CELL & GENE THERAPY INSIGHTS

EDITORIAL

Adherent versus suspension-based platforms: what is the near future of viral vector manufacturing?

"Therapeutics developers are searching for a viable viral vector manufacturing platform as the industry is at the inflection point. Paraphrasing Tolstoy, while successful manufacturing platforms are all alike, every unsuccessful manufacturing platform is deficient in its own way."

RAMIN BAGHIRZADE, Global Head for Cell & Gene Therapy, AGC Biologics

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The first sentence of Tolstoy's opus Anna Karenina starts by declaring that "All happy families are alike; each unhappy family is unhappy in its own way." Popularized as 'Anna Karenina principle', and applied in multiple scientific and social disciplines, the concept suggests that successful endeavors all share a common set of main traits, while there are many routes to misery if there is a deficiency in any of the key attributes. Paraphrasing Tolstoy, while successful manufacturing platforms are all alike (e.g. with regards to titers and yield), every unsuccessful manufacturing platform is deficient in its own way.

With over 1,200 clinical trials globally in cell and gene therapy, the field is reaching an inflection point with maturing late-stage pipelines and upcoming wave(s) of commercialization. Over two-thirds of all clinical trials in this area are currently in Phase 2/Phase 3 [1]. Using oft-cited numbers, FDA predicts that by 2025, it will be approving 10–20 cell and gene therapy products per year, with over 200 INDs filled annually [2].

As the field matures, so does the demand for viral vector manufacturing, in particular for lentivirus (LV) and adeno-associated virus (AAV) production, two dominant vectors used for *ex vivo* and *in vivo* gene therapies. While searching for a successful manufacturing platform, the ultimate objective of therapeutics developers is to make the manufacturing process commercially viable. Commercial viability is tied to both quality of manufacturing process acceptable for filing by the regulators and cost of virus per patient justifiable from business point of view as a percent of the overall COGS. The latter aspect is closely tied to process scalability.

When addressing the question of commercial viability, therapeutics developers face a crucial question: do you opt for adherent or suspension based viral vector manufacturing process? The article summarizes the pros and cons of each approach, and concludes that there is place for both.

The case for adherent based platforms:

Dominant approach in the industry, 'good enough' to commercialize at least some products while not letting perfection to be the enemy of progress

In biologics, two techniques of growing cells in culture can be distinguished: adherent and suspension. In adherent cell culture, cells are grown while attached to a substrate as monolayers. In suspension cell culture, cells are free floating in the culture medium. Currently, adherent cells are used in the manufacturing of about 70% of viral vector products [3]. The most common mode of manufacturing AAV and LVV vectors is by using adherent human embryonic HEK293 cells. Typically, human embryonic HEK293 cells – or HEK293-derived 293T cells, are transfected with a vector construct (containing GOI) and helper/packaging plasmids.

Traditionally used adherent culture system units include the likes of roller bottles, flasks, Corning's HYPERStacks® and Thermo Fischer's Nunc™ Cell Factory™ systems. Relying on 2D adherent plasticware platforms as a starting method of choice for upstream manufacturing, is easily understandable from at least three angles [4]:

1. They can be readily procured off the shelf
2. They are relatively easy to cultivate at lab scale
3. They require less expert bioengineering know-how compared to three-dimensional platforms

Moreover, basic adherent culture system units require low upfront CaPex investments and are hence practical starting points for (early) research purposes and beyond. Heavy CaPex investments is hardly a priority – or an option, for, say, an academic player or a fresh university spin-off. Considering that much of the innovation in cell and gene therapy comes from smaller sized biotech companies [5], not uncommonly, cell and gene therapy therapeutics developers inherit the process developed in and/or licensed from academia.

Probably one of the most documented examples of an adherent based process making it to market is that of Luxturna® (voretigene neparvovec), that uses AAV2 to carry a functional copy of the *RPE65* gene into the retinal pigment epithelial (RPE) cells. The product was developed by Spark Therapeutics (now part of Roche), and received an FDA approval in Dec 2017. Luxturna's AAV upstream manufacturing process relies on a roller bottle – basic 2-D cell culture system, using adherent HEK 293 cells process. Classic

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'scale-out' approach applies here – the only way to increase a manufacturing output is to add more roller bottles – rather than increase the volume of the vessel ('scale-up'), which could have been an option if this was a suspension-based process. Diane Blumenthal, at the time (2019) Spark's Head of Technical Operations, argued for the principle of "don't let perfection be the enemy of progress" [6]. What clearly made adherent platform viable enough is a relatively low dosage required (sub-retinal injection) and relatively low number of target patient population.

