contamination could condemn the field to regulatory oblivion, particularly if the European regulatory authorities continue their predilection for the precautionary principle and zero risk.

With advances in autologous stem cell technology and artificial organs gathering pace, xenotransplantation companies need to start making progress, and fast. The knockout cloning technology should enable a more systematic and rapid investigation of the antigenic targets involved in immune rejection. But will that be enough? Seven years ago, xenotransplantation pioneer Imutran (now out of business) predicted animal organs would be clinically available by 2002. If transgenic pig organs take another seven years to reach the clinic, stem cells and tissue engineering will be providing alternatives and the patience of those bankrolling xenotransplantation may have run out.

I'll be back

Platinum eyes, an iron grip, muscles of steel, and a wooden delivery. Arnold Schwarzenegger truly was The Terminator. In the eponymous film, The Terminator is an abject baddie, a killing automaton from the future sent to extinguish the leader of the rebellious forces by killing his mother before she conceives. Naturally, The Terminator fails in his task, as anyone familiar both with the imperturbable nature of timelines and the conventions governing movie storylines could have forecast. In the sequel, however, the roles would be reversed, The Terminator turning out to be a goodie, a robotic guardian sent back to protect the teenage rebel leader from an adversary of pure liquid metal evil.

The story of the Terminator gene looks set to follow a similar path as one environmental representative tentatively proposes that there may be a role, after all, for genes that limit the fertility of GM crops (see p. 212). Inattentive readers may need to be reminded that the Terminator technology was, in essence, a molecular switch that prevented the germination of seeds. Crops containing the Terminator technology were never marked. However, the mere concept of a gene that rendered plants unable to provide seed was sufficient to foment revulsion in the breast of those already discontented with the idea of GM plants. Gordon Conway, head of the Rockefeller Foundation, argued that it was unethical to deprive developing world farmers of the potential benefits of GM plants in the cause of corporate profitability and called upon the agricultural seed industry to "disavow the use of terminator technology." Monsanto capitulated to the pressure in October 1999. The 2000 corporate pledge of a born-again and humbler Monsanto affirmed its commitment "not to pursue technologies that result in sterile seeds." Other seed companies have made similar commitments.

The new Terminator technology, like the robot in Terminator II, would, it is envisaged, be a humbler, kindlier beast. Its role would not be to prevent resource-poor farmers from gaining illegal access to GM crops. It would be an environmental control mechanism—a way of reducing the unwanted spread of transgenes in field situations. English Nature, environmental advisors to the UK, have expressed concerns about the "stacking" of genes for herbicide tolerance in crops, such as oil seed rape. It believes that the environmentally designed GM crops of the future—developments which it favors—may depend on incorporating genetic incompatibility into crops. The genetic constructs may not be those for which DeKalb/Monsanto/USDA still holds a substantial IP portfolio, but with hesitant support from the informed end of the environmental movement, it looks as if the Terminator may well be back.