CELL & GENE THERAPY INSIGHTS

SCALING UP/OUT: COST-EFFECTIVE & ROBUST TRANSITIONIONING THROUGH THE CLINIC TO COMMERCIAL MANUFACTURE

SPOTLIGHT

INTERVIEW with:
Ryan Cawood, CEO and Founder, OXGENE

Ryan founded Oxford Genetics in 2011, after earning a first class degree in genetics and a PhD from Oxford University. The idea behind the company was to simplify and standardise the process of DNA engineering using a proprietary DNA plasmid platform called SnapFast™ that allowed researchers – for the first time – to assemble complex sections of DNA as simply as 'molecular Lego'. Ryan used his background in genetic engineering and virology to guide and grow the business through a series of strategic changes that explored how further development of the SnapFast™ platform through in house research and development could help overcome multiple challenges in the development of new biologics.

This culminated in a rebrand to OXGENE in 2019, as the company redefined itself as a leading solutions provider, using a combination of proprietary technologies to address multiple pinch-points on the journey through design, discovery, development and manufacture of a novel biologic.

A necessary transition: why viral vector production for gene therapy needs to evolve

Cell & Gene Therapy Insights 2020; 6(8), 1163–1170

DOI: 10.18609/cgti.2020.127

Q Can you give us some background on the story of OXGENE, and its technological focus to date?

RC: I founded OXGENE, rebranded to as Oxford Genetics, nine and a half years ago. I was doing a PhD in gene therapy at the University of Oxford and making some quite complicated gene therapy vectors, which got increasingly challenging to construct as they got bigger and involved larger and larger pieces of DNA. By the time I finished my PhD, I was convinced that there was better way to build DNA. I founded the company on that central premise. The concept was to build DNA like Lego; to build consistent DNA blocks that had reproducible behaviors, and then assemble more complicated pieces of DNA from those predefined blocks.

At that point, I didn't know much about running a business, or how the industry worked. I originally thought we'd be a product-based company that would sell the pieces of DNA we made. That worked pretty well, but it became clear that the company was never going to grow much beyond that unless we changed the business model. We started to use the pieces of DNA we made to custom-build larger sections of DNA for customers. And then behind the scenes, we also started using that same platform to build our own technologies, and to invest in our own research and development. This grew into three different areas: antibody discovery, gene therapy manufacturing strategies, and CRISPR engineering. We have evolved as the markets have moved, and today most of our research is in the gene therapy area.

Q Why the strong focus on gene therapy, and why now? What is your take on how viral vector manufacturing needs to evolve, particularly in terms of its scalability demands?

RC: It's a really exciting time for gene therapy. When I was doing my PhD, the industry was struggling. There weren't many clinical trials going on, and there was little investment in the sector. I'm really pleased that as we've been developing our business, the industry has completely changed, thanks in large part to some clinical success stories in patients, which is excellent to see. We've followed the industry as it's expanded, which is also why we've invested more heavily in that part of our business.

> "...the industry is essentially using technologies – for the most part – that were established 20 or even 25 years ago."

In terms of the current state of play in gene therapy manufacturing, the industry is essentially using technologies – for the most part – that were established 20 or even 25 years ago. It's almost like making a cake. Every time you want to make a cake, one person puts all the ingredients in, mixes them up, and hopefully the cake comes out well in the end. Right now, to make a gene therapy vector, you have to transfect multiple plasmids into the cells, add the transfection reagents and hope for the best. It's not particularly reproducible; sometimes it

doesn't work, sometimes the yields are low, and it's very difficult to then scale the process up.

Our approach to viral vector manufacture needs to change.

> "Our approach to viral vector manufacture needs to change."

Q Turning to adeno-associated vector (AAV) manufacture in particular, what do you see as the critical factors in achieving this field's twin key goals of improved yield and quality, and how is this reflected in OXGENE's platform?