Another well-documented example of an adherent based process making it to market is that of Zolgensma® (onasemnogene abeparvovec-xioi), AAV9-based gene therapy used to treat spinal muscular atrophy (SMA). The product developed by AveXis (now part of Novartis), received an FDA approval in May 2019. Zolgensma's AAV upstream manufacturing process relies on an iCELLis® fixed bed bioreactor (FBR) adherent platform. iCELLis FBR platform has been cited as the 'most cost-effective option' for adherent cell culture [7], and has been used as a commercially viable solution without the need to switch to a suspension platform. There is extensive data available to demonstrate how one may scale a, say, 48L Cell Factories based process to a 200L iCELLis® FBR without changing critical quality attributes (CQAs) of the product [8].

Apart from commercial launches of Luxturna and Zolgensma, there is documented evidence of some developers making an explicit decision of intending to commercialize part of the pipeline on an adherent platform, and part in suspension, as well as gradual transition to suspension. To note, in its SEC filing back in 2013, Bluebird Bio, one of the gene therapy pioneers, explicitly pointed out that they intend to 'continue manufacturing' its Lenti-D vectors (SKYSONA™ – approved by EMA in July 2021) on an adherent platform, while adapting its Lenti-Globin (ZYNTEGLO™ – conditionally approved by the European Commission in June 2019) vectors in suspension [9]. Interestingly, in its SEC filing in 2020, BMS disclosed that it would assume the contract manufacturing agreements for ide-cel (ABECMA® – a CAR-T product approved by FDA in March 2021) on an adherent platform, while 'over time' the manufacturing will be performed in suspension [10].

The case for suspension based platforms:

Well-established in traditional biologics, still in early stage of maturity in cell and gene therapy industry, though viewed as "must have" for certain types of products/ indications

Adherent manufacturing mode typically implies that to increase the manufacturing output, one has to 'scale out', rather than 'scale up' – well established in traditional biologics and typically, though not exclusively, associated with stirred tank bioreactors (STRs).

Frequently cited limitations of basic 2D upstream manufacturing units include limited options for scale – which can make manufacturing prohibitively expensive, and batch-to-batch consistency, which may pose regulatory challenges. Adherent based manufacturing process also tends to be performed using fetal bovine serum (FBS) – that may pose safety, consistency, and ultimately, regulatory challenges [11]. On the other hand, switching to serum free, suspension platform is not always a viable solution and is far from being a failure free endeavor. While, for example, HEK293 cells have been adapted to grow in suspension [12], and there are alternative suspension-based cell lines, these are not without their challenges – with regards to timelines, costs, quantity and quality of viral vector. Moreover, as 'the product is the process', switching the process may mean that the product is no longer the same, and may require e.g. bridging/comparability studies. A dilemma frequently facing therapeutics developers is whether the existing (adherent) process is 'good enough' for commercialization and how much they are willing or able to wait and invest to try switching to suspension.

Perhaps one of the best-documented cases for the suspension-based process making it to market is that of Glybera, AAV1-based product, launched by UniQure, and widely dubbed as the 'first gene therapy' in the Western world [13]. The drug was approved by EMA in October 2012 to treat hereditary lipoprotein lipase deficiency (LPLD). While adherent HEK293 process was used for the pre-clinical studies and the first clinical trial in 2005, as higher quantity of vector was needed, HEK293 platform was changed to suspension based on baculovirus production system [14]. NIH scientists first demonstrated the suitability of this method by infecting *Spodoptera frugiperda* (Sf9) insect cells with three different baculoviruses – used both as a 'helper' virus and as the vehicle for AAV genetic material [15]. The baculovirus based manufacturing platform is not without its own challenges. For example, in the case of Glybera, while switching the platform helped with generating higher quantities of vector, the impurities profile was viewed as 'unacceptably high' in the assessment report by the EMA [16]. To note, the carryover of the baculovirus DNA was highlighted as a 'major concern', and the therapeutics developer was requested to perform a detailed risk assessment. A comparability study also had to be conducted comparing plasmid based adherent HEK293 process vs. suspension baculovirus based platform [14].

