CELLSERIES

Cell Culture & Bioprocessing | Stem Cell & Regenerative Medicine | Cell & Gene Therapy

Pre-Event Newsletter Sept 2019

FEATURING

Automated Mammalian Colony Picking

Molecular Devices' ClonePix™ 2 Mammalian Colony Picker

A Stay at the Stem Cell Hotel

Community of experts unraveling the mysteries of stem cells

Q&A with Ralph Kern

Chief Operating Officer & Chief Medical Officer at Brainstorm Cell Therapeutics

NOVOTEL LONDON WEST

29 - 30 OCTOBER 2019 | LONDON, UK

OXFORD GLOBAL CONFERENCES

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Hayley Watson
Portfolio and Client Engagement Director

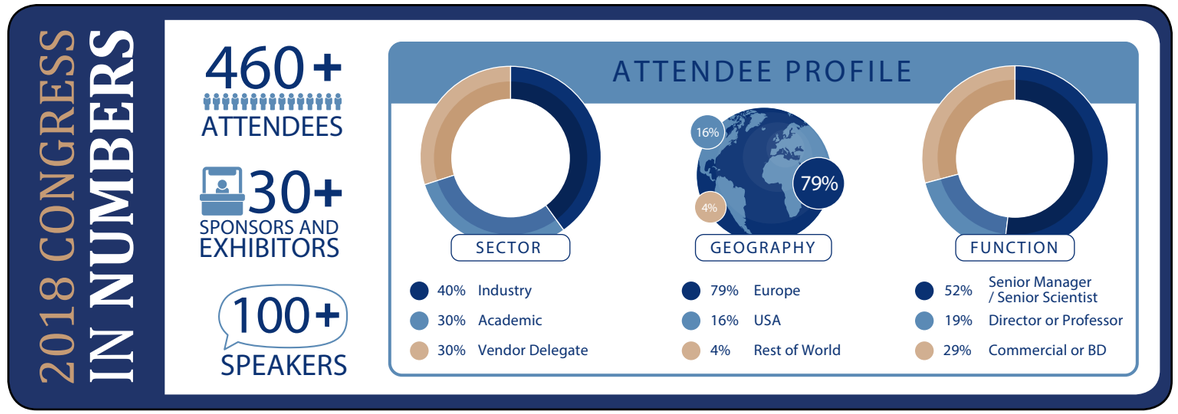
Rimsha Raza
Senior Operations & Events Executive

Jess Thomson
Senior Producer and Team Leader

Guillaume Alonso
Marketing & CRM Manager

Jamie Gordon
Delegate Sales Team Lead

TEAM MEMBERS:
Tim Richters: Sponsorship Portfolio Manager
Ryan Leahy: Conference Producer: Cell & Gene Therapy
Emma Richardson:
Associate Producer: Stem Cell & Regenerative Medicine



WELCOME TO OXFORD GLOBAL'S CELL SERIES PRE-EVENT NEWSLETTER!

With Oxford Global's 2019 Cell Series taking place in October in London, I am delighted to bring you news of key features & exciting additions for this year's congress.

The 2018 congress was our most popular yet, bringing together over 400 attendees in London to discuss collaborative solutions, challenges and the latest developments within the Cell Culture, Bioprocessing, Cell & Gene Therapy and Stem Cell field. The feedback concerning the high-level talks and seniority & diversity of the attendees was overwhelmingly positive.

If 100+ presentations isn't enough to choose from, this year's congress will feature a highly anticipated Regenerative Medicine Stakeholder Session bringing attendees together through a mixture of roundtables followed by a panel discussion, to having engaging and innovative discussions with peers regarding challenges, solutions and key developments within the field. Alongside there will be a number of panel discussions featured on the programmes addressing topics such as Improving Efficiency In CHO Cell Line Generation, Cost-Effectiveness In Cell & Gene Therapy and Gene Therapy Regulatory Pathways For Rare Disease.

2018 saw the launch of the congress dinner which was very well received, and we're pleased to announce its return this year. After a busy first day of learning, sharing and networking, we'd like to invite you to join us for a three-course meal and a glass of wine on us!

Welcoming over 400 attendees, the 2019 co-located programmes will feature 100+ presentations on key topics including;

Cell Culture & Bioprocessing: Shaping the cell culture and bioprocessing field, such as 3D Cell Culture applications, technologies and innovations, cost effective processing methods, high yield cell lines and novel large scale production tools and technologies. New for 2019, we are also pleased to announce a special one-day focus session on Cell Banking, providing delegates with a vital opportunity to dive deeper into the challenges of utilising cell banks, and their vital role in the development of cell-based therapies.

