Thank you for asking me to be part of your project on thoughts from the ancient modern history of xenotransplantation. Before I start, I ask your forgiveness for what are sure to be inaccuracies in my recall of detail and timing of events and my sketchy knowledge of events since my retirement 10 years ago.

I became involved in the late 80's early 90's when I moved from managing a clinical renal transplant service to a Clinical Immunology Department and wanted to continue my research interest in transplantation but without patients. At that time xeno was largely a surgical endeavour involving primates and it was unable to make any meaningful progress despite several hubristic surgeons who were willing, nay enthusiastic, to actually transplant a few unfortunate patients. (Think here of the litany of setbacks to progress of the field: #1: early surgical hubris).

David White, then of Cambridge, made the first really meaningful advance by showing that the complement cascade regulator Decay Accelerating Factor or DAF, could inhibit various in vitro models of hyperacute rejection (HAR) in the pig-human combination. To my mind this was beginning the modern era of xeno research. David and co went on to generate DAF transgenics and showed some inhibition of HAR and early graft survival. He was so enthusiastic that he stood on the stage at the Rome Transplantation Society meeting and called for a move to the clinic (#2: early scientific hubris). Despite the Pope who came to the meeting and addressed us in the hot sun, inter alia supporting xeno, there was no enthusiasm for immediate clinical application because the preclinical data did not support it. On its own, inhibition of the complement cascade was not sufficient because xenoantibodies continued to cause havoc.

Initially two scientific groups tackled antibody. David Cooper's and our group showed by different routes that Gal was the main target. However, Uri Galili in a completely separate literature had already shown that Gal was the major target of human anti-pig antibodies. This prompted a series of efforts to remove the Gal target. The main effort was to inactivate the a1,3 galactosyltransferase gene in the pig. The other from Ian McKenzie's group was to compete for the enzyme's substrate using transgenic expression of human H-transferase. This was at least partially and often very effective but the appeal of elimination was greater. In those days, gene KO was performed in embryonic stem cells which were then used to generate hybrid blastocysts and finally hybrid animals which could then be bred on to become full KO animals. The only problem was that pig ES cells did not exist and we spent several years unsuccessfully trying to generate them. It was only when Dolly the cloned sheep came along that a technique that worked in pigs was found and there was a race to produce GalKO pigs. Our group won the patent race but not the actual race to first on the ground and within a couple of years at least 5 groups had made GalKO pigs. This was the second major advance toward clinical xenotransplantation. The GalKO and complement regulator transgenics were soon interbred and hyperacute rejection was overcome.

The next and overlapping era was that of the companies, the likes of Sandoz, Baxter and so on who had an interest in either immunosuppression or dialysis and so a position to protect. Their hope was a rapid translation to the clinic and / or to protect existing stakes such as dialysis, and they invested heavily in the various groups. The companies were very rough bedfellows and we were naïve virgins (setback #3). However, when the hope (or threat) of rapid clinical exploitation faded, they lost interest and the survivors went back to academic funding sore but experienced.

The cause of this fading of hope was two-fold, one from each side of the equation that needed solution for clinical application to be realistic. First, efficacy had to be shown. While there were plenty of signs of progress and indicators of imminent success in preclinical models, it was like reading a horror story. With each new page there was a new barrier, a new failure and new problem

that required another genetic modification for solution. This is still the problem today with the two major clinical applications that were the main focus at that time; renal and cardiac transplantation – both solid organs. The focus was yet to seriously turn to pancreatic islet cells. The companies probably could have coped with this but when the other side of the equation raised its head, the bell started to toll. Safety had been discussed widely, pigs were dirty but could be cleaned up and isolated in expensive barrier facilities and the companies threw money at the problem and built them. But then came the stain that could not (at that time) be scrubbed clean: porcine endogenous retrovirus (setback #4). The horror was compounded by the fact that it was a retrovirus (think AIDS) and that one of the most prominent medical (and xeno) researchers of the time, Fritz Bach of Harvard, rose up to his full height and declared xenotransplantation to be dead (setback 4.1 – God had spoken, even the Pope's prior blessing was now null and void). This was met with incredulity by xeno researchers many of the most prominent of whom were his friends and colleagues at Harvard Medical School. Bach's intervention was denounced as treachery (and nest fouling) and he was until his death persona non grata to many around him. The companies were faced with efficacy still needing more money and an unknown amount more time and now a seeming insoluble problem with safety. They backed out of funding but still held tight to their grips on our IP via our patents…..just in case we were able to make something work. We bound up our wounds, took antibiotics and slowly recovered, finding some dignity and sufficient dollars in old fashioned academic funding. Interestingly the (continuing) progress of xeno to the clinic has been so slow that all the primary patents that these companies coveted so much are all now time expired, like me.

When I retired 10 years ago, PERV was the only significant known barrier to safety of xeno that could not be managed. At that time, and correct me if I'm out of date, as far as I know there is still no direct evidence of a human being infected with PERV let alone it causing any illness. So, all the concern is based on a theoretical possibility. However, I understand that a US company has edited out all the PERV sequences along with Gal and other transferases and inserted many complement and other regulators. This holds great promise for xeno. However, a minor word against premature adulation (potential setback #5). Building a stable pig herd for clinical use from a founder can be very difficult because of inbreeding issues, as hybrid vigour and fecundity suffer. Old-fashioned pig husbandry is still relevant.

I wish you all good luck and patience in what I hope are the last stages (of still indeterminate duration) before clinical application of xeno is a reality. The virtues in xeno are patience, constancy, self-reliance, doggedness, resilience, avoidance of hubris (and companies), a healthy dose of cynicism, self-deprecation, humility, humour and excellent science to justify academic funding. If anyone has these virtues, I have yet to meet her.