APRIL 2021 Volume 2, Issue 2

IMMUNO-ONCOLOGY INSIGHTS

SPOTLIGHT ON:

Modelling the I-O manufacturing facilities of tomorrow

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IMMUNO-ONCOLOGY INSIGHTS

Volume 2, Issue 2

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FOREWORD

Modelling the I-O manufacturing facilities of tomorrow

JOHN LUNGER has been Adaptimmune's Chief Patient Supply Officer since August 2019. John leads the teams responsible for producing and delivering products to patients, accelerating supply execution, and optimizing the supply chain to be ready for commercialization. Previously, John was Head of Supply Chain and Commercial Product Supply at Merrimack Pharmaceuticals where he led clinical and commercial supply chain as well as the cross functional supply team for Merrimack's first commercial product launched in October of 2015. Earlier in his career, he held various senior manufacturing, operational, and strategy roles with VWR International, Pfizer and Wyeth Pharmaceuticals. In his nearly 10 years with Wyeth he held a number of leadership positions, including operations and supply chain strategy, supply management, procurement and strategic sourcing, business systems implementation, generic pharmaceutical business management, and site operations management in a pharmaceutical manufacturing plant in Ireland. John began his career serving as a nuclear trained officer on a U.S. Navy submarine followed by strategic consulting with Accenture. John holds a Bachelor of Science degree (with distinction) in Ocean Engineering from the U.S. Naval Academy and an MBA in economics and operations management from the University of Chicago's Booth School of Business.

Immuno-Oncology Insights 2021; 2(2), 85–87

DOI: 10.18609/ioi.2021.018

Over the last 5 to 10 years, immuno-oncology (I-O) therapies have moved from 'bench to bedside', with exciting clinical outcomes leading to wider adoption of checkpoint inhibitors and new CAR-T products hitting the market across the globe. For those of us charged with manufacturing and delivering these therapies, this growth is both exciting as well as a bit daunting – as we consider the myriad of challenges we must address to achieve our ambition for these therapies to be curative and mainstream.

'Curative' means deep and durable responses for people with cancer. While this is typically the remit of research and product development teams, with cell therapies the effectiveness of the product is often influenced by the manufacturing process itself. For example, while an engineered T-cell product is dependent on genetic modification of the T-cell receptor, the effectiveness of the cellular product may be also be driven by the cell phenotype, culture, and expansion, as well as the cryopreservation processes. Changes in the manufacturing process, therefore, can improve the product. Manufacturing facilities and processes must be able to manage these potential improvements.

'Mainstream' refers to access to I-O therapies, and this is where manufacturing and supply organizations can have the most impact. One key element to make cell therapies mainstream is capacity. How can we provide enough drug product manufacturing capacity to deliver tens, hundreds, or thousands of treatments per year globally? A second element to consider is cost. How can we become more efficient and reduce manufacturing and supply costs to a level that supports ongoing innovation while ensuring price is not a barrier to treatment access?

Potential solutions to these challenges are the topics for this month's Spotlight on Modelling the I-O Manufacturing Facilities of Tomorrow. Xiuyan Wang and Isabelle Riviere from Memorial Sloan Kettering Cancer Center review the progress of I-O therapies and some of the areas the industry will need to address, from gene transfer technologies through standardization of new analytical techniques. They also cover future challenges with allogeneic process development that may mean both allogeneic and autologous platforms co-existing for quite some time.

Anthony Welch, Marc Ernstoff, and Jason Yovandich from the Frederick National Lab for Cancer Research (FNLCR, part of the National Cancer Institute) describe the way they are working with both industry and academic organizations to investigate cutting edge manufacturing and analytical technologies for cell therapy with the goal to advance I-O therapies. From the Biologics Innovation Facility at the University of Technology in Sydney, Edwin Huang reminds us that I-O is not only about cell and gene therapies but also biologics, and he covers the work being done on training the next generation of manufacturing professionals.

Finally, we are introduced to John Powderly and the unique operating model of the Carolina BioOncology Institute, where they have a 'point of care' facility for product manufacturing and treatment for patients enrolled in a number of early phase I-O clinical trials. Concluding our Spotlight is the transcript of a roundtable with leaders from three companies preparing to commercialize I-O products. I was delighted to be part of the roundtable as we discussed our perspective on the 'real world' challenges of late stage and commercial I-O manufacturing.

While the challenges facing the manufacturers of I-O therapies are complex, the ambition to make these therapies curative and mainstream keep the scientific and manufacturing teams pushing forward with innovative ways to solve these problems. Harnessing the power of the immune system to cure cancer is within our reach, and I couldn't help but think that the real I-O Manufacturing Facility of Tomorrow already exists in our own bodies. Our individual immune system produces elegant cures every day, so – while we have to develop external manufacturing facilities now – I can imagine a future where

DOI: 10.18609/ioi.2021.018

the real 'factory' is our own adaptive immune system finally being able to identify and kill cancer cells.

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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 stock and stock options for Adaptimmune.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited.*

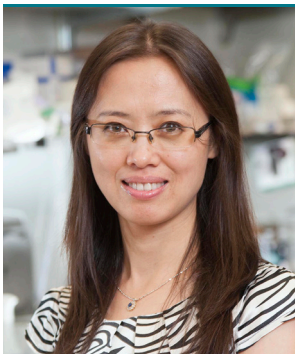
Revised manuscript received: *Apr 16 2021;* ***Publication date:*** *Apr 23 2021.*

MODELLING THE I-O MANUFACTURING FACILITIES OF TOMORROW

SPOTLIGHT

EDITORIAL

Outlook on manufacturing adoptive cell therapies for cancer immunotherapy



"The next logical and important question for the field is how to increase manufacturing capacity and access while bringing down the cost."

XIUYAN WANG
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Immuno-Oncology Insights 2021; 2(2), 69–72

DOI: 10.18609/ioi.2021.016

Science magazine named cancer immunotherapy as the breakthrough of the year at the end of 2013, recognizing the promising clinical outcomes following treatment with immune checkpoint inhibitors and innovative chimeric antigen receptor (CAR) T cell therapy. In 2018, James Allison and Tasuku Honjo were awarded the Nobel prize for conceptualizing cancer immunotherapy by targeting the immunosuppressive signal mediated by Cytotoxic T Lymphocyte-Associated Protein 4 and demonstrating Programmed Cell Death 1 (PD-1) activation-induced cell death in lymphocytes. Antibody immunotherapy has become part of the standard of care with 11 immune checkpoint inhibitors drugs in the market. As of today, four CD19- and one BCMA- targeted CAR T cell products have been approved by the FDA (Tisagenlecleucel, Axicabtagene ciloleucel, Brexucabtagene autoleucel, Lisocabtagene maraleucel, and Idecabtagene vicleucel), for the treatment of relapsed or refractory B cell malignancies and multiple myeloma, respectively. Thousands of autologous and more recently allogenic early phase adoptive cell therapy clinical trials are ongoing [1]. Oncologists and patients have more treatment options than ever before – cancer is no longer the terminal disease it used to be.

Among the most common types of adoptive cell therapies, namely tumor-infiltrating lymphocytes (TILs), engineered T cell receptor (TCR) T cells, CAR T cells or natural killer (NK) cells, CAR-T cells have opened up a new era in synthetic cancer immunotherapy. CAR-NK cells have also recently promoted encouraging clinical results. These cellular therapies have either extended the life or cured patients who have failed all conventional chemo/radio therapies and surgeries. Despite their promising clinical efficacy, gene-modified cells are complex products manufactured according to elaborate procedures that are responsible for the high cost and have limited accessibility to a wider patient population [2]. The manufacturing process for gene-modified cell product starts from either patient-specific or donor cells, followed by genetic modification, expansion, end of process cell harvest and formulation [3]. To ensure that the product is commercially viable and can be widely distributed to larger patient populations, the formulated product need to be cryopreserved and stored for future distribution.

The steadfast dissemination of adoptive cellular therapy applications demands efficient process development, effective tech transfer to CMOs, reproducible manufacturing according to cGMP and efficient distribution of clinical-relevant products such as autologous CAR T cells. To be competitive in the fast-paced market, it is critical to qualify and standardize all ancillary and chemically defined reagents whenever possible. It is equally important to standardize the manufacturing process and use tools such as quality by design (QbD) and process analytical technology (PAT) early on [4]. Status, scalability and availability of manufacturing instruments play a key role at every step of manufacturing, not only for processing but also for product and process qualification. The choice of instrumentation not only dictates the design of manufacturing process itself but also likely impacts the design of the manufacturing facility.

Needless to say, it is a huge undertaking to conceive and build new instruments for cellular therapy, especially those aiming to integrate all steps of the manufacturing procedure. One major challenge of such an endeavor is that the technologies used to generate cellular therapies are constantly evolving and improving. For example, more investigators have chosen to use specific subsets of T cells now instead of the whole T cell population; CRISPR/Cas has joined meganucleases, ZFNs, TALENs and has become a gene editing tool of choice for gene modification, all requiring the incorporation of an electroporation step in manufacturing platforms workflow [5]. More potent synthetic receptors and enhanced cell phenotypes are enabling treatments with lower cell doses resulting in the ability to shorten manufacturing processes; these successes beget the necessity to modify

DOI: 10.18609/ioi.2021.016

manufacturing platforms and formulation schemes and to adapt analytics and sampling plans to limit the number of cells required or enable surrogate measurements [6]. Moreover, instrument makers also need to navigate and meet the complexity of regulatory requirements. Therefore, there is often a gap in the instrumental availability and technological advancement in the cell therapy field. Nonetheless, there is a growing number of devices that can be used to perform unit operations, such as CliniMACS® Plus for cell selection; COBE® 2991, CellSaver® 5, Sepax™ C-Pro and LOVO for cell washing; Maxcyte and Nucleofector™ for electroporation; G-Rex® and Xuri™ for cell expansion; CryoMed™ and VIA Freeze™ controlled-rate-freezers for cryopreservation; and CliniMACS Prodigy®, and Cocoon® that have integrated the majority of manufacturing steps and support a higher degree of automation [7].

Highly integrated, automated instruments have the advantage of process control and standardization, one caveat for such devices being the limited flexibility in adding new functionalities. In order to adapt to the fast pace of scientific discoveries and prolong the life cycle of such commodities, instrument designers and engineers are required to look beyond the current status quo and keep the evolving nature of cell therapies in mind. Miltenyi Biotech has given us an excellent example for such a vision by recently adding the CliniMACS® Electroporator to facilitate fully automated cell electroporation to the existing CliniMACS Prodigy® to meet the growing need for electroporation in the manufacturing process design. On the other hand, a modular design of the manufacturing process certainly affords cell manufacturers a higher degree of flexibility and lower the risk of chain supply disruptions. It also likely allows more efficient use of any given piece of instrumentation perhaps at the expense of more intervention from manufacturing operators. It is noteworthy that the four types of adoptive cell therapies mentioned above all have specific manufacturing requirements that differ from each other. Therefore, both the modular and integrated manufacturing process designs are required and will likely co-exist in the near term. Nonetheless, high degrees of automation are eventually desired and will be required to meet increasing centralized manufacturing needs and to potentially enable more decentralized or point-of-care manufacturing applications, for example for rare diseases.

The next logical and important question for the field is how to increase manufacturing capacity and access while bringing down the cost. Although there is significant existing knowledge on scaling up biopharmaceutical processes and recently gained knowledge on scaling out autologous cell therapies, it is noteworthy that shifting adoptive cell therapy from the autologous to the allogenic setting is not just a matter of increasing the dimension of the bioreactors; the cells must retain their appropriate phenotype and function upon large scale expansion for multidose manufacturing in order to ensure their efficacy. Alternative cell types such as viral specific T cells or iPSCs may be used as source material instead of blood draws or leukapheresis products [8]. Development of in-process control and effective product qualification methods are paramount to maintain a product's critical attributes, safety and potency during manufacturing processes that are likely to be lengthier and at much larger scale when compared to autologous products. It is also more important than ever to build flexible manufacturing and testing facilities to meet growing and changing needs. Additionally, by-passing *ex vivo* manufacturing through targeting and engineering cells in situ is already in the early stage of development. Although the potential of this approach remains unknown, early studies using γ-retroviral vectors, adenoviral vectors and lipid nanoparticles suggest the feasibility of transducing T cells *in vivo* which could change dramatically the paradigm in cell therapy.

Numerous challenges remain to be addressed in order to ensure efficient and affordable cell product manufacturing while new challenges will arise as adoptive cell therapies mature. Scientists will continue to make

ground-breaking, seminal discoveries and translate these discoveries into clinical applications while engineers will have to adapt manufacturing and analytic instrumentation to new requirements, and industrial partnerships will continue leading these new applications towards commercialization. Novartis, Gilead, and Bristol Myers Squibb are temporarily the pioneering companies who have successfully obtained FDA approval for CAR T cell therapies. These successes have driven the establishment of a wealth of new companies with diversified portfolios. With the rapid rise of personalized cell therapy/medicine, potent and cost-effective adoptive cell therapy will hopefully become a widespread therapeutic modality across a range of therapeutic areas in the near future.