Cell & Gene Therapy: Companies pioneering this field, including Kite Pharma, GlaxoSmithKline, Cellectis, Pfizer and Juno Therapeutics, will present case studies on recent approaches to the commercialisation and manufacturing of cell and gene therapies. New for 2019, we are also pleased to introduce a second dedicated clinical development stream, enabling discussions across both days on the development of cell and gene therapies for oncology, rare diseases and other difficult indications.

Stem Cell & Regenerative Medicine: Addresses key challenges in stem cell bioprocessing, development, and clinical trials, as well as the current advances in cell based therapies for regenerative medicine, iPSC characterisation, technologies in stem cell drug discovery and development.

Read on for a range of interesting interviews and insights with some of our industry-leading speakers and participating sponsors, and I look forward to welcoming you to the 2019 Congress. **- Hayley Watson, Portfolio Director**

CELL SERIES

UK 2019

NOVOTEL LONDON WEST

29 - 30 OCTOBER 2019 | LONDON, UK

8TH ANNUAL
CELL CULTURE & BIOPROCESSING CONGRESS

6TH ANNUAL
STEM CELL & REGENERATIVE MEDICINE CONGRESS

5TH ANNUAL
CELL & GENE THERAPY CONGRESS

WHO IS ATTENDING?

For the full attendee list please contact marketing@oxfordglobal.co.uk

- **400+ senior level delegates** representing global pharmaceutical organisations, leading biotech companies and internationally renowned academic institutions.
- **Directors, VPs, CEOs and Heads** working in bioprocessing, cell line engineering, CAR T-Cell therapy, manufacturing, gene therapy development, stem cell technologies, regenerative medicine and biobanking.

These companies and many more:

UCL, Merck, Biogen, Kite, CATAPULT Cell and Gene Therapy, UCB, Bayer, Boehringer Ingelheim, GlaxoSmithKline, The University of Edinburgh, Sanofi

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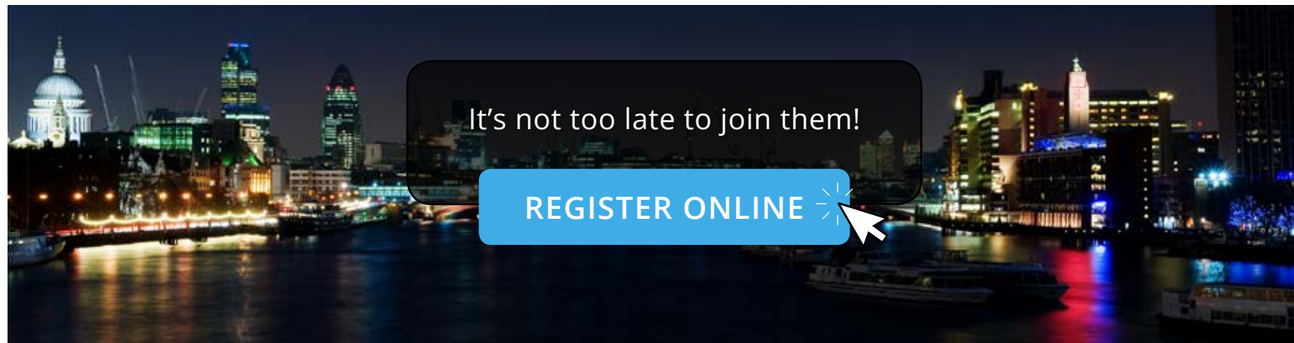

APPLICATION OF FISH TO VALIDATE GENOME ENGINEERING TECHNOLOGIES

REMBEN TALABAN

Remben Talaban,
Investigator, GSK Associate Fellow

Prior to joining GSK in 2014 as a member Biopharm Process Research (BPR) Disruptive Technologies Team, Remben worked at Great Ormond Street Hospital NE Thames Regional Genetics Service looking into patients with various genetic disorders such as Duchenne Muscular Dystrophy, cystic fibrosis, cases of aneuploidy, fertility problems and children with development delay. Remben joined GSK in 2014 bringing with him extensive experience in molecular cytogenetics from his previous employment at St. Luke's Medical Centre's Cytogenetics Department, Great Ormond Street Hospital and also at The Institute of Cancer Research. His efforts in establishing a cytogenetics laboratory and subsequent embedding of conventional and advanced fluorescence in situ hybridisation (FISH) techniques have afforded us an unparalleled and in-depth understanding of our cell lines aiding the rational design of our next generation CHO platforms. Building on this, he is currently exploring new novel technologies using other microscopy methods and develop innovative new ways of working ensuring BPR is fully equipped in the ever-changing bioprocessing landscape.