The challenges associated with switching to suspension still seemed to have paid off in the case of Glybera – despite the voluntary market withdrawal of the product in 2017. Depending on the indication/dosage/quantity of vector required, therapeutics developers may feel obliged to opt for suspension, as the only sustainable option. For example, while assessing viral vector needs for muscular myopathies, Salabarria *et al.* (2020), concluded that adherent platforms are 'simply not feasible' for AAV manufacturing for scales exceeding 1–5E+10^{15} vg, and hence would not be suitable for late phase/commercial applications in these indications [17]. For these particular cases, it is suggested that alternative, suspension-based methods are to be used, such as HEK293 adapted in suspension, infection based platforms (e.g. using baculovirus), or a stable producer cell line, with the upstream scale to 500L and beyond. In a similar vein, Pfizer announced ramping up its AAV upstream manufacturing to 2,000L to support its late phase AAV9 trial to treat Duchenne muscular dystrophy – DMD [18].

While, as highlighted earlier in the article, fixed bed bioreactors (iCELLis®) have been assessed as the 'most cost effective' solution on an adherent culture, the same study found suspension based STR manufacturing as the most cost-effective technology "…when a suspension-adapted cell line was available" [7]. The availability of a suspension-adapted cell line, and its characteristics, is a critical qualifier. For example, as has been argued elsewhere [19], producer cell lines in suspension are superior to transient transfection methods – when it comes to cost, reproducibility and scalability, though can be 'cumbersome' and time-consuming to develop, with no guarantee of success.

While there are documented examples of successful manufacturing in suspension, including successful adaptation to suspension from the adherent process [19], in my professional career in the industry, I have also come across multiple cases where a therapeutics developer tried moving to suspension, failed to do so, and focused the efforts on optimizing the adherent process instead. While not uncommon, failures to move to suspension is not something therapeutics developers readily and openly advertise.

CONCLUDING REMARKS: THERE IS NO SILVER BULLET

It is clear that there are challenges associated with either adherent or suspension method of manufacturing. What is also evident is that adherent mode of manufacturing can be a viable solution in certain cases, and not in others. At the same time, suspension-based

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manufacturing, while viewed as a 'must have' in certain cases, might not always be the way to go. There is no silver bullet, and the manufacturing strategy has to be evaluated on a case-to-case basis. As therapeutics developers bring cell and gene therapies to market, they tend to juggle among multiple and at times conflicting dilemmas, including:

- Is the manufacturing process 'good enough' for commercialization in the given disease indication?
- How much process development/ optimization is needed to make the process commercially viable?
- What is the right tradeoff between speed to market and time to develop a manufacturing process?
- At what point (if at any at all) is it wise to switch to suspension: before the market approval or after? (with all associated implications – e.g. comparability/bridging studies).
- If suspension-based process is sub-optimal, should one opt for adherent process instead or continue investing in developing a suspension process?
- What type of suspension-based process should one opt for? (e.g. HEK293 transient transfection, co-infection, stable producer cell line)

While juggling among these and other dilemmas, it is critical for therapeutics developers not to get sidetracked by a mammalian bias – as sometimes, less is more. Depending on the process productivity (total transducing unit (TU) or vg/batch), one might be able to treat more patients from, say, a 48L adherent platform, than from a 200L suspension-based STR.

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BIO

Ramin Baghirzade

Dr Ramin Baghirzade is the Global Head for Cell & Gene Therapy (C&GT) at AGC Biologics. With 15+ years of experience in life science and healthcare, Dr Baghirzade previously held roles of increasing responsibility at Roche and Lonza in global business development, strategic marketing and market intelligence functions. He holds a PhD Degree in Medical Sciences, as well as an MBA.

AGC Biologics provides world-class development and manufacture of mammalian and microbial-based therapeutic proteins, plasmid DNA (pDNA), viral vectors and genetically engineered cells. AGC Biologics' global network spans three continents, with cGMP-compliant facilities in Seattle, Washington; Boulder and Longmont, Colorado; Copenhagen, Denmark; Heidelberg, Germany; Milan, Italy; and Chiba, Japan

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