REFERENCES

1. Clinical Trials database
2. Ten Ham RMT, Hovels AM, Hoekman J *et al.* What does cell therapy manufacturing cost? A framework and methodology to facilitate academic and other small-scale cell therapy manufacturing costings. *Cytotherapy* 2020; 22: 388–97.
3. Wang X, Riviere I. *Manufacturing of CAR-T Cells: The Assembly Line*. Gene and Cellular Immunotherapy for Cancer: Springer (In press).
4. Bure KYL,Lowde ll M, Wall I, Zandstra P. Quality By Design for Advanced Therapies: An Informed Route to Enhanced Late-Stage Clinical Success and Empowered Process Flexibility. *BioProcess Int.* 2020; 18.
5. Eyquem J, Mansilla-Soto J, Giavridis T *et al.* Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature* 2017; 543: 113–7.
6. Feucht J, Sun J, Eyquem J *et al.* Publisher Correction: Calibration of CAR activation potential directs alternative T cell fates and therapeutic potency. *Nat. Med.* 2019; 25: 530.
7. Wang X, Riviere I. Clinical manufacturing of CAR T cells: foundation of a promising therapy. *Mol. Ther. Oncolytics* 2016; 3: 16015.
8. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. *Nat. Rev. Drug Discov.* 2020; 19: 185–99.

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 authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

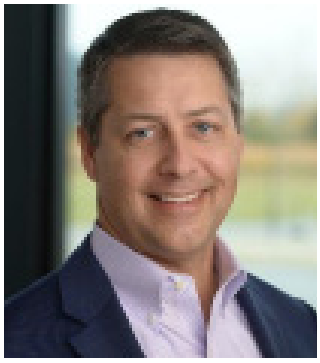
Article source: *Invited.*

Revised manuscript received: *Apr 14 2021;* ***Publication date:*** *Apr 21 2021.*

DOI: 10.18609/ioi.2021.016

EXPERT ROUNDTABLE

How to increase I-O manufacturing efficiency, flexibility, and productivity in line with expected future trends in supply and demand?



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John Lunger has been Adaptimmune's Chief Patient Supply Officer since August 2019. John leads the teams responsible for producing and delivering products to patients, accelerating supply execution, and optimizing the supply chain to be ready for commercialization. Previously, John was Head of Supply Chain and Commercial Product Supply at Merrimack Pharmaceuticals where he led clinical and commercial supply chain as well as the cross functional supply team for Merrimack's first commercial product launched in October of 2015. Earlier in his career, he held various senior manufacturing, operational, and strategy roles with VWR International, Pfizer, and Wyeth Pharmaceuticals. In his nearly 10 years with Wyeth he held a number of leadership positions, including operations and supply chain strategy, supply management, procurement and strategic sourcing, business systems implementation, generic pharmaceutical business management, and site operations management in a pharmaceutical manufacturing plant in Ireland. John began his career serving as a nuclear trained officer on a U.S. Navy submarine followed by strategic consulting with Accenture. John holds a Bachelor of Science degree (with distinction) in Ocean Engineering from the U.S. Naval Academy and an MBA in economics and operations management from the University of Chicago's Booth School of Business.

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Vijay Yabannavar is the Executive Vice President and Manufacturing and Technical Operations Officer at Gritstone Oncology. Before Gritstone, he served as vice president of global technical operations – vaccines, biologics and sterile operations at Merck. Prior to that, he was senior vice president of manufacturing & technical development at Emergent BioSolutions, focusing on biodefense. Dr. Yabannavar also held process development and manufacturing roles at Trubion Pharmaceuticals, Novartis Pharmaceuticals, Chiron Corporation and Schering-Plough Corporation. He has served as a member of the Advisory Board for the Chemical Engineering and Materials Science Department at the University of California, Davis. Dr. Yabannavar obtained his Ph.D. in chemical engineering from the Massachusetts Institute of Technology (MIT) and his B. Tech. in chemical engineering from the Indian Institute of Technology Bombay.

DONNA RILL
Chief Technology Officer, Triumvira Immunologics USA, Inc

Ms. Rill is currently serving as the Chief Technical Officer of Triumvira Immunologics, USA, Inc. She has extensive manufacturing, clinical and translational research laboratory experience in cell and gene therapy, monoclonal antibody production, and protein production. She has setup and managed core development laboratories covering a large range of testing services to facilitate research, core drug development activities as well as manufacturing and quality control laboratories. With her expertise in the areas of laboratory construction, project management, development and operations, cGMP, cGTP, and GLP regulatory compliance, quality control/assurance system, database development, and clinical standards of practice, she has designed and qualified cGMP Cell & Gene Therapy Laboratories, cGMP Vector Production facilities, core service laboratories, and Translational Research Labs. Ms. Rill has previously held the positions of Vice President of Manufacturing for Cell Medica, Chief Development Officer for Opexa Therapeutics, Laboratory Director of Cell and Gene Therapy, Translational Research Laboratories for Cell and Gene Therapy, Baylor College of Medicine; Associate Scientist/Lab Manager of the Bone Marrow Transplant Research Laboratory, and the GMP Cell and Gene Therapy Laboratories, St. Jude Children's Research Hospital; Education Coordinator and Clinical Instructor, Department of Clinical Laboratory, LeBonheur Children's Medical Center and University of Tennessee Center for the Health Sciences.

Immuno-Oncology Insights 2021; 2(2), 89–102

DOI: 10.18609/ioi.2021.019

Q Can you each set the scene by introducing us to your respective company's platform and pipeline in the I-O space, and the associated manufacturing considerations and strategies?

JL: Adaptimmune is an integrated cell therapy company, with a mission is to transform the lives of people with cancer by designing and delivering cell therapies. Over the next 5 years, we will be executing on what we call our '2-2-5-2' strategy: at the end of that period, we aim to have two marketed SPEAR T cell therapy products (MAGE A4), two further BLAs targeting additional indications, five more autologous products in the clinic, and two allogeneic products entering the clinic.

Based on that strategy, you will note that we have multiple cell therapy platforms. The first is our autologous platform, for which we currently have multiple clinical trials in progress involving both first-generation and next-generation SPEAR T cells. Secondly, we are developing an allogeneic, off-the-shelf platform based on induced pluripotent stem cells.

From the manufacturing perspective, in addition to working with some contract manufacturers, we have built the internal capability to deliver our drug products both for our current and planned clinical trials, and for a first commercial launch we are targeting for 2022 (MAGE A4 against synovial sarcoma). We have a SPEAR T cell manufacturing facility here in Philadelphia, where I am based, which supplies our current clinical trials in both the US and Europe. Additionally, we developed and now produce our own lentiviral vector internally, utilizing dedicated space at the Cell and Gene Therapy Catapult Manufacturing Centre in Stevenage, UK.

DR: Triumvira Immunologics has a T cell platform, T cell Antigen Coupler (TAC). It differs from CAR T approaches in that it actively engages the natural T cell

receptor to activate T cells leveraging all-natural activation and regulatory mechanisms. Our initial autologous clinical trial with the TAC technology will target HER2 for solid tumors.

Triumvira is also developing an allogeneic program targeting HER2 positive tumors and a couple of other yet-to-be-disclosed targets. The allogenic platform focuses on γδ T cells and invariant NKT (iNKT) cells.

We currently outsource manufacture to a CDMO in Montreal. However, as we build out our clinical trials in the future, we will be looking at the best option to move forward, whether that is to go to a larger, more robust CDMO environment or to build our own facilities.

We outsource production of GMP viral vector after early internal development, followed by transfer of targeted construct(s) to the selected vendor. In support of the allogeneic program, we are establishing a robust biorepository for the master cell banks.

The culture phase of the autologous TAC program is an automated manufacturing process and will be fully automated, with the addition of an automated fill-finish device in the near future. Automation is also being incorporated into the allogeneic program.

VY: Gritstone Oncology is a clinical-stage immunotherapy company based in the San Francisco Bay area and in Boston.

Cancer vaccines were our initial focus. We had the proprietary technology to identify neoantigens and so we started making immunogenic vaccines with that. However, since then we have progressed to also working on infectious disease vaccines.

Today, we have three different programs of our own: Granite, which is an autologous cancer therapy; Slate, an off-the-shelf shared neoantigen; and COVID-19 vaccines. We also collaborate with other companies, including Gilead Sciences with whom we are developing HIV vaccines.

The pipeline is driven in part by our proprietary EDGE™ technology, which is a powerful machine learning-based platform for initial target prediction. Our immunogenic vaccines are then designed based upon two platforms: an adenoviral vector for priming, and a self-amplifying mRNA (SAM) delivered via lipid nanoparticle (LNP) for boosting. We believe both of these can get the T cells to the level where they can be therapeutically important.

Q Ensuring flexibility in manufacturing strategies and capabilities in what is still a rapidly evolving field is clearly key. Firstly, how do you seek to ensure the necessary level of flexibility and future proofing on the strategic side – for example, in terms of scale/capacity needs, or potential future directions in overall I-O platform evolution?

VY: It's a great question. I mentioned we have three different modalities we are working on internally – an individualized therapy and an off-the-shelf approach, both in cancer, and a third in the very different arena of mass vaccination for COVID-19. We need the flexibility to be able to adapt the manufacturing platform to each one of these.

For the individualized vaccine therapy, we need to have a productive system at the miniaturized scale that we can automate. And at the other extreme, we have the need to produce large numbers of vaccine doses for COVID-19, at a much larger scale and with high productivity.

There are individual considerations and technological advances happening for both

the adenoviral vector and mRNA/LNP technologies we use. We need to be looking at high productivity for the adenoviral vectors, with higher throughput and higher yields in the purification. And with the mRNA/LNP, we are dealing with automating the nucleic acid synthesis and LNP production and also scaling up as required.

JL: We have about 150 people working in the CMC function and even though we are a late-stage company with a Phase 2 asset, we still have many people working on the early-stage process and analytical development. They are in place to take the lessons from the translational work and quickly get it into a new process. As a matter of fact, I think we have had up to six different autologous manufacturing processes in the clinic at any one time. Different media, different bioreactors, different technology and automation… It is very challenging to manage that at speed when you are dealing with third parties.

It's not that the third parties, the CDMOs, aren't developing those capabilities, too. But as we all know, the cellular immunotherapy market is just exploding, and it is tough to make rapid changes when you are waiting in the queue. I think the sector will evolve towards using CDMOs more in the future, but right now, many of the small companies are doing the same thing as us, which is building internal capabilities in large part to ensure that flexibility.

Turning to scale, for autologous products, that is about constantly watching the science, seeing where your commercial patient population may be located, and making sure you are in position to respond to that. Of course, with allogeneic therapies it's a very different story – being able to reduce the pressure on manufacturing capacity by stockpiling product is what makes allogeneic approaches so interesting at this stage.

However, having both autologous and allogeneic platforms in our pipeline, it is by no means cut and dried. I really do believe there is a driving down of cost happening for autologous products at the moment. You have four autologous T cell immunotherapy products on the market now, and operationally, we are getting better all the time. Plus, the science with allogeneic products is not for the faint of heart. So while we are still going to work on that one, I think autologous products will be in the market for a while yet. Therefore, we need to make sure we plan for that scale and to try to cover all the bases so we can respond as the technology and science evolve.

DR: I agree wholeheartedly with what both John and Vijay have said, but I also think there are a couple of other considerations.

For a small biotech company like Triumvira Immunologics – much smaller than either Adaptimmune or Gritstone Oncology – it is very difficult to drive funding towards a facility early on when you don't have your proof of concept. Identifying a CDMO that can at least help you with that very early proof of concept work is important. However, you must be cognizant of what the future holds for your company, your platforms, and the future of cell and gene therapy in general.

What we know today is not what we will know tomorrow, but we need to design facilities to meet today's needs, today's flexibility. For me, that means going with 'flex wall' systems that you can basically adapt on the go, and in a manner that enables and allows

"...we need to design facilities to meet today's needs, today's flexibility. For me, that means going with 'flex wall' systems that you can basically adapt on the go..."

- Donna Rill

DOI: 10.18609/ioi.2021.019

for upstream/downstream processing that is more akin to the established biologic modalities, which the big pharma companies are used to. For example, having core areas for the common process steps (e.g. seed material processing, fill-finish, cryopreservation) and then having the more product-specific steps in focused production areas within the facility. I think it is hugely important for the field to consider that.

As the demand for marketed cell and gene therapy products increases – for instance, as the move from hematological to solid tumors is achieved – then the field has the potential to really explode. When that happens, we are going to need the flexibility to schedule on demand, and that is extremely difficult to acquire from CMOs right now. That is a future key consideration for the sector as a whole.