Chinese hamster ovary (CHO) cells are the preferred host cell line for the commercial production of recombinant therapeutic proteins due to its high adaptability to various culture conditions, ease of genetic manipulation, ability to produce vast amount of recombinant products and proven safety record. The most common strategy in cell line development for expression of biopharm assets relies on random integration of the gene of interest (GOI) in particular mAb-expressing vector into the CHO host genome followed by selection and identification of top performing lines from a myriad of less suitable phenotypes. However, this approach of clone generation suitable for commercial manufacture is time-consuming, laborious and oftentimes, results in high degree of phenotypic heterogeneity and unpredictable expression due to the variability of the genomic site of integration. In order to mitigate the effect of uncontrolled transgene insertion specifically position effect-induced instability, we are evaluating two targeted integration approaches to our CHO CLD processes that will deliver consistent and stable transgene expression. In order to identify the targeting profile of the two approaches and assess the extent of off-target integration, we employ FISH (fluorescence *in situ* hybridisation) on a number of genome-engineered cell lines using specific in-house designed probe sets.

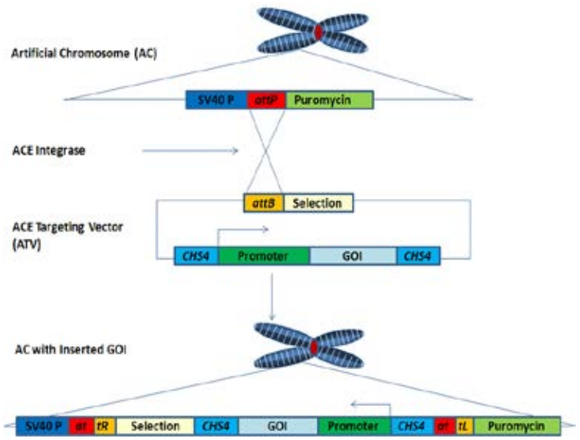

Figure 1: Components of the Chromos Artificial Chromosome Expression (ACE) system. The Platform ACE containing multiple attP recombination acceptor sites; ACE Integrase, a modified DNA integrase derived from lambda integrase which can catalyse the site-specific integration of the genes of interest onto the Platform ACE; and an ATV, which serves to shuttle genes of interest onto the Platform ACE in a site-specific manner (Lindenbaum et al., 2004).

The first approach in our targeted platform is through the use of our proprietary Chromos Artificial Chromosome Expression (ACE) system. The ACE system allows targeted insertion of the gene of interest into the cell in an autonomously replicating, non-genome integrating format. This system is comprised of four main components: the Platform ACE, the Platform ACE Host Cell Line, the ACE Targeting Vector (ATV) and the ACE Integrase plasmid (Figure 1). The platform ACE is a mammalian artificial chromosome that contains multiple integration acceptor sites based on the lambda phage integration system, which allows for the site-specific insertion of multiple copies of DNA sequences (Kennard et al., 2009).

The second approach in our CLD toolbox employs genome engineering through site-directed integration by clustered regularly interspaced short palindromic repeats/ CRISPR-associated RNA guided nucleases (CRISPR/Cas9) technology. This technology allows specific integration into a precise genomic locus by creating DNA double-strand breaks (DSBs) induced by engineered RNA nucleases (Lee et al., 2015). The induced break can then be repaired by one of two major DNA damage repair pathways: non-homologous end-joining (NHEJ) or homology-directed repair (HDR).

Rapid-FISH to assess On vs. Off-target Artificial Chromosome integration in Chromos cell line generation

The development of the Rapid-FISH screen is an integral step in Chromos CLD protocol in order to rapidly screen cell lines and determine those with high level on-target artificial chromosome integration for subsequent progression, ensuring Chromos technology is dictating cell line performance. With the use of a fully automated cytogenetic workstation coupled with expertise in FISH analysis, this step decreases analysis time by 50% and increases accuracy in clone selection.