RC: Yield and product quality are inextricably intertwined. Some diseases require systemic gene therapy treatment, meaning that you need extremely large quantities of AAV per patient. But if you're going to deliver large quantities of virus systemically, you need the quality of your viral vector to be very high and very potent. Otherwise you're delivering material that either doesn't work or may cause toxicity. If you can improve yield and quality together, you can reduce cost of goods and have a product that's more active on delivery.

Q Can you go deeper into OXGENE's philosophy and approach to simplifying AAV production processes – for example, in terms of reducing the number of transfection steps required?

RC: Our philosophy is that the only way in which you will truly be able to scale AAV manufacturing is to completely remove the dependency on plasmids and the transfection process. This is partly because of the number of input ingredients you need, but also because the process itself is limited by cell density, is hard to scale, and comes with prohibitively high costs. We've been developing multiple technologies that all focus on reaching that objective.

One particularly exciting new technology we're bringing to market is TESSA, which stands for Tetracycline Enabled Self Silencing Adenovirus. If we think of how AAV is produced in nature, it only replicates when it's in the same cell as an adenovirus; that's why we call it an adeno-associated virus. In this natural setting, the AVV produced is of exceptionally high quality; almost every AAV particle has an AAV genome packaged inside it. But when we produce AAV using plasmids, for some serotypes only 2–5% of the particles actually contain a genome.

We wanted to reproduce 'natural' AAV replication, and to do that, we needed to use an adenovirus. But why aren't people doing that already? The main reason is that when you use adenovirus to manufacture AAV, you make about as much adenovirus in the end as you do AAV. This is potentially a major safety issue, and means you have to work really hard to purify the AAV and remove all the adenovirus. We knew this was the challenge we'd have to overcome, so we developed a way to halt the adenovirus lifecycle halfway through. This means that the adenovirus can go into a cell, convert that cell into a viral vector manufacturing machine,

> "...we've managed to get the yield from our lentiviral packaging cell lines pretty close to that of our transient transfection process."

then shut itself down. It can provide the help to make AAV, but doesn't make any more adenovirus. In terms of suppressing adenovirus production during an AAV manufacturing run, TESSA is somewhere in the region of 99.999% to 100% effective.

Once we developed this adenovirus, we thought about how to use it to manufacture AAV. We could replace the helper function in the AAV manufacturing process, but you'd still have to deliver two other plasmids: one with Rep and Cap and one with the ITR-flanked gene of interest. So we thought perhaps we could add the AAV Rep and Cap genes into the adenovirus as well, thereby removing another plasmid from the process. That has been tried before, but without success. However, because of our molecular Lego platform, we could make lots of different viruses in different configurations to find the one that worked best.

We can now deliver everything you need to manufacture AAV, with the exception of the ITR-flanked gene of interest, in a single virus; and there are many other transfection-free methods of delivering this.

Q Are there any aspects or features of the OXGENE platform that are designed specifically to solve bottlenecks in large-scale AAV vector manufacture?

RC: As we discussed before, the main challenges for AAV manufacture are maintaining – or improving – AAV yield and quality in large scale production. So far, the degree to which our TESSA platform improves AAV yield is serotype dependent. For some serotypes we've observed a ten-fold improvement, and for other serotypes we've seen a 100-fold improvement; that's just in the number of virus particles that are coming from the cell. What is almost more interesting is that when we look at those particles, they're also in some cases up to 2,000-times more infectious. As well as these improvements to yield and quality, we've also seen a significant increase in packaging efficiency. For AAV2, this has increased from about 2–5% to around 70%. Going back to how much AAV you'd then need to deliver to the patient, there may potentially be significant safety benefits to this as well.

Q Shifting the focus to lentiviral vectors (LVVs) production, can you outline this particular platform and how it addresses issues that relate to LVVs specifically?