Q ...And how about on the technological side, in terms of enabling bioprocess and bioanalytical tools?

DR: Another factor that plays into facility design of how, when, and where to do your process development and manufacturing is the fact the field is ever-changing.

The techniques we will have tomorrow will be different to the ones we have today. That means we must have an environment where we can do the appropriate comparability testing. If one is already in a clinical trial with a set process and needs to conduct rigorous comparability studies in order to advance, one must establish and understand what contributes to critical process parameters and critical quality attributes and material attributes in order to effectively incorporate new technology into the manufacturing process. It requires good integration between R&D and process development, followed by translating into the GMP manufacturing environment.

Another important consideration is vein-to-vein turnaround time. In diseases with high relapse rates, it is important to improve and optimize processes – to look at technology that can potentially decrease the culture time, as well as improve the turnaround time for release testing. It is key for us to gain a better understanding of what it takes to do this around the autologous program, but also for the allogeneic. When it comes to off-the-shelf, I think there are more unanswered than answered questions from a technological standpoint, and in particular, in terms of what it takes to establish your cell banks – what is expected by regulators, the long-term stability of both the cell banks and the product, etc.

Due to the number of unanswered questions in this field in general, one must stay abreast of the best techniques and methods and have very aggressive quality control laboratory and quality assurance programs underway.

VY: Donna covered it really well with respect to both throughput and turn-around time. Both are extremely important, so your process has to be ready for that.

For personalized/autologous therapy in particular, we need to be able to handle the product for multiple patients all at once. As I mentioned previously, that demands high productivity at a miniaturized scale, and the automation to go along with that. Regarding turnaround time, I would echo the comment that it is not just about the processing, but also the testing aspects as well – can we find abbreviated testing in some cases, so we can do the release testing and get the product out to the patient really quickly? Of course, doing this will help to lower Cost of Goods (COGs) as well. Staying on the analytical side, regulators are increasingly looking for advanced assays. For example, it is not enough just to say

"For personalized/autologous therapy in particular, we need to be able to handle the product for multiple patients all at once ... that demands high productivity at a miniaturized scale, and the automation to go along with that."

- **Vijay Yabannavar**

you have the infectivity of your viral vectors anymore – what about the antigen expression? Do you have ways to quickly get to that?

For our other product, which is a self-amplifying mRNA, we need to be looking at the potency of the product, the size of the lipid nanoparticles, and the stability of the product itself. All of these are evolving areas for analytical tools, which we need to make sure we can meet the regulatory requirements moving forward.

Q ...And finally, at the regulatory level: how to ensure flexibility in what is still an evolving regulatory CMC environment for cell-based therapies in particular?

VY: I think when it comes to the regulatory side, comparability is actually going to be your friend. If you have a platform process where you have used the same type of vectors or other products before, can you bridge back to that, both on the analytical side and perhaps also on the stability side, so that the regulators can feel confident and you are able to introduce you next product more easily? We tend to use that approach to cut down the development time in this area.

JL: We retain the ambition while we are still in a Phase 2 trial to have a process lock – so ideally, we will change nothing in Phase 2. The fact is that with most cell therapies at the moment, when moving into a regulatory enabling trial, you can often do that based on very few patient runs in your Phase 1. That forces us to really think about what we need to do to process lock.

It also prompts the question of where to do early-phase trials? We are currently doing trials in the US, Canada, France, Spain, and Italy - those are the markets we're currently in. And everybody knows they all have different requirements in terms of what you need for a Phase 1. So we might make a change to a media, or a change to a bioreactor, for example, and we will have one country that says 'that's fine, you are in Phase 1, your process knowledge is still quite light, so go ahead and make your change'. But another will be looking for comparability even in a Phase 1. So then you have to decide where you are going to go for your Phase 1 studies to get those initial signals. European countries may have easier access to patients because there might be less competition, but perhaps the reason there is less competition is because it's harder to run a trial there.

Our approach is to look for signal finding in the US, or perhaps some European countries that don't have quite such varying and uncertain requirements relating to Phase I information.

DR: On a related point, even if you don't have the means to completely lock down your process as you go into Phase 1, it's hugely important for you to ensure you have residual material to do that development work, and to encourage your people to develop internal process

DOI: 10.18609/ioi.2021.019

control. For instance, build a bank of seed material that is predictable in terms of how it will react in a given circumstance, so that you can just directly parallel that against a new technology, a new approach, or streamlined processing further down the line. Even with the lentiviral vectors you use, as you change the construct, the target antigen, there will be variability. You have got to know what can drive that variability and have some consistency that you can bridge across into your next studies.

In the early stage biotech environment, being able to know where you want to end up for product launch and building that in early is critical. This includes the aspects of automation, flexibility, and turnaround times. And even if you have got tight scheduling scenarios, where can you cryopreserve a product in process if you need to, and pull it back out?

Living in 'Hurricane Alley' in the US, we must be cognizant of the potential need to shut down a production mid-process for an extended period of time and be able pull back up for completion at a later time. And we must be able to validate such exceptions in advance of need, assuring the yield of an equivalent product.

Q Let's go into a few of the overarching manufacturing trends and talking points impacting the cellular immunotherapy field in particular. Firstly, we have touched on personalized versus off-the-shelf cell therapy – what is your take on the manufacturing business models and likely trends we will see moving forward?

JL: This is a topic we could talk about for hours! Those of us who are working to optimize autologous therapies will probably have a greater desire to say autologous is going to win, because that is where we spend our time. But again, I honestly believe that while we might not get to the 90% gross margin number you see in small molecules or biologics, continued reduction in COGs will mean we will get to a place where there will be a long-term market for these products. For example, since our first manufacturing run with the original academic process, we have seen our vector costs drop per patient by more than 90%. We have seen our total COGs drop by more than 40%. And I expect this to continue as we gain economies of scale, and we introduce electronic batch records and other systems that will cut down on QA time.

On the allogeneic side, I'm not sure how many true cost estimates we have seen thus far. There is an assumption it's going to be off-the-shelf and therefore cheaper. But in our experience, media is not cheap, custom media is not cheap, cytokines are not cheap. You are looking at what are your yield is of your process, how many patients per run are you going to get, whether you will need to re-dose a patient versus giving them, a one-time therapy – all of those things are going to factor into the final cost of the product. I'd love to see a little bit more detail out there on allogeneic COGs: exactly how expensive on a per patient cost basis do we think it's going to be, or can we get to? Finally, there is gene transfer technology to consider. We as a field need to move towards higher payloads and of course, the much faster turnaround times of transposons, Sleeping Beauty, CRISPR, etc. will all factor in to COGs.

In short, I think autologous is going to get less expensive than people think it will, and I also think allogeneic is currently more

expensive than people think. It's probably more of an even playing field in terms of COGs right now than many suspect.

VY: From the Gritstone perspective, if we are able to detect the neoantigens on specific tumors, then we pursue a personalized therapy for that patient, and we have to be ready in manufacturing for that scenario. In order to do this, we obviously need to be prepared to handle small-scale and keeping in mind COGs, the only way to do this effectively is to have a high throughput – if you are able to manufacture products for hundreds of patients a month, rather than just a handful. It is also necessary to be able to manufacture the personalized product quickly, which means accelerating not only the bioprocessing but also the release testing. If we can do both these things, I think that handling autologous products becomes feasible.

However, in cases where there is the possibility of producing an off-the-shelf product – for example, we do have certain cases where a KRAS mutation allows us to produce the same kind of vaccine for hundreds of patients – then that is easier and economically more viable for us. So where possible, we would prefer to develop the off-the-shelf product.

DR: From my perspective, I think it is a matter of keeping an open mind about the field and where it's going. We have also seen the diseases themselves evolve over the course of time, and the prospect of successful treatment for various diseases has changed, too. This presents new challenges including, as Vijay mentioned, the fact you might not always be targeting the same antigen for a given disease entity. It's important to be aware of the potential need for the autologous side of this story to be able to assess the uniqueness in a given patient, and that a different target may be required for them than an antigen more commonly associated with a disease.

I think we can learn a lot from the autoimmune disease world where the peptide repertoire that generates a specific disease path varies greatly. If that sort of approach becomes more of a modality for the cancer immunotherapy field moving forward, then we will need to have rapid screening methodologies available to screen patients. And at that point, if you are looking at an autologous approach, you will need to be able to figure out when is it appropriate to screen those patients, and whether a given target antigen will prove to be beneficial for each one of them.

I just think there is a lot we will need to be cognizant of in terms of the future of personalized cancer immunotherapy. But I agree with John that there will be a place for autologous for quite some time to come, even as we continue to push allogeneic approaches forward – in fact, I'm not so sure autologous will ever completely go away, as some envision it will.

Q The centralized versus decentralized manufacturing debate continues to gather pace. What have been the key recent developments for you, and what are the key considerations behind selection of either model?

DR: At the end of the day, nobody cares for your product, no one understands your product, like you and your company does. It is your product, you own it, you have to perfect it, you have to optimize the process. I think there is a time and place

DOI: 10.18609/ioi.2021.019

for decentralized manufacturing, but I don't think it's early on in development. I think you can move out too soon.

Once you decentralize, you have less control over the product. It means you cannot do an immediate in-process assessment as soon as you encounter any variability, to further understand what makes the best product. That is critical for me because but when you come to the final analysis, you sometimes find that some cell therapy products that were supposedly manufactured in the exact same way are more efficacious than others. You have to be able to understand those nuances and build out from that. Additionally, it is very hard to build process improvement and automation when you are decentralized – it's even hard to transfer new changes from process development labs into a CMO with efficiency and certainty.

I do think there are a lot of pros to being centralized, and definitely at the early stages of development. It does add some complexity – in terms of being able to reach some markets, for instance – and it is a little less convenient for the patients. But I think there are technological advances that can help address those issues. For example, while you have limited turnaround time for getting a leukapheresis or whole blood in for primary processing, there are now a number of stabilizers being developed and assessed that could potentially increase seed material stability prior to it entering the manufacturing process. And for those who can pursue it, having a cryopreserved final product delivers a lot more flexibility.

So, I'm not sure that there is going to be a huge need for decentralization in the long-term. And in any event, I personally would not push manufacturing out to a decentralized model until the process was well-defined and 100% locked-down. Even then, you would still have to exercise tight controls and tremendous oversight over that decentralized manufacturing.

> "...while we might not get to the 90% gross margin number you see in small molecules or biologics, continued reduction in COGs will mean we will get to a place where there will be a long-term market for these products."
>
> - **John Lunger**

VY: I agree with Donna: when the technology is very complex, when the analytical methods are also very sophisticated, it makes sense to have all that expertise in one location. For the vaccines our company makes, that is very much appropriate.

However, over recent times I am seeing with certain product types, especially nucleic acid-based vaccines, exploration of 'GMP in a box' approaches. That is because it will be faster just to make the product on site at many locations. It's early days – I think we are still 3–5 years away from the point when those technologies will be sufficiently well developed and it is possible to use them reliably. I would anticipate that centralized manufacture will remain the preferred model for the next 3–5 years at least.

Q Sourcing and managing raw and starting materials and consumables/plastics has come under particular scrutiny during the COVID-19 pandemic – can you talk about the steps your companies have taken to manage risk in this department over this challenging period?

JL: We have taken to begging!

But in all seriousness, clearly the primary need from a public health perspective is manufacturing the COVID-19 vaccines. It's a little hard to argue with that, although of course, the diseases we all work with don't stop just because of the virus.

We have just had to work closely with the suppliers. We have taken safety stocks down to a level we are frankly very uncomfortable with. We've had to prioritize internally, and to focus on quickly qualifying new sources if and when we can find them.

So it's the blocking and tackling of supply-based management that is really what we're having to do at the moment. Moving forward, all those suppliers are trying to add capacity, which will help, but I am not sure there is a magic bullet in this particular area, other than constant communication with the vendor base: making it clear what your products are used for and where you fall in their list of priorities. And micro-managing inventory - we have people who know down to the small numbers how many pipette tips we have, for example, because even those were becoming tough to find.

Q Looking to the future, what are some of the key opportunities for improving IO therapeutic manufacturing and supply chain models and strategies? Firstly, in terms of increasing productivity and decreasing turnaround time?

DR: I think it boils down to a couple of things I alluded to earlier. Firstly, being sure you have a step-through process that means you can build out your facility in a way that you can stage production, but you also have the flexibility to move in and out of those production suites. It is key that you develop a platform, especially as you move towards automation, to be more cost-effective and improve turnaround times, and ensure you have the ability for large capacity within a contained footprint (which also helps contain costs, of course).