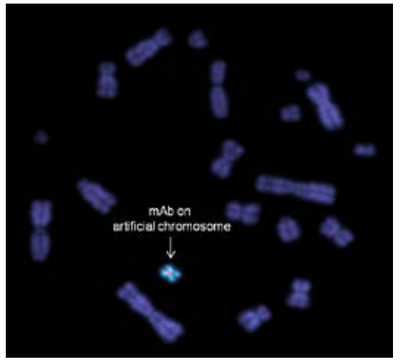

Figure 2: Transgene (mAb) integrated into the AC. This signal pattern was observed in 31% of the cells analysed on Bulk1-1B cell line:cell line progressed

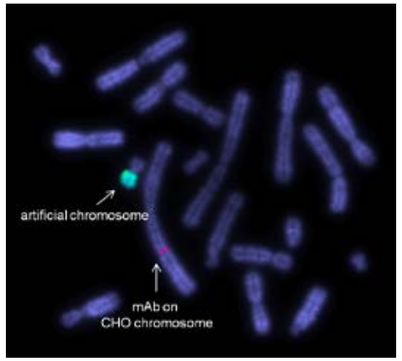

Figure 3: FISH analysis of PS8 p6 cell line showing off-target integration of the transgene (mAb) into CHO host chromosome: cell line terminated.

FISH analysis to validate Genome Engineering technologies

CHO-K1 mAb-expressing cell line was subjected to tandem locus amplification (TLA) analysis to determine transgene genomic integration site (termed FD5-8 locus). This locus was subsequently targeted in CHO-K1 using CRISPR/ Cas9 technology to deliver a GFP reporter gene. FISH analysis showed localisation of the GFP to the expected locus and identified successful mono- and bi-allelic genome targeting.

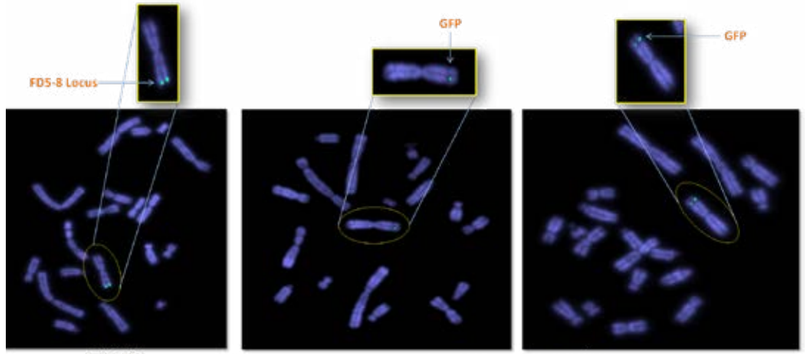

Figure 4: FD5-8 locus (A) of CHO-K1 genome was targeted using CRISPR/ Cas9 technology to deliver GFP. FISH analysis revealed monoallelic (B) and biallelic (C) integration of GFP at the pre-defined locus.

The establishment of molecular cytogenetics facility within GSK allows us full autonomy and control over our mammalian cell line characterisation efforts. This facility supports diverse workstreams within our team, including validation of the targeting profile those based upon targeting technologies such as CRISPR/Cas9-mediated genome engineering and GSK's proprietary Chromos Artificial Chromosome Expression (ACE) platform. The use of novel FISH techniques is an integral step in cell line development process for both the targeted integration approaches that will allow accurate selection of clones for subsequent progression. Molecular cytogenetics has indeed enhanced our capability in cell line selection and has provided us insights underpinning biopharm cell line performance through rational design of our next generation targeted platform.

I hope to bring you this and more in the upcoming Cell Series 2019. See you there!

Remben Tablan will be presenting on Day Two of the 8th Annual Cell Culture & Bioprocessing Congress with his talk *'Leveraging Molecular Cytogenetics in Mammalian Cell Line Development'*

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STEM CELL THERAPIES IN REGENERATIVE MEDICINE **VASILIKI KALODIMOU**

Stem Cell research has become an integral component of regenerative medicine as stem cells can repair or replace old cells and can heal the recipient. Major influences in the growth of stem cell research include the development of cellular therapy for neurodegenerative disorders such as Parkinson's and Alzheimer's, while further treatment for spinal cord injury, type 1 diabetes, cardio and oncology therapies have played a crucial role. The rise of therapeutic success is assisted by innovations in the manufacturing of stem cells, with bioprocessing methods and techniques in tissue engineering playing a pivotal role in stem cell development. Experts are vigorously working on cutting-edge therapeutic innovations and clinical procedures to identify new targets for stem cell development.

What project are you currently working on?

My work is focusing on adipose mesenchymal stem cell research and clinical trials for patient care.