RC: We've been developing packaging and producer cell lines for LVVs for about three and a half years, and we're now offering these out for evaluation. These cell lines allow you to

reduce the number of plasmids you need to transfect into the cells from four down to one in the case of the packaging cell line, or none for the producer cell line. The market expectation is that lentiviral packaging and producer cell lines will be the solution to scalable lentiviral vector manufacture, which is why we've focused our attention on this, perhaps more conservative, approach to manufacturing lentivirus than we've taken for AAV.

The fact that there are clinical products that use lentiviral vectors being used to treat patients speaks to the success of the industry. However, you need significantly fewer lentiviral particles per patient than you do for AAV, because lentiviral vectors are most commonly used for *ex vivo* cell therapies. And because you're then transducing the cells *ex vivo*, you don't require the 1 x 10^{12} viral particles per kilogram you might need to treat a patient with an AAV based gene therapy.

Lentiviral production is also slightly different, because we have a precedent to follow, in that retroviral packaging and producer cell lines have been around since the mid-to-late 1990s. Creating a stable producer cell line means that all the genetic components of the viral vector are integrated into the cell's own genome, so you no longer need to perform a transfection step to produce lentiviral vectors expressing your gene of interest. Now this is much simpler for retrovirus than lentivirus, because there aren't that many genes, but the number of genes in HIV-based lentiviral vectors – some of which are toxic to cells – make this a bit more challenging.

Q The traditionally high cost of LVV remains a major concern for the cellular immunotherapy field in particular – how does OXGENE's platform seek to aid in cost of goods reduction?

RC: If you run a bioreactor to produce lentiviral vectors, about 40% of the cost of goods comes from plasmids and reagents. If you can cut that cost by using a producer cell line, then you immediately make a significant saving on production. That's just in terms of your costs going in, not even considering the process improvements. For example, transfection limits batch size, and increases the complexity involved in actually making the virus. Simplifying this process improves reproducibility. That said, the main challenge for lentiviral packaging and producer cell lines is that viral yields are generally slightly lower than with the transfection process, leaving a trade-off between scalability and overall yield. So far, we've managed to get the yield from our lentiviral packaging cell lines pretty close to that of our transient transfection process. It's slightly lower for the producers, so we're busy optimizing and improving that now – but it's already at the point of commercial viability, because it would be cheaper to use this cell line than consistently produce large quantities of lentivirus by transient transfection.

> "...we are doing really exciting things in terms of genetic engineering and developing new approaches..."

Q Why is collaboration so important in viral vector bioprocess development, and how is this reflected in OXGENE's approach?

RC: We've been fortunate to have a number of different collaborations with some significant players in the gene therapy industry over the last 3–4 years. We might think we are doing really exciting things in terms of genetic engineering and developing new approaches, but end-user companies have a different perspective, and their feedback has been invaluable. If you're going to attempt to throw out the existing process, it's crucial to understand just how far you can push the boundaries, and the only people who can tell you that are the therapeutic companies. They've taught us a huge amount, and we hope to have many more collaborations in the future. It is the best way to learn what the industry needs, and the best way to make progress.

Q Can you sum up both your own and OXGENE's chief goals and priorities for the coming 12 months?

RC: We have just been through the process of refitting a new facility of around 7,000 square feet, which will allow us to expand our process development capabilities. Bringing our new viral vector manufacturing technologies to market is our number one priority for the year ahead. We want to get these to the point where we've done all the validation our customers will want to see, and made sure that the data is available for them in the event that they want to file those technologies with regulatory bodies.

Beyond that, we want to continue to grow the company. For the last 3 or 4 years we've been growing at around 160% a year, which has been great. This year is obviously going to be more challenging than others, due to the Covid-19 pandemic, but so far we are optimistic that we can continue our progress.