Secondly and most importantly is having the flexibility of integrating new technology that can improve turnaround times. For so many of the tumors we work with, cell therapy's advantage and opportunity is as a post-frontline treatment – I think it will be a long time before cellular immunotherapies become frontline treatments. That means we are going to get compromised patients; we are going to get patients already into the more rapid relapse phase of their diseases. And we do not benefit that patient by increasing the time to give them another treatment option. Being able to turn it around fast and avoid bridging treatments during that gap when the product is being manufactured (because a patient had a short-term relapse, for instance) changes the degree of complexity of the whole treatment program.

It is key that the technical staff are on board with this, because automation will have a big role to play in both increasing productivity and reducing turnaround time. It will be important to ensure you have the right integration of technical input from them, but with a minimal amount of hands-on time involved. Currently, even with functionally

> "...most importantly is having the flexibility of integrating new technology that can improve turnaround times."
>
> - **Donna Rill**

DOI: 10.18609/ioi.2021.019

closed systems, there is still a lot of hands-on technical time, which also makes a huge contribution to COGs.

JL: Regarding turnaround time, I think it is important for us to begin to really focus on the patient journey.

Some refer to turnaround time as their manufacturing time - from the start of manufacturing to release of the product. Others talk about vein-to-vein time for autologous products, which is from apheresis onwards (some include lymphodepletion in that process, others don't). We decided to focus on 'I to I', which is identification to infusion. This approach gets you into places that are outside the control of the company – for example, in terms of screening time: we have two companion diagnostics that are part of our process. How quickly those tests happen, how easy they are to conduct and process, becomes part of this effort. One of those tests is a biopsy, so we are looking to move to a liquid biopsy to reduce that timeframe. I think some of the innovation will move towards enabling that 'I to I' timeframe reduction.

Donna raised a good point that we probably won't see a first-line cellular cancer immunotherapy treatment for quite a while. However, perhaps we could collect the apheresis starting material as soon as a patient's diagnosis is made and they screen for the particular product we offer? Then we could manufacture product at risk and to all intents and purposes, have an autologous off-the-shelf product.

If possible, that would be a business decision we would have to work out, which would be based on how much manufacturing the patient's product costs and how much capacity we have available. But nonetheless, that for me is an interesting place we have started to explore, although not by design. We had a patient who was stable on a product and didn't needed the cell therapy we had made for them, so we stored it. Then when their disease did progress, we were able to get the cells to them within several days.

I do think that for autologous products, turnaround time is one of the areas where we are making really considerable advances, both technically and operationally.

VY: One thing that becomes clear when you hear all of these different suggestions is that all of our individual technologies are going to be somewhat unique. So we need to be very much technology-oriented. In this particular case, I think a good guideline is to ask what we will need in the next 3–5 years, and what we might need beyond that.

The continuous improvements to which both John and Donna referred are critical: in order to deliver in the 3–5-year timeframe, we will need to continuously improve both the production and the analytical technologies to get where we want to be. But beyond that, we also have to be looking at newer technologies that are emerging.

DARPA (Defense Advanced Research Projects Agency) recently gave a $41 million dollar grant to GE Research and their consortia to make nucleic acids on demand. DARPA is funding this for 5 years to see if it can be done. If it were to come to fruition, then you wouldn't necessarily need to separately manufacture the plasmid, or to use *E. coli* bacteria – you could perhaps simplify making the mRNA. That is the sort of potential future breakthrough we have to include in our longer-term planning considerations. Of course, it is challenging to have to produce our products today with current methods whilst also thinking about what is to come several years from now.

Q How about in terms of Cost of Goods reduction?

"...it's not only the product flow logistics, but the actual patient journey logistics that are going to need streamlining..."

- John Lunger

VY: As I mentioned earlier, controlling COGs for our vaccines really comes down to high throughput. We will make COGs improvements primarily through having miniaturized, automated process that allow production of hundreds of vaccines a month. The second major point of focus is if we can get some of the testing done in an automated fashion as well. This will mean inline testing, if we can achieve it, which avoids the need to send out the sample to a lab and wait for them to send back the results.

Those are the innovation areas where the throughput is really the key to allow us to get to lower cost. And that is what we will need to make our autologous therapy or personalized vaccines feasible from a business perspective.

Looking to the other end of the spectrum, and to further make the point that this is very much a technology platform-/indication-specific question, we will need to make our COVID-19 vaccine in very large quantities. Once again, high productivity will be central to COGs control. There is a lot of innovation happening in that area - both on the upfront synthesis side and the downstream purification. In a number of cases, the purification yield may only be at 30–40% at the moment, though, so if we can get that up to maybe 90%, it would make a big difference to productivity and COGs control.

JL: I think one further lever for overall COGs control that I think will come increasingly to the fore is material costs.

Right now, we are still using a lot of consumables, media, cytokines – various items that are still at the lab-scale level. The supply base is engaged in trying to scale both the quality and to gain those efficiencies. I absolutely agree with Vijay that serving more patients pushes your cost per patient down no matter how many you do, and I know when we look at our own COGs breakdown that material costs certainly represent a bigger proportion of our supply base than was my experience previously in either small molecules or biologics. So I think that's another area that is an opportunity to reduce costs, and I think it will come about naturally through market pressure and increased demand for all the materials we are using.

Q Can you comment on creating fluid logistic pathways in this context?

JL: I suppose the first answer would be choose allogeneic – that is clearly the answer in terms of logistics because if you didn't have to worry about rapid turnaround, then fluid logistics would become a little bit less of a risk. But let's assume we are still where we all think we will be for a while yet – in the autologous space.

Firstly, get through COVID and get more flights. Certainly, the approximately 50–60% reduction in air travel during the pandemic has impacted our ability to respond. Instead of an hour between flights, it might be 6 hours. That has been something that has surprised us a bit in terms of impacting our ability to meet our supply chain needs.

DOI: 10.18609/ioi.2021.019

Cryopreservation on the front end and back end definitely gives you additional flexibility. I think many companies' clinical sites are now introducing cryopreservation – I know that is the case with both Kymriah® and Yescarta®, for example. I think if you can introduce that to your process and avoid the urgent need for fresh starting material on the front end in particular, that can help streamline the logistics or at least give you some improved flexibility so you don't lose products at that stage.

It is also critical to look at logistics from the patient's perspective and try to streamline the patient's journey, whether that means apheresis in the community, treatment in the community. Right now, there is a lot of logistics involved for the patients as well as their caregivers in just getting to and from the clinical centers of excellence. I think apheresis is something that is a little bit easier to solve than treatment in this regard – it is a market that is steadily growing in the community. The treatment side is more complex, of course, given the need to be able to manage side effects. In our particular case, the patient needs to stay within the treatment area for at least 10–14 days.

So I think it's not only the product flow logistics, but the actual patient journey logistics that are going to need streamlining in order to make these therapies more mainstream.

VY: Again, I think for each of our different technologies, mapping of end-to-end flow is very important.

For me, if I am banking on getting the oligonucleotide from another company, if they are delayed I cannot do the manufacturing. And at the other end of the supply chain, where you are trying to get your final product to the clinical point of care where they will be administering the vaccines, convenience is the key. If you have already made the vaccines in such a way that they don't require freezing and thawing, where they can be stored at or close to room temperature, then the logistics obviously become far less complex. That is an area where technology has a key role to play. For example, more and more people are exploring lyophilizing their vaccines to try to make them stable at room temperature.

Q Finally, can we go deeper on how to enable and expand patient access to cutting-edge cancer immunotherapies moving forward?

DR: I think one of the most important things to keep in the forefront or our minds is the fact that more and more private practice groups are handling disease entities. And their abilities are different to the hospital/academic environment and the classical cell and gene therapy labs within that environment.

That being said, and as has been mentioned before, there are probably easier ways to handle the seed material collection – working with your community blood centers, setting up contracts and agreements, etc. I have done that myself with the American Red Cross and Blood Groups of America in previous studies. And standardizing that material collection process is important. Make sure you have a process that is amicable with

> "...more and more people are exploring lyophilizing their vaccines to try to make them stable at room temperature."
>
> - Vijay Yabannavar

different platforms – different institutions and their apheresis centers, and their various operations. And in every case, you need to be concerned with whether the primary processing of the leukapheresis is done it in a way that allows it to go straight into your manufacturing process.

Returning to my first point, though, I do think the considerations for private practice groups must be brought into play. Even with the big academic centers, if their routine visits or routine treatment is done at private practices located within them, it becomes problematic and disruptive for all involved.

Obviously, most of those centers already have the ability to do the infusion but being able to develop the capability to handle the cryopreserved product coming into their facility might be a different story, currently. I think that will become a larger market as we move forward.

I am located in the Houston area, where we have quite a large cancer center. They have their own pilot facilities in the outskirt towns around Houston that do the actual treatment, meaning patients don't have to come all the way into the city. I think that community outreach to and through those smaller, rural communities is something we must consider - how we can engage that type of environment and broaden access to the seed material at a more convenient location for patients? As John emphasized, you cannot forget the patient journey itself.

VY: From my perspective, I think one of the key aspects is whether we can make the therapy available in a very timely manner to the patients. For example, in the scenario where a patient is undergoing surgery and you want to treat them quickly after that before they progress, then it is going to be really important to partner with that surgical center. We have to look into the logistics in detail, because treating the cancer at the various stages of the disease is going to require different approaches – we will need a collaborative approach to ensure the patients can benefit.

We hope you enjoyed this transcript of the roundtable
You can also watch the recorded roundtable here:

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DOI: 10.18609/ioi.2021.019

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: *Dr Yabannavar owns Gritstone stock and stock options. Dr Lunger owns Adaptimmune stock and stock options. Ms Rill owns Triumvira Immunologics stock and stock options.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Roundtable recorded: *Mar 23 2021;* ***Publication date:*** *Apr 23 2021.*

IMMUNO-ONCOLOGY INSIGHTS

MODELLING THE I-O MANUFACTURING FACILITIES OF TOMORROW

SPOTLIGHT

COMMENTARY/OPINION

Future-proofing cell therapy manufacturing capability: lessons from the NCI

Anthony Welch, Marc Ernstoff & Jason Yovandich

The current state of cell therapy manufacturing includes centralized and decentralized models, autologous and off-the-shelf approaches, and various viral and gene editing methods for engineering the cell product. The National Cancer Institute's (NCI's) Division of Cancer Therapy and Diagnosis (DCTD) supports a cGMP pilot plant for manufacture of cell and gene therapy products including cGMP lentivirus and gamma-retrovirus vectors for use in cell therapy production. DCTD offers these resources and know-how to innovators through the NCI Experimental Therapeutics (NExT) Program and public workshops with the goal of optimizing and standardizing raw material selection, quality attribute testing, and product formulation.

Immuno-Oncology Insights 2021; 2(2), 51–55

DOI: 10.18609/ioi.2021.015

The National Cancer Institute (NCI), part of the National Institutes of Health, is involved in a range of initiatives to enable innovation in cell therapy and to address the manufacturing challenges facing the field. One specific initiative funded by the NCI Division of Cancer Therapy and Diagnosis (DCTD) includes support for a cGMP pilot plant for manufacture of biologics, including manufacture of cell and gene therapy products. The goal of this DCTD investment is to bridge the gap between scientific innovators and early clinical trials. This manufacturing resource is called the Biopharmaceutical Development Program (BDP) and is part of the Frederick National Lab for Cancer Research in Frederick, MD (FNLCR). The scientific staff of the Biological Resources Branch (BRB) are responsible for technical and scientific oversight of the BDP projects.

It is a dynamic period in cell therapy manufacture, and debates over centralized versus decentralized models, and autologous versus off-the-shelf approaches, are ongoing. Ultimately, in order for innovation to continue, these debates must be solved by the underlying science. There will likely be both allogeneic and autologous products available to patients based on factors including the disease in question, the opportunity to use cell therapy as a bridge-to-transplant, and the toxicity of these products in different indications.

Centralized versus decentralized manufacturing capabilities will be dependent on the ability to manipulate and reproduce the product attributes that lead to predictable efficacy. Until the field develops a deeper understanding of these critical quality attributes, innovation will stall. As such, a suite of assays and standards that support the identification and quantification of appropriate quality attributes will be the driver for future manufacturing advances. Currently, the quality of these assays, and the current absence of appropriate standards, limits manufacturing innovation.

In addition, there is a technology gap between the innovator academic scientists involved in mechanism-based discovery research of novel cell therapies for cancer and the clinical production capabilities at various clinical centers or industry. This is due to the fact that the cell therapy products manufactured in support of academic preclinical efficacy and discovery studies frequently require significant process changes when transferred into a cGMP manufacturing setting to support clinical studies. These process changes can, and will, effect the quality attributes of the product. Affordable, small-scale manufacturing technologies that accurately scale to cGMP production levels might be of great value for these innovators.

