# Our experiences of the "Dark Days" of Xenotransplantation

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The impact of the current COVID-19 pandemic is having on xenotransplantation research, or research in general, in unprecedented. Here in Australia many laboratories have had to shut down for some periods of time, as only essential work was allowed.

We are not virologists, so other than showing that CD46 acts as a receptor for the measles virus, we were just observers for the PERV and BSE stories. The field is fortunate that the cow was not the species of choice for human xenotransplantation, otherwise it may have stopped the field indefinitely. The first examples of "mad cows disease" occurred in the mid 1980's, and "infection" to humans in the early 1990's. Yet, research quickly identified the agent, and measures put in place to stop further spread.

PERV is another chapter in the history of xenotransplantation. When it was shown that, *in vitro* at least, the PERV could infect human cells, the opponents of xenotransplantation believed that if the field was to progress to human trials it would bring a global pandemic, similar to what we now see. In an opinion article, we suggested that if PERV was to be such a problem, then we should have seen it already. Unfortunately, the fear of PERV was one of the reasons for a "moratorium" on xenotransplantation trials. Most in the field remained optimistic that ways to prevent this cataclysmic pandemic, and continued with their research. Indeed they were right, using CRISPR every copy of PERV can be removed from the pig genome. We were impressed with the boldness of the German group, who injected huge amounts of PERV into monkeys- it was harmless! Is a bit more boldness is needed today?

Another potential set back result for the field, was trying to modify Gal expression in pigs. We were among the first to show that Gal was the major target for human antibodies causing Hyperacute Rejection-HAR. But, what to do about lowering/ ablating Gal from pig tissues to decrease/avoid HAR? Gene Knockouts could be done in the 129 strain of mice due to multiple embryonic stem cell lines derived from this strain. It was suggested that 129 mice could be unique, due to the high incidence of spontaneous testicular teratomas in this strain. These ES cells that could be grown in vitro, modified and then used to produce viable mice. Several groups tried to isolated pig ES cells to no avail. We didn't have the expertise to try this in pigs- thus tried several other approaches. Firstly, we tried the glycosyltransferases to compete with the galactoslytransferase, then galactosidases, enzymes which cleaves Gal from the next sugar in the chain- leaving N-acetylgalactosamine exposed. We used these to produce low Gal cells in vitro,& in transgenic mice , Gal was significantly reduced , in our *in vivo* model of HAR, graft rejection was delayed, but not to such an extent to be of practical value. It was only with the advent of animal cloning, starting with Dolly the sheep, that the field again went forward.

The thing is our frustration with the relative SLOW PACE that clinical research is moving now - just as well the first allotransplants were done in the 1950s, where there were fewer theoretical objections to trying something new and potentially life saving. We give several examples: (1) our CD46 Pig Islets grafted into immunosuppressed diabetic monkeys; --monkeys were sacrificed at 3 month intervals--the last at 15 months; none were diabetic or required insulin, and all were healthy until sacrificed. This may be the FIRST example of both surviving and functional pig tissue in primates. Surely this could have then been tried in volunteer diabetic patients; the only drawback being a requirement for immunosuppression--used widely in humans for a variety of conditions. Secondly - in the early 1990s, Swedish scientists attached normal pig kidneys to human AV shunts (in situ for haemodialysis) and observed HAR - as expected. Surely GKO pig kidneys could have been used in patients to see what happened? If the kidney did not undergo HAR & produced urine, a lot could have been learned. If it failed - back to the drawing board! Such studies could easily have been organized with due monitoring of side effects (one of the Swedish patients had an anaphylactic reaction-easily treated) and care to avoid infection. Currently, we hear that Ig-ve pigs have been produced with most of the Human Ig genes inserted. Hopefully such HuIg pigs would also be Galo/o to avoid their antibodies being neutralized in vivo in humans. Immunising these pigs with COVID-19 virus would lead to a polyclonal antibody to use, passively, in patients (better than multiple Mabs) - while we await a vaccine, or the trials of infusing sever COVID-19 patiens with serum form individuals who were COVID-19 infected and recovered. (Yes, we know that pigs will have a different ”repertoire” of genes to humans—but—so what at this difficult time? ie time for a bit of boldness!).

However bleak the future looks at the present, we are encouraged that it has been in similar situations before, and always managed to find a solution. We are also encouraged by the new generation of researchers that now take up the challenges.