AFFILIATION

Ryan Cawood
CEO and Founder, OXGENE

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author holds patents or has patents pending relating to TESSA technology. The author has no other conflicts of interest.*

Funding declaration: *The author is an employee of and shareholder in Oxford Genetics Ltd.*

ARTICLE & COPYRIGHT INFORMATION

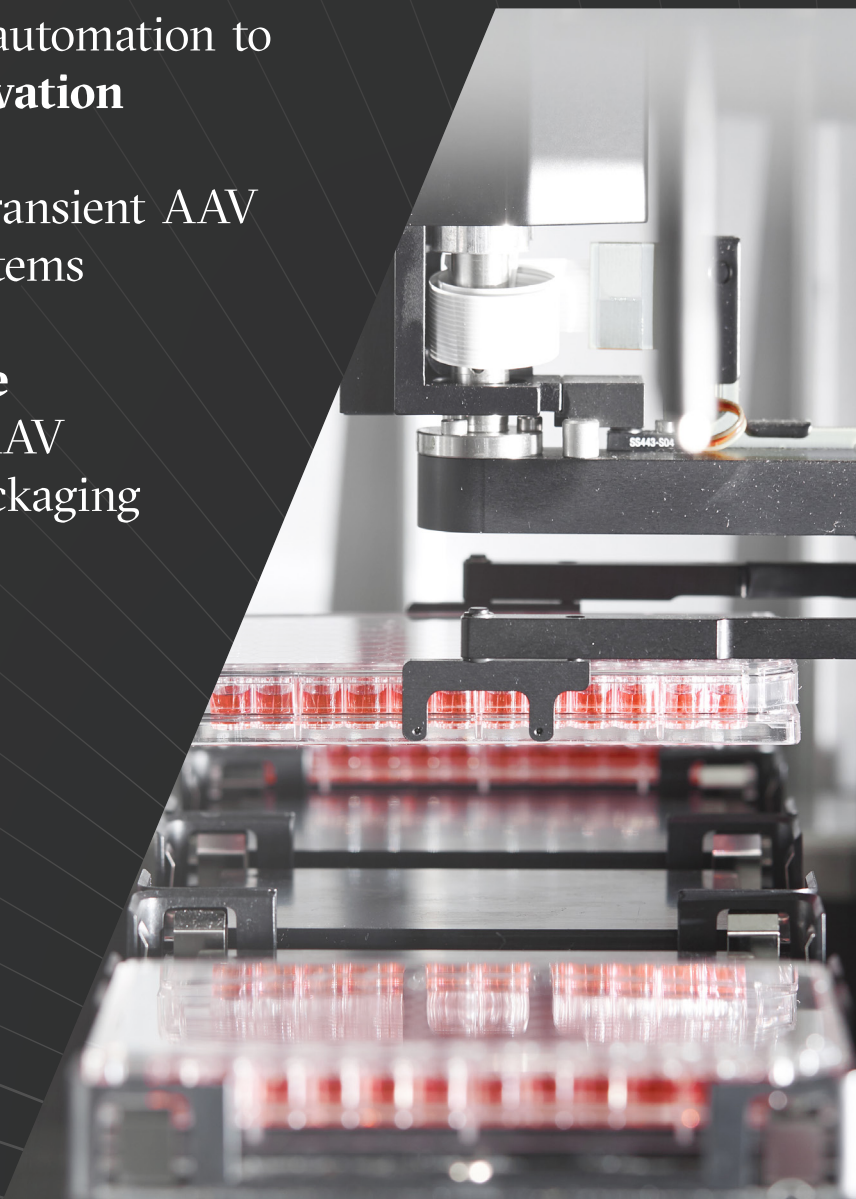
Article source: *This article is sponsored by OXGENE.*

Interview conducted: *Aug 20 2020;* ***Publication date:*** *Sep 29 2020.*

OXGENE

GENE THERAPY AT THE EDGE OF IMPOSSIBLE

- At OXGENE, biology meets automation to deliver groundbreaking **innovation**
- We offer **industry leading** transient AAV and lentiviral production systems
- Now launching fully **scalable technologies**: plasmid-free AAV production, and lentiviral packaging and producer cell lines



Accelerate your discoveries at
www.oxgene.com