NCI EFFORTS IN CELL & GENE THERAPY

The FNLCR, under contract from the NCI DCTD, is currently manufacturing CAR T cells to support a multi-center pediatric trial in acute myeloid leukemia (AML), and have FDA approval to initiate manufacturing in support of another multi-center pediatric trial in sarcoma and glioblastoma. In addition, FNLCR has the capacity to manufacture cGMP lentivirus and gamma-retrovirus vectors for use in cell therapy production. Finally, the NCI/FNLCR development laboratories are working on non-viral CRISPR/Cas genome editing processes that will be appropriate for cGMP manufacturing.

The NCI/FNLCR is working on increasing capacity by evaluating current cGMP workflows for CAR T production, in order to identify improvements. Facility capabilities are being expanded at FNLCR to include three additional manufacturing suites that can support either cell therapy or viral vector production.

To address lymphocyte trafficking, tumor microenvironment immunosuppression, and immune target identification, the NCI's Immuno Oncology Branch (IOB) NCI grant portfolio includes extramural efforts to develop small molecules as immune modulators and epigenetic control of antigens, and MHC and immune checkpoint expression. In addition, as part of the Cancer Moonshot℠, IOB is supporting an effort to develop and use canine pet patients in advancing the development of immunotherapeutic approaches.

ADDRESSING CHALLENGES IN GENE EDITING

The NCI is currently evaluating non-viral methods for genome engineering and cellular immunotherapy applications. Improvements in those areas present the possibility of reducing raw material costs, as no cGMP viruses are required, and shortening the time from bench to bedside.

Recently, activities have been initiated to develop processes that support CRISPR/Cas gene editing and non-viral transduction, and small-scale work is underway to evaluate knock-out, knock-in, and off-target effects.

DOI: 10.18609/ioi.2021.015

BOX 1

The NExT program.

The NCI Experimental Therapeutics Program (NExT) program aims to advance and supporting promising new drug discovery and development projects. Only applications with a clear path to the clinic, or potential benefit to patients, are accepted. The NCI may allocate various contract resources to awardees in order to assist in the implementation and development of projects.

The NCI Division of Cancer Treatment and Diagnosis (DCTD) is currently seeking proposals for viral vector and cell therapy production, and investigators seeking production of clinical grade vector and/or cell therapy products can submit proposals through the NExT Program. Application details can be found at [1].

CRISPR/Cas and template DNA (dsDNA or ssDNA) is being delivered via electroporation – in general, electroporation reduces T cell viability more than traditional virus transduction, which presents a challenge for optimization. The assay development required to evaluate off-target effects of genome editing presents another challenge. For a genome editing approach to be of value, it must have high efficiency, low impact on cell viability, and low or no off-target effects. A suite of assays is required to support development from small-scale to cGMP-scale process development. With respect to raw material sourcing, NCI is identifying partners to assist with sourcing cGMP ssDNA templates for use in knock-in cellular editing.

SUPPORTING INNOVATORS

The NCI oversees a program for manufacture of autologous cell therapy products to support Phase 1/2 multi-center clinical trials. The program is funded by the DCTD to support extramural innovators in academic or small-company settings in translating their novel cell therapy concepts to early clinical trials in cancer patients. Access to manufacturing resources is available to the extramural community through a peer-review process called the NCI Experimental Therapeutics (NExT) Program (Box 1). In addition, to better position manufacturing innovation for the future, the NCI is looking for opportunities to collaborate with groups and entities that are establishing assays standards to support cross-lab quality attribute testing.

To facilitate translation and innovation for early-stage cell therapy, developers should define and articulate their production and testing. Therefore, early interactions between innovators, experienced manufacturing teams, and the Food and Drug Administration will help position novel products for both first-in-human and then expanded Phase 2/3 studies, that could provide data in support of regulatory approval.

Some of the common issues that delay translation include:

- **Raw material selection:** for example media, viruses, nucleic acids, apheresis formulation and cryopreservation.
- **Quality attribute testing specifications:** justification for specifications must be supported by data, and address questions such as whether CD4/CD8 ratio, percentage of CAR T cells in the product, non-CAR T cells in product, and vector copy numbers (VCN) value are important.
- **Formulation challenges:** this includes considerations such as frozen versus fresh product, and whether the product is dosed as a flat dose or dose per kilogram.

To better understand the challenges hindering progress in the field, to date, the NCI has also held two workshops on cell-based immunotherapy for solid tumors, in order to

BOX 2

NCI workshops on cell-based immunotherapy for solid tumors.

Following a successful first meeting in December in 2018, the second NCI Workshop on Cell-Based Immunotherapy for Solid Tumors was held on December 10–11, 2020. The workshop reviewed technologies for the development of autologous and allogeneic cell-based therapies for solid tumors, and brought together researchers, industry scientists, FDA representatives, and NCI staff to identify challenges currently facing the field, and potential solutions (Table 1) [2,3].

TABLE 1

Workshop highlights.

Scientific challenge	Potential solutions
Paucity of appropriate tumor-specific targets	Screening approaches and strategies to identify individualized and 'public' neoantigens
Insufficient expansion and/or persistence of cell products	Strategies to overcome T-cell apoptosis, exhaustion and/or dysfunction due to chronic antigen stimulation
Limited understanding of how T cells behave *in vivo* after transfer	Enhanced imaging approaches and other monitoring strategies
Inadequate T-cell homing to tumor	Strategies to improve cell trafficking and tumor penetration
Immunosuppressive effects of the tumor microenvironment (TME)	Enhanced understanding of how metabolic factors and other aspects of the TME affect immune cell fitness and function
Lack of informative animal models	Early-stage 'proof-of-concept' testing on small cohorts of human subjects
Clinical challenge	**Potential solutions**
Low availability of GMP reagents	Improved investigator access to reagents
Inability to compare between different cell products	Standardized methods and assays
Lack of harmonization in IND-enabling studies, especially with new non-viral approaches for cell engineering	Improved dialogue with the FDA to reassess required testing, streamline the regulatory process and reduce cost to investigators
Limited funding and infrastructure for small first-in-human clinical trials	Enhanced support for early phase clinical studies

The workshop identified several major scientific and clinical challenges in the field. NCI will be working to address these barriers and meet the needs of the extramural research community.

engage and interact with extramural researchers (Box 2).

TRANSLATION INSIGHT

In order to further advance the field – and prepare it for an increasingly stringent regulatory environment – the continued identification of the quality attributes of cell and gene therapy products that correlate with efficacy will be crucial to drive innovation. Any innovation proposed in manufacturing must be directed toward preserving or enhancing those attributes and so improved assays and standards that support measurement of those attributes are of critical importance. In terms of scientific understanding of which quality attributes in cell therapies are responsible for efficacy, the field is still in its relative infancy. To achieve shorter manufacturing times, point-of-care manufacturing, and other important goals for the field, the science must always come first.

REFERENCES

1. NCI NExT
2. 2nd NCI Workshop on Cell-Based Immunotherapy for Solid Tumors
3. NCI Workshop on Cell-based Immunotherapy for Solid Tumors

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DOI: 10.18609/ioi.2021.015

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 authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Revised manuscript received: *Mar 24 2021;* ***Publication date:*** *Apr 21 2021.*

INTERVIEW

Driving a new model for point of care cellular cancer immunotherapy manufacturing

JOHN POWDERLY is Founder and President of Carolina BioOncology Institute (CBOI), PLLC in Huntersville, NC. Dr. Powderly attended Georgetown Medical School from 1991–95 and was awarded the Lawrence Dean Scholarship. His 4th year of Med School was spent at National Cancer Institute (NCI) as an internship on the Immunotherapy Service. From 1995–1999 he attended the University of Texas, at Houston Health Science Center for a combined Medicine/Pediatrics Residency. In 1999–2000 he was appointed faculty at MD Anderson Cancer Center and in 2000–2002 performed his oncology fellowship at University of North Carolina, Chapel Hill while focusing on immunotherapy. He founded CBOI in 2005 as a community-based clinic and the only independent cancer center on the East Coast that investigates Phase 1 clinical trial drugs. CBOI has opened more than 100 early phase clinical trials and serves as a regional referral hub for Phase 1 access. In addition, he founded a Human Applications Lab called BioCytics, whose purpose is to perform basic and translational research in immunological treatment of cancer.

R. BRENT DIXON, PhD, is Laboratory Director of the Clinical and Human Applications Laboratory of Carolina BioOncology Institute, PLLC. He previously directed the South Carolina DHEC Public Health Laboratory in Columbia, SC. He has a PhD in analytical chemistry and a BS in chemical engineering from North Carolina State University. He has a master's degree in clinical management and leadership from The George Washington University School of Medicine and Health Sciences. Dr. Dixon is a fellow of the American Association of Clinical Chemistry Academy, the Association of Clinical Scientists, and is board certified as a

high-complexity clinical laboratory director by the American Association of Bioanalysts and the National Registry of Certified Chemists.

JENNIFER MONTAGUE, PhD, is Director of Scientific Business Development for Carolina BioOncology Institute, PLLC and facilitates collaborative relationships with other cell therapy-focused biotech and pharmaceutical companies. She has also held positions in the fields of Medical Information and Clinical Research. Jennifer has a PhD in Biochemistry and Biophysics from the University of North Carolina at Chapel Hill, where she studied the process of apoptosis, or programmed cell death.

STEVE BRADLEY is Facility and HAL Business Manager for Carolina BioOncology Institute, PLLC. He previously worked in various manufacturing roles, most recently as Plant Manager for Mohawk Industries largest production site in Thomasville, NC. Steve has performed multiple facility startups, has a BS in Chemical Engineering from North Carolina State University, and an MBA from the McColl School of Business at Queens University in Charlotte.

Immuno-Oncology Insights 2021; 2(2), 57–67

DOI: 10.18609/ioi.2021.011

Q Can you briefly introduce us to the Carolina BioOncology Institute – its background and current activities?

JP: We have a simple vision: our model is to be a single facility to leukapheres, grow immune cells, and dose patients in Phase 1 studies.

We are actually two organizations under one roof – a clinic (Carolina BioOncology Institute) and small biotech company. (BioCytics) Carolina BioOncology Institute is the larger organization and it incubates BioCytics. Both were founded in 2005 as a reflection of the same vision but they are separate organizations, each with a different purpose.

Carolina BioOncology Institute is a PLLC based in Huntersville, North Carolina – a suburb of Charlotte. It's a cancer research clinic focused on Phase 1 immuno-oncology trials, whose mission is to provide patients with access to the Phase 1 immuno-oncology pipeline with the highest level of comprehensive and quality medical care. The clinic has seen over 4,000 patients and has opened more than 100 Phase 1 trials since 2005. Currently, the clinic occupies 31,000 square feet, and we recently completed a 15,000 square foot buildout of a human applications

lab, transforming prior warehouse space into multiple large cGMP ISO 7 cleanrooms for Phase 1 development.

BioCytics is incorporated in Delaware. It's a small biotech company with a mission to develop effective, affordable, and accessible autologous cellular therapies to treat solid tumors. ("BioCytics" translated literally means "living cells, applications of"). We have a clinical trial, BioCytics 0001, which began accruing in 2007. It is a phlebotomy, tumor biopsy and leukapheresis study, which allows our clinic to collect patient samples from healthy volunteers, convalesced patients, and cancer and autoimmune disease patients for our in-house research and development. To date, it has accrued 605 patients.

Carolina BioOncology Institute has a Human Applications Lab (HAL) that also performs development and manufacturing services along with translational laboratory research to help Phase 1 sponsors develop their cell therapies.

Additionally, we are also developing our own in-house proprietary cell therapy pipeline, which comprises fractions of different immune effector cells.

We are the only such model within the United States where an independent human applications lab is adjoined to a privately held Phase 1 clinic.

Q Tell us about the transition to a point of care cGMP biologics production facility – what have been the key steps on this pathway, for you?

JP: I can thank my prior mentors at the National Cancer Institute, where I first conceived of the model over 25 years ago. I initially thought all cancer immunology research labs had a clinic down the hallway. I then realized during my career the translational highway from bench to bedside was often a major bottleneck, especially in academic centers.

So when I founded both companies in 2005, I designed the Phase 1 clinic to be a gateway to a future cell processing facility as a means to an end – the end being to eventually apply immune cellular research directly to patients. Over the years, the model has slowly been adapted as I have learned about the full value and efficiencies of being independent, nimble, and at the point of care, with access to patient biospecimens for translational research and GMP optimization.

Some of the key steps or drivers were actually market indicators and opportunities that helped accelerate our transition into a GMP facility. For example, there was a 233% increase in IO agents in development between 2017 and 2020, of which 34% were cell-based. And soon the number of cell therapies in development will surpass the number of drugs [1].

On the technology side, multiple new benchtop devices can perform cGMP processing for single patients, so that technical feasibility to grow cells at the point of care has now become a reality.

I still believe there are currently too many bottlenecks for centralized manufacturing, which is why I think decentralized manufacturing, such as our point of care model, will soon leapfrog it. Taking patient access as one example, with the point of care model you can have a collection site dedicated to patients who are familiar with their oncology nurse – and that nurse is also cross-trained in leukapheresis.

> "...there are currently too many bottlenecks for centralized manufacturing, which is why I think decentralized manufacturing, such as our point of care model, will soon leapfrog it."
>
> - John Powderly

A further example is the entire paradigm shift I see towards patients being able to retain custody of their own cells. Their leukapheresis cells can be banked and/or used for different immune fractions or constructs without having to undergo repeat pheresis, or delays waiting for a particular centralized manufacturing run for a given sponsored trial.

There are also cost savings to be made through point of care manufacture – for instance, you don't need a courier or other third-party logistics supplier.

The removal of complexity in the supply chain in general addresses a key bottleneck for centralized manufacturing. If the autologous pheresis occurs at the point of care alongside the manufacturing, you avoid many of the delays with scheduling, for example. In the CDMO model, just rescheduling a patient for a suite that has been reserved can cost up to $15,000. We simply reschedule the patient at no cost because we have plenty of cleanroom space and chairs available to conduct the leukapheresis. And of course, with advanced cancer patients, scheduling logistics are very difficult to begin with due to the need for multiple visits to the doctor.

Large, open ISO 7 rooms enable multiple benchtop cell processing and culture devices per room, avoiding the antiquated 'one suite, one patient' process. And our small size means we can integrate our collection data and metrics with cGMP processing and dosing data all on the same informatics platform, which leverages modern bioinformatics and captures capabilities for future AI. We are currently interfacing our EMR and EDC and human applications lab with our clinical trials.

There is the time factor to consider. Terminal cancer patients cannot afford to await pre-authorization and suffer insurance denials and delays.

An additional positive for our 'in-house' cell therapy fractions at the point of care is there should be a reduced risk of cytokine release syndrome, so we would avoid agonistic gene inserts unless there is an 'off' switch in the construct.

Finally, patient consumerism demand is a key driver that will enable future patient-funded point of care cell therapies, ultimately decreasing cost versus the current big pharma/centralized manufacturing model.

Q Turning to the design of your facilities, what were the initial chief considerations and decisions for you?

SB: When Dr Powderly planted his flag in 2005, he did it in such a way that he was able to pick up the adjacent space each time it became available. So he steadily built from 1,000 square feet to 30,000 square feet today that includes the additional 15,000 square feet that just built out.

DOI: 10.18609/ioi.2021.011

We did over 20 revisions in planning the ISO 7 cleanrooms. Key considerations were that they needed to be large enough to handle very high volumes, but also flexible enough to handle different devices – whether that's a CliniMACS Prodigy®, or one of the other devices on the market for cell expansion, such as the Quantum or Xuri. We wanted the flexibility to be able to keep those together or separate. The result of this process is that we now have three large rooms and two smaller rooms.

Another important consideration was that we needed the cleanrooms to integrate with a nearby USP-797 and USP-800 pharmacy, a GLP translational lab, a CLIA High Complexity Clinical Lab, an analytical lab space, and plenty of freezer storage. Ensuring everything was well designed at those points of interconnectivity was crucial. There are pass-throughs in key places, and everything is basically adjacent, so a patient's cells can literally be leukapheresed, walked down the hall, and grown in a cleanroom within sight of that patient.

Our model focuses on Phase 1 trials, which by definition have an experimental or development component. Having a larger cleanroom allows for good interaction (assuming the need for many re-qualification 'bridge' runs) whilst enabling closed system processing with separate, dedicated areas for incubation. This allows us to utilize the space available to its maximum.

Another key was affordability and this was a big focus. Our recent build-out in an adjacent warehouse space was done on a limited budget (<$1.5 million) but we used the best materials we could find under that budget and carefully managed the project. The project engineering and project management was all done in-house, which helped keep costs down and quality up.

Our concept was further refined when David James, PhD (founder of Scinogy's Rotea) visited our site in 2018 and discussed his recent publication, 'How short-term gain can lead to long term pain', which included discussion of cleanroom building [2].

Q What is your approach to ensuring optimal flexibility/future-proofing and efficiency moving forward – in terms of both evolving bioprocessing tools and technologies, and being able to cater for novel/emerging therapeutic modalities?

JP: Being in the Phase 1 trial space, it is imperative that we cater to the shifting sponsor market and also to patient consumerism demand. That is why we built our model for the cellular future.

We envision that many cell and gene therapy trial sponsors will need our in-house backbone of autologous cell therapy fractions to combine with their viral or non-viral constructs, antigens, cytokines, and reagents.

Cell and gene therapy sponsors also may leverage our knowledge – for example, of cultures and other methodologies for the various immune cell fractions and how to upscale

"Our model focuses on Phase 1 trials, which by definition have an experimental or development component."

- Steve Bradley

them, or regarding the many device options available for cell selection and culture, including manual/semi-automated/automated, viral or non-viral, magnetic or flow sorting, etc.

We are early adopters of many of these new device platforms (the aforementioned Rotea, for example) which will attract sponsors who want different options in terms of platforms to compare and try to optimize. It is noticeable that many manufacturers are currently aligning themselves with certain devices, but we have a different approach. We want to have a multitude of different devices that are configurable, so that we can maximize efficiency and have different pathways available to optimize various cell products depending on the different devices they may need. That allows C&GT sponsors to switch devices, re-optimize and requalify in the same cleanroom during a Phase 1. Again, we see this as a means of avoiding or removing the bottlenecks in centralized manufacturing, competing on cost of goods (COGs) and to leverage the efficiencies in point of care manufacturing.

Many of our current Phase 1 drug sponsors – those making cytokine analogues, novel checkpoint inhibitors, or agonists, for example – are also beginning to develop their own cell therapies. That gives us the advantage of being able to leverage long-standing relationships with those sponsors and accelerate development accordingly.

SB: I mentioned the two smaller cleanrooms early. Those were designed specifically as smaller project rooms that could potentially be dedicated to a single sponsor, or dedicated to our own IP for something specifically tailored. They were also set up and qualified to run at either positive or negative pressure, and we have further flexibility in terms of being able to run emergency power, CO2, nitrogen, or medical air to any table or other location within the space. So I won't say those rooms can be reconfigured on the fly, but they can be set up quickly for a specific purpose without having to gut the infrastructure.

BD: It is also key to think about how we train our staff. To teach our growing workforce, we have designed a thorough 'onboarding' process to orient and train staff as part of our competency evaluation across the variety of devices in our cellular therapy laboratory.

The other tools we use are process development and continuous improvement (such as Plan, Do, Check, Act). These enable consistency and optimization of critical steps, in conjunction with standard operating procedures and change control processes.

Operationally speaking, effective communication is important and we utilize team huddles to allow for real-time decision making and input from all staff.

Medical technologists and biomedical technicians are highly skilled in clinical labs and clearly understand regulatory environments, so we've helped cross-train them for competency and proficiency in both the clinical laboratory and the cGMP processing. That also helps future-proofing with optimal flexibility, as it gives us a multiple-disciplinary team of scientists, technologists, and staff working together in a dynamic fashion to quickly apply new technology.

Q Cell therapy is clearly a key area of focus for you moving forward, but it is an area that is still relatively immature, at least in cGMP manufacturing terms – what do you see as the remaining challenges in this regard, and what is your approach to addressing them?

DOI: 10.18609/ioi.2021.011

JP: One of the most common bottlenecks is access to starting materials, such as autologous cells. Typically, centralized CDMOs will perform tech transfer and optimization on healthy donor cells. But we know from our in-house results that healthy donor cells have very different immune cell counts, phenotypes, and function (e.g., exhaustion and anergy) compared to those from cancer patients.

> "Operationally speaking, effective communication is important and we utilize team huddles to allow for real-time decision making and input from all staff."
>
> - R. Brent Dixon

Our model leapfrogs over those challenges because being at the point of care, we have many cancer patients willing and motivated to donate their autologous cells. Those can then be used for optimization and qualification runs, whilst also allowing us to engineer ways to expand exhausted but cancer-specific T cells.

Another huge challenge is affordability and accessibility, which our model addresses by vertically integrating the manufacturing at point of care using automated, single-patient closed system platforms, whilst still maintaining the rigorous QC/QA requirements of cGMP manufacturing.

It is also challenging being the first to do our particular model. The current stakeholders, such as large CDMOs and big pharma, don't want to lose control of patient cell custody, but our model turns that on its head by declaring that the patients should keep custody of their own cells. A single patient's leukapheresis could be used for multiple immune fractions, or different constructs from different sponsors, or different cell products simultaneously, whether they are from our own in-house sponsored research, or from multiple other collaborating biotech sponsors manufacturing at our site or elsewhere.

The big question there is, if we do it, will the patients come and pay for it? I can only quote my patients who ask me every day 'when can I get my own T cells back?' I tell them we are almost there.

Q How do you view the remaining obstacles to a widespread migration towards point of care bioprocessing and QC of autologous cell therapy products?

BD: The biggest hurdle is efficiently managing the unique complexities of clinical practice, the diagnostics laboratory, and cell manufacturing, each to their respective quality and regulatory standards. Because small companies working in the cell and gene therapy development space invest so much in identifying and optimizing their biological product, the steps needed to bring it through the regulatory framework to scale-up, and then to cGMP development steps such as qualification runs, CMC, and the IND process itself, can be overlooked until relatively late in the day. We can leverage our expertise and flexibility to work with small biotech sponsors to support tech transfer, optimization, and upscaling, which is key to our future growth.

It is key to have a strong quality management system. We have developed ours over time – we recently completed our 300th SOP clinic-wide. We have GMP cell therapy software with Title 21 Software solutions, which interfaces with our EMR, and we will be able to collect the quality metrics just as well as a centralized facility. We have the added advantage that our leukapheresis collection efficiencies, cGMP cell health metrics, batch processing, and release potency assays can all be correlated with patient labs and adverse events, tolerability, efficacy, and survival, in real time across one enterprise scalable model. This also allows for feedback algorithms and predictive biomarkers.

We are proud to have recently passed our first GMP vendor quality audit, and we're currently working on our first sponsored GMP qualification runs for a Phase 1 cell therapy.

Q Tell us more about the starting material procurement/management side – can you share any keys to ensuring robustness and efficiency, and how the testing and analytics piece in particular is evolving to provide insights that can be leveraged downstream in bioprocessing?

JP: We have performed 138 leukapheresis over the past 3 years – that is approximately one to two per week. Half of those patients are stage IV solid tumor cancer patients. We track the leukapheresis-related adverse events and we perform complete blood counts with five-part diff on each patient pre- and post-pheresis, and on the leukapheresis product itself, in order to calculate hematocrit and lymphocyte collection efficiency for all of our patients.

To date, all leukapheresis adverse events have been mild grade one, so performing this on advanced stage IV patients is feasible. We have also validated an 8-color flow cytometry panel and identified major differences in the starting material of cancer patients compared to healthy volunteers, such as lymphopenia and exhaustion markers.

We have learned ways to improve venous access and optimize lymphocyte collection efficiencies based on leukapheresis parameters, total blood volume, flow rate, and other Spectra Optia® settings, after reviewing our data with TerumoBCT, who have been very helpful.

Most importantly, cancer patients who are severely lymphopenic, which is common after chemo and/or checkpoint inhibitor treatment, will need additional attention downstream with their cultures and expansion time, and potentially other cytokines and agonists.

"Today, we are increasingly seeing the introduction of non-viral transduction..."

- John Powderly

Q Solid tumors include a diverse collection of cells with distinct signatures and different levels of sensitivity to treatment. How is your IP method or model suited to address this heterogeneity?

DOI: 10.18609/ioi.2021.011

JP: Metastatic cancer has intra-patient heterogeneity, which makes it difficult to target. Often, each tumor may be an entirely different mutant clone, with its own immune escape mechanisms (i.e., mutational antigenic shift, checkpoint shift, HLA/MHC down regulation).

By performing leukapheresis for CTCs, we gain access to the entire heterogeneity of patients' clones. We are able to identify these immune escape mechanisms and choose various fractions of immune cells that have the best ability to kill escaped tumor clones. We have built our model around this concept: to engineer adaptable autologous cell therapies with perpetually feedback-learning algorithms using tumor lymphocyte killing assays. The beauty of this model is that it also can be 'front-loaded' into both neoadjuvant and adjuvant settings, because we know CTCs are abundant in diagnostic leukapheresis (DLA) in these settings.

My vision is surveillance by DLA to select and engineer immune cell fractions before the patient relapses radiographically or develops any symptoms. We can monitor whether our fractions are working by the DLA CTC count. This can only be optimized at a point of care model with fresh DLA CTCs because CTCs are extremely hard to grow and don't survive freeze/thaws.

Q What are the chief trends you have seen from manufacturing tool providers in recent times: what have been the key breakthroughs for you, and what would be top of your wish-list in the way of future innovation to come through?

JP: Firstly, on a general note, I think that viral vectors are last decade. Today, we are increasingly seeing the introduction of non-viral transduction, which is going to be this decade: better electroporation and mechanoporation methods and tools; chemical, nanoparticle and microfluidic mechanisms are all coming fast, are affordable, and are being built and automated with Quality by Design methodology for in-component QC/QA.

There have been improvements in cell selection and enrichment, such as closed sorting – for example, the Tyto™ device, which we are using. Other improvements in automation coming through include in-line culture biosensors for cell health, phenotyping differentiation markers, and even cell counts in real time.

Improvements in primary tumor culture are key to us because we are point of care. These include primary tumor organoids, hydrogels, matrices, and hypoxia (including culturing leukapheresis CTCs, which is one of our core points of research focus).

We have also seen improvements in adult induced pluripotent stem cells (iPSCs), which are following similar trends to the tumor organoids.

Q You work with tool providers and help them to development their technologies – are there any best partnering practices from the tool provider side that you would highlight? Any advice on how to optimize such relationships?

JP: I am used to being the squeaky wheel because we are an early adopter. So we love tool and device makers who listen to us. And a big issue is configurability.

If a device maker has an automated, closed system cell culture device or cell selection device, we want it to be configurable enough that the end user can use it through their pre-GMP optimization and qualification runs and tweak it along the way so it can be continuous with the pre-Phase 1 and Phase 1 process. That is a learning curve that device makers have to change: we want them to be more adaptable and configurable with their devices. It is coming now… But we had to yell that from a building.

Q Can you distill our discussions today into a vision for the cellular immunotherapy bioprocessing model of the future?

JP: I predict decentralized, regional Human Application Labs with clinical doors for patients will eventually surpass centralized models, bring costs down while increasing access, and usher in a new market of patient consumerism to cure their own diseases, from cancer to autoimmune diseases, and from diabetes to hemoglobinopathies.

The question is, who will lead the new decentralized market: hospital systems? Academics? Device manufacturer? Legacy CDMOs? Or new players like ourselves? I think we are 15 years ahead of others who have not seen the advantages of the decentralized market opportunity for patient access and demand. I have spent the past 15 years designing and building it.

REFERENCES

1. Upadhaya S, Hubbard-Lucey VM, Xin Yu J. Immuno-oncology drug development forges on despite COVID-19. *Nat. Rev. Drug Discov.* 2020; 19: 751–2.
2. James D. How short-term gain can lead to long-term pain. *Cell Gene Ther. Ins.* 2017; 3(4): 271–84.

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DOI: 10.18609/ioi.2021.011

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: *Dr Powderly has received grants or contracts from Nucleus Inc, MT Group, STEMCELL, BMS, Roche, AstraZeneca-MedImmune, EMD Serono, Lilly, Macrogenics, InCyte, Top Alliance BioScience, Seattle Genetics, AbbVie, Corvus, Curis, RAPT Therapeutics, Alkermes, Arcus BioSciences, Tempest Therapeutics, Calico Life Sciences, Apros, Jounce, Atreca, BioBank Online, Sequenom, RepLimmune, Engineered Pharma, CEM Corporation, Therapeutic BrainPower LLC, Merck, Conjupro, Repertoire Medicines. He has received consulting fees from TerumoBCT. He holds joint IP with Atreca Inc. and is developing IP for BioCytics and CBOI for cell therapies. He is on the Data Safety Monitoring Board of AbbVie, a stock holder of BioCytics Inc. and CBOI PLLC, and is currently speaking with venture firms, stake holders and oncology practices to raise capital for equity stake in BioCytics Inc. to clone facility network enterprise model.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

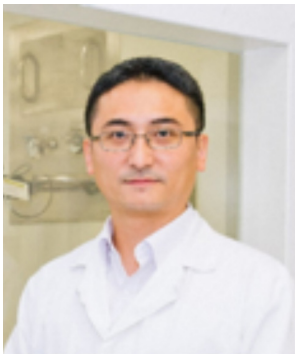
Interview conducted: *Feb 11 2021;* ***Publication date:*** *Apr 16 2021.*

MODELLING THE I-O MANUFACTURING FACILITIES OF TOMORROW

SPOTLIGHT

INTERVIEW

Key considerations in delivering tomorrow's biopharma manufacturing facilities and workforce



EDWIN HUANG has over 15 years of research and commercial experience in bio-processing technology, covering microbial, plant cell and mammalian cell systems for various bio-production purposes, with a strong focus in mammalian application for biologics production to support pre- to early stage of clinical studies. Edwin has held various technical and managerial positions with local and oversea, private and publicly-listed start-ups, like Acyte (Sydney), Apollo Life Sciences (ASX:AOP), Biosceptre International (UK), and brought therapeutic antibody products into first in human use. A cross discipline background in engineering, life science and commerce has enabled Edwin to provide sound business and investment consulting in areas of biopharmaceutical and life science to management, existing and prospective investors and other research firms. Edwin is currently managing the $11.5M Biologics Innovation Facility at UTS, and also a retained expert with multinational patent law firms to work with global biopharmaceutical companies.

Immuno-Oncology Insights 2021; 2(2), 73–81

DOI: 10.18609/ioi.2021.010

Q Can you introduce us to the UTS Biologics Innovation Facility and its activities?

EH: The Biology Innovation Facility (BIF) is a relatively new capability at the University of Technology Sydney (UTS), having opened its doors in the latter part of 2019. It represents an AUS$11.5 million investment, mainly by UTS but also partly funded by the New South Wales State Government's Department of Industry. The staff in the facility are sponsored by National Collaborative Research Infrastructure Strategy (NCRIS) programs, through the funding management body of Therapeutic Innovation Australia (TIA).

The facility is 430 sq.m. and is intended for research, training, and early-stage manufacturing of biologics. It was designed and commissioned to fully comply with GMP requirements. As it is primarily a university research and teaching facility we are not going to seek ongoing GMP accreditation, mainly due to the financial implications of doing so.

Thanks to a strategic partnership with Cytiva (formerly known as GE Healthcare) the facility is equipped with state-of-the-art, single-use technology. And through further partnership with other vendors, like Pall Filtration and Sartorius, BIF is available to provide a full range of pharmaceutical manufacturing best practice and techniques.

There are four main goals for the BIF:

1. To become a regional and global training hub for bio-manufacturing
2. To provide bioprocessing capabilities and support to translational research at preclinical and early clinical stages
3. To provide a 'sandbox' for start-ups or SMEs to develop innovative and fast-to-market solutions, with reduced cost and risk
4. To promote connections to and awareness of the biopharmaceutical industry in general

BIF actually provides hands-on teaching and training experiences by being the only institute in the southern hemisphere that delivers training programs from the Dublin, Ireland-based National Institute of Bioprocessing Research and Training (NIBRT). NIBRT is an Irish Government-funded facility that provides training to support biopharma companies seeking to establish manufacturing facilities in Ireland. However, NIBRT programs have now gone global to meet the needs of trainees who wish to develop their career in the bioprocessing industry wherever they may be located. By bringing this program to Australia, BIF can now train people not just from science and biologics backgrounds, but it can also re-skill or up-skill those from other manufacturing industries, such as the automobile and mining sectors. These trainees gain unrivalled access to full, operational manufacturing plants in simulated GMP environments, giving them hands-on experience with both GMP equipment and operations.

BIF also provides process development, manufacturing, and analytical services to the biotech industry and academia, as a platform for scalable, cost-effective, and reproducible biologics manufacturing.

The facility has upstream process bioreactors ranging from 250 ml to 200 l, working with different mammalian cell expression systems to deliver recombinant proteins in quantities

DOI: 10.18609/ioi.2021.010

"Once the antibody production cell clones or cell lines are developed, the Biology Innovation Facility can assist with process development and can then do the scale-up batch production. We also can go into the analytical side to support the final product characterization for quality control and quality assurance purposes."

ranging from milligrams to kilograms. We have industrial-scale infrastructure, such as a 2000 l decontamination system and a 4,000 l water purification system, and BIF is future-proofed by the ability to incorporate an additional 1,000 l bioreactor.

Q Can you go into more depth on your work specifically relating to immuno-oncology modalities and platforms?

EH: The majority of projects BIF currently deals with are in the protein therapeutics field, partnering with academics and start-ups as the manufacturing partner for their therapeutic antibody programs. Once the antibody production cell clones or cell lines are developed, BIF can assist with process development and can then do the scale-up batch production. We also can go into the analytical side to support the final product characterization for quality control and quality assurance purposes.

However, BIF also provides a sandbox to help people to explore technologies and regulatory pathways for the manufacture of other innovative products. For example, BIF recently assisted a local Australian start-up to do scalability and workability assessments for its proprietary oncolytic immunotherapy, which have previously proven to be a potent system for targeting advanced melanomas. We do have other companies talking to us about a range of product types – for example, exosomes – but they are all in the planning stage at present.

Q With global demand for cutting edge, increasingly complex biologics set to increase dramatically in future, what do you see as the key trends and advancements that can help meet this growing need?

EH: A number of industry trends over the past decade have contributed to the current strong desire for production facilities that are able to accommodate multiple product operations, and that also have fast change-over, which means short

downtimes between production runs. The rise of personalized medicines and niche drugs gaining momentum in the marketplace are two examples.

Furthermore, thanks to advances in various technology areas, we now see increasing volumetric output of the bioreactors we use in terms of grams per liter quantities. Therefore, more product can be made with smaller bioreactors.

The third key trend for me has been the huge drive in pushing the application of Quality by Design principles as early as possible in product development. Scalability is being built in from very early stages of process development.

These three things combine to form a growing understanding that to ensure the future success of bio-manufacturing facilities, they need to be both adaptable and responsive to industry needs, whilst maintaining quality, flexibility, and efficiency. At BIF, we believe the modular and configurable type of facility design and fit-outs, largely based on single-use technologies, present an answer to this.

Over the last couple of years, I would say the vast majority of new biologics facilities have been based on some form of single-use technology – usually the combination of easy-to-clean stainless steel for the external housings, with pre-sterilized, pre-validated, low-leaching disposable material for product contact interfaces.

Configurable single-use unit operations allow process lines to be modified or extended quickly by simply moving or adding production units at the required locations in the facility. Compared to the traditional GMP facility, which is based on large stainless steel tanks hard-wired with piping for water and steam, we find that single-use unit operations-based facilities reduce time to market as well as capital expenditure. In other words, the time period from the day you design the facility, through construction, to the day when you actually start pumping the product out is much shorter, with lower investment.

Additionally, these new facilities benefit from reduced operational costs because they require less labor to clean and sanitize. And the changeover between batches and different product runs is much faster because equipment doesn't require the same degree of cleaning and validation between runs. Basically, one production run finishes, you put in new bags or consumables, and then you start the next production run. Most importantly, because you have this operational flexibility, you don't necessarily need a big facility. You wheel in/wheel out the unit operations as required. Of course, smaller facilities mean fewer utilities and infrastructure required to support them.

We believe this lighter capital investment combined with a greater operational agility and ability to respond to changing market requirements means this single-use technology-driven model represents the future of the bio-manufacturing.

Q What other technological aspects or advancements will play a critical role in the future of bioproduction, for you?

EH: Clearly, automation will be crucial. Biologics are still produced very much in a manual or semi-manual way.

Within each individual unit operation, we are starting to see more automation happening – for example, in cell counting. The next step is to start connecting different unit operations

DOI: 10.18609/ioi.2021.010

together in an automated manner. Currently, the main advancements are probably more on the QC side – we are seeing more in-process automated sampling and analysis. I think these are good starting points. Automation will come in more and more, as there will always be the drive to minimize human involvement and thus further improve the safety, speed, and robustness of the entire production process.

We are also beginning to see more incorporation of cloud-based and other big data-related technologies – so-called Industry 4.0 – which is providing a degree of real-time surveillance and feedback in biomanufacturing facilities. Bioprocessing equipment is starting to feature wireless data transmission capabilities feeding into process control. This allows operators to have more real-time visibility of what is happening during bioprocessing, and to have the capability to make process adjustments as required.

That goes hand in hand with automation, of course. We believe the third piece that will integrate everything is artificial intelligence and machine learning. This innovation will drive in-process efficiency improvements through the real-time leveraging of decades of accumulated biopharma industry knowledge.

Ultimately, we would all like to see biotherapeutics being manufactured as reproducibly and precisely as small molecule drugs, but at the end of the day, we are talking about biological systems: there will always be some intrinsic variation between batches and production runs. However, the application of AI can assist our forward-thinking and help us to respond to process needs and events – it is necessary to not only improve process efficiency, but to ensure we have the optimum strategy in place to deliver the best possible product quality. We anticipate that this sort of technology will be built into the facilities or unit operations of the future to help operators, engineers, and scientists to better manage production on a day-to-day basis.

Q Regarding your role in helping train the industrial biologics manufacturing workforce of the future, can you comment on the nature of this challenge as you perceive it at the 'coalface', so to speak, and how all stakeholders could evolve and contribute moving forward to help alleviate this considerable issue?

EH: Before setting up the BIF, UTS ran some industry-wide surveys to understand the skills requirements to support the biopharmaceutical industry, and the biotech industry in general, in Australia. The overall feedback they received was that Australian biopharma and biotech SMEs were kept very busy trying to recruit to fill skill gaps, because they frequently couldn't find the right person for the job. This was something of a surprise because the Australian Government does generally seek to

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enable scientists and engineers to settle in the country.

UTS then approached NIBRT in Ireland regarding their bioprocess training curriculums. We wanted to understand why this industry-specific training should be required – why traditional university training proved to be insufficient enough to prompt the Irish Government to set up NIBRT in order to support the industry in Ireland. We discovered that NIBRT had conducted a similar survey to ours back in 2018, and that they also found a very high percentage of biopharma industry responders (about 86%) were experiencing difficulty in filling one or more positions within their organization. So it seems that this is not just a problem we have in Australia, it is a global thing.

We decided to dig deeper in order to better understand why people think traditional university training, which after all prepares students with good knowledge, training, solid theories, and understanding, is not sufficient to meet the skills requirements of the biopharma industry. We wanted to identify the missing elements, and we learned two things – two misconceptions that people have about this industry, essentially.

Firstly, people think that to work in industrial biopharmaceutical manufacturing, you require very highly specialized skills and very specific training. We believe that is not necessarily the case.

Of course, there is a requirement for highly specialized scientists and engineers in the R&D space. But when it comes to manufacturing, we think it is a much more multi-disciplinary environment: it is important that staff have some understanding of the science, of clean-room operations, and of quality systems for documentation. However, each of these skillsets does not necessarily require very in-depth theoretical understanding. What is more important is to understand how the knowledge from each of these different areas is brought together and integrated within the facility.

This helps to explain why some industry partners state that while their staff might have very in-depth, specific scientific or engineering training, they tend to feel there is still something not quite right. We now understand that if you want to send those people into the industrial manufacturing environment, where many different specialized areas are married together, they may need to receive broader training. That is a key benefit of NIBRT's industry-focused training – we prepare scientists and engineers based on what they already know, but we also share some of the things they might not be so familiar with.

It is also important to recognize that the skills required are not as highly specialized as people tend to believe. While some people regard being highly specialized as a positive thing – allowing them to work with cutting-edge technology, for example – others do not. For many, specialization comes with a concern that their skillset may not be easy to transfer into other areas later on, should they wish to move on to a different role or a different industry at some point in their career.

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We set out to encourage and help our students understand that while they are being trained to work in the biopharmaceutical manufacturing environment, we hope to provide a multi-disciplinary experience for them – that they will be able to move on to other opportunities later, if they wish, because many of their skills are highly transferable. For example, understanding how to operate in the cleanroom, or how to manage GMP documentation, are readily applicable skills in numerous other manufacturing industries – pharmaceutical packaging, for example. So we don't want people to have this idea that they must be very determined to get anywhere in biopharma manufacturing, and that they will then be stuck there – we prefer to look at it as a good starting point for any career in manufacturing.

The second misconception is that many people worry about long-term employment prospects in the Australian biopharma industry. That is common feedback we receive when talking to people who are considering training with us – they are unsure whether it is the right investment, because they might not end up with a job. However, if you also talk to industry people, they will say they don't want to invest in setting up manufacturing facilities because they are not sure they will be able to find a sufficient workforce. So it becomes a 'chicken and egg' situation.

Another key lesson we have learned from NIBRT is the importance of encouraging people to take a long-term vision of how to build the industry. And that isn't just about individuals or the industry, it is also likely to require Government intervention. If you look at the Irish model, the Irish Government set up NIBRT to deliver a workforce first, which then allowed them to actively encourage biopharma companies to come to Ireland and set up manufacturing there. We think this is a model we can learn from and leverage in Australia.

Australia, traditionally speaking, has very high-quality medical research. There are a lot of smart scientists and engineers here, who already help us to move bench research towards commercial manufacture. What we need is someone to kick start the cycle. The industry may think that if they come to Australia, they will have to invest both in setting up a manufacturing facility and in training the local workforce. If government can assist with this whole process and alleviate that concern, it will encourage industry to invest here and by doing so, they will also create more job opportunities for Australians. All three parties have to review this situation and find a way to support each other to make the industry happen.

While COVID-19 has undoubtedly been a very unfortunate thing for many, many people, there is some small silver lining in that it has prompted a review of so-called 'soft infrastructure' and sovereign manufacturing capabilities. I think that has brought about a realization that there are certain manufacturing sectors, certain skillsets we would like to have in Australia, and we believe that the Government is now taking the initiative to make that happen. There is a lot of talk and funding now going into soft infrastructure preparation, and we believe more will follow. We hope that UTS and the BIF can play a part by ensuring that our students have open minds so that when the opportunity comes, they can work together to build the industry.

Q How would you sum up the current state-of the art in strategies to optimize the flexibility of biotherapeutic manufacturing facilities – and where do you expect to see continued progress in this regard over the mid-to-long-terms?

EH: We have entered a new era of single-use technologies that has been driven by the need to generate more personalized biologics and therapeutics, and fewer global blockbuster drugs. In addition, the capability to run multipurpose facilities is enabling the modular construction of facilities, a reduction in raw materials used, less cleaning and validation between batches and ultimately, lower costs whilst generating greater production efficiency. This is enabling both small and large biotherapeutic companies to produce multiple therapies in parallel and at various stages of development: in essence, having the right capacity in the right location for patients they want to treat.

Continuous progress is needed, as it is in any industry, and there are still a lot of efficiencies to be gained across development, design, clinic, and manufacturing. Within development and design, the idea of using scalable technologies reaps rewards when transitioning from the bench to the clinic. Using scalable bioreactors, cell lines, chromatography techniques, filtration media, and reagents that can all be used from research through to production can keep costs down. The adoption of mAb and recombinant protein workflows, which are now widely accepted by the regulatory bodies, and using their fundamentals for the purification of new therapeutics like viral vectors and mRNA vaccines will shorten time to market.

Further improvement of process economics, by getting more output per unit of time, volume, or dollar, may be achieved through integration of technologies, operation simplification, and insights gained via analytics. Leveraging this extended connected platform with increased data sharing could revolutionize biomanufacturing, providing a route to risk reduction and economic global manufacturing in an increasingly fragmented market. Additionally, from a facility management perspective, addressing the aging infrastructure and outdated equipment of legacy facilities has also long been an issue for biomanufacturers trying to move into the next era of manufacturing. By updating through single use systems and adaptability techniques, smarter processes can be created that offer more flexibility in this new era of manufacturing.

A great facility needs a great team to operate it – soft infrastructure ultimately determines the fate of the capital investments. Skill gaps and shortage in the biomanufacturing industry can be overcome when a shared vision is held between Government, industry, and individuals. Flexible, transferable skill sets and opportunities to earn competency-based qualifications are the answers for the biomanufacturing industry to build the critical workforce of the future.

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DOI: 10.18609/ioi.2021.010

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 declares that they have no conflicts of interest.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *Feb 4 2021;* ***Publication date:*** *Apr 20 2021.*

Introduction to live-cell analysis for cytotoxicity

Live-cell analysis offers a powerful technique for monitoring cell proliferation and death. By acquiring images at regular intervals, cell proliferation can be measured in real-time for days or even weeks, and overall cell health can be evaluated based on morphology and growth. This information is vital for robust, reliable assay development, and allows experimental conditions to be easily optimized prior to cell treatments. The non-perturbing nature of live-cell imaging also allows these assays to be used alongside other techniques to maximize the information gained from precious samples. The Incucyte® Live Cell Analysis System offers a range of tools to visualize and quantify cell proliferation, identify specific subpopulations, and measure cell death.

Immuno-Oncology Insights 2021; 2(2), 83
DOI: 10.18609/ioi.2021.017

UNDERSTANDING CELL BEHAVIOR AND FUNCTION

The Incucyte Live-cell imaging system acquires Phase HD, brightfield and fluorescent images of cells, cells from within an incubator, so the entire process is non-perturbing. Cells can be quantified and imaged in real-time across the entire workflow. During culture, cell health, morphology and plate uniformity can be examined. Kinetic assays can then be performed to monitor the effects of treatment – for example, cytotoxicity. Images can be acquired regularly for as long as is required, with no need to define an endpoint. After every image acquisition, images are analyzed using integrated software.

LABEL-FREE MONITORING

Three main label-free methods are available for measuring cell growth and proliferation **(Figure 1)**. Confluence provides a percentage of the field of view that is covered by cells. Brightfield analysis allows for measurement of the area of 3D objects such as spheroids and organoids. Using cell-by-cell analysis, individual cells can be identified in 2D phase images, allowing cell count to be accurately measured over time.

FLUORESCENCE LABELLING

Fluorescent imaging can provide even more information on cell subpopulations and cytotoxicity **(Figure 2)**. Again, three main approaches to fluorescent labelling of cells are available. The first is to generate a stable cell line that expresses a fluorescent reporter. Secondly, a range of rapid dyes and reagents are available, which have been validated as non-perturbing, making them ideal for use in live-cell assays. These include rapid nuclear dyes, as well as reagents for detection of surface markers using live-cell immunocytochemistry. In particular, rapid dyes and reagents are useful in systems where generating a stable cell line is too time-consuming, or not possible. Lastly, a suite of cell health reagents can be used to measure cell viability and apoptosis. By combining these readouts, complex and translational assays can be developed to provide meaningful insights into cell behavior and function.

VALUABLE INSIGHTS IN REAL TIME

Label-free imaging and quantification using the Incucyte® Live Cell Analysis System enables kinetic, non-perturbing analysis of cells in monolayer culture, along with analysis of more complex cultures, such as spheroids and organoids. Fluorescent labelling adds insight into cell subpopulations including analysis of cell cycle stage, identification of cells in co-culture, and real-time apoptosis measurements. Together, these tools provide valuable real-time insight into kinetic cell behaviors in both simple and complex multi-cellular cultures, with applications across the whole cell analysis workflow.

Figure 1. Label-free proliferation.

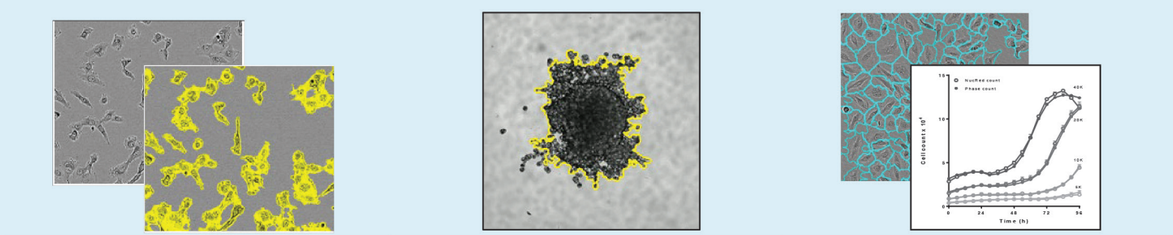
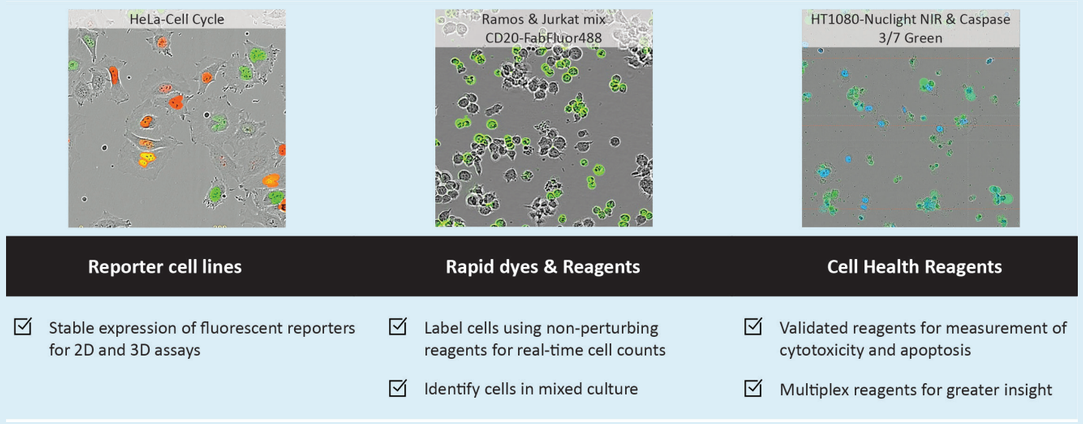


Figure 2. Fluorescence labelling.



IMMUNO-ONCOLOGY INSIGHTS

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