The aim of our latest study was to assess the histological and morphological effects of autologous infusion of adipose-derived stem cells (ADSC) on an established and chronic vocal fold scar in a rabbit model comparing it with an untreated scar and the traditional treatment of hyaluronic acid injection.

Recently, several studies have been performed on animal models regarding the effect of cell therapy on scarred vocal folds. Human embryonic stem cells, human mesenchymal stem cells from bone marrow or autologous adipose stem cells in scarred vocal folds, with very promising results. The experimental model in the vast majority of studies is based on creating a trauma on the vocal fold and within 7 days maximum, there is implantation of stem cells. Our protocol is based on creating a trauma on the vocal folds of an experimental model (rabbit) and infusing adipose-derived stem cells (ADSC) on the scar after 18 months.

We conclude that autologous injection of adipose-derived stem cells on a vocal fold chronic scar of a rabbit enhance the healing of the vocal fold and the reduction of the scar tissue, even comparing with other treatments. (https://www.ncbi.nlm.nih.gov/pubmed/26933440)

Vasiliki Kalodimou, Lab Director at the Flow Cytometry-Research and Regenerative Medicine Department of IASO Maternity-Pediatric and Research Hospital

Vasiliki Kalodimou is the Lab Director at the Flow Cytometry-Research and Regenerative Medicine Department of IASO Maternity-Pediatric and Research Hospital in Athens. Before this, Kalodimou served as a haematology research associate at General University Hospital of Heraklion, and as an instructor at both the General University Hospital of Heraklion and the Ipokratios Medical-Technical Institute of Athens. Kalodimou received her bachelor's degree in Human Physiology and her master's degree in Human Molecular Genetics from the Imperial College University of Medicine. She completed her PhD at the University of Medicine in Athens.

Since 2006, her research interests include: stem cells in everyday practice and their applications in regenerative medicine and Flow Cytometry, human genetics & population genetics as well as cellular standards. In collaboration with the Hellenic Flow Cytometry Society, Kalodimou has developed quality control schemes for stem cell marker enumeration. In addition to collaboration with state universities and pharmaceutical companies on research projects, Kalodimou frequently publishes her findings and has 2 patents. She is in the editorial board and a reviewer in several international journals as well as board member in scientific organizing committees for medical conferences.

What do you expect to be the biggest news to come out of stem cell research in 2020?

I believe that in the years to come stem cell research will reach its peak in several areas such as:

- Stem cell blood products will be widely available.
- Stem cell scientists will advocate for evidence-based medicine more routinely for patient safety, and for the field.
- Several major academic hospitals will offer formal physician training in cellular and stem cell-based regenerative medicine.
- Diabetes will be partially controllable in some patients by stem cell-produced mini pancreases in clinical trials.
- A stem cell-based ALS treatment will show signs of efficacy in clinical trials and raising hope of significantly extending lifespan and quality of life for patients.
- Clinical trials will suggest that arthritis can be partially treatable with stem cells that will have been proven to effectively and safely regenerate cartilage.
- Replacement of entire organs will seem more realistic in the years following 2020.

Major influences in the growth of stem cell research include the development of cellular therapy for neurodegenerative disorders such as Parkinson's and Alzheimer's, while further treatment for spinal cord injury, type 1 diabetes, cardio and oncology therapies have played a crucial role.

You are giving a talk on cellular standards. What are the major issues you have encountered?

Many labs are facing problems on how to apply and follow the standards in a daily routine, as well as quality controls and enumeration schemes for cellular therapies. This results in bad quality service for the product and for the potential patient care.

We need to keep in mind that standards are designed to provide minimum guidelines for organizations, facilities, and individuals performing cellular therapy product collection, processing, or administration or providing support services for such procedures. They represent the basic fundamentals of cellular therapy that can be applied to any cell source or therapeutic application and are intended to be used throughout product development and clinical trials. Cellular therapy is an emerging and evolving field and standards are required for the establishment of a quality management program in order to promote quality medical and laboratory practice in a broad range of cellular therapies. We need standards and guidelines in order to enhance and improve patient care and the healthcare system sufficiently in the field of cellular therapies.

Who are you most excited to see in the upcoming 6th Annual Stem Cell and Regenerative medicine Congress?

As a speaker of last year's 5th Annual Stem Cell and Regenerative Medicine Congress in London, I am looking forward to interacting with fellow researchers, exchanging ideas, discovering industry innovations and interacting with leading biotech companies, global pharma organizations in the field of stem cells and regenerative medicine.

I'm also looking forward to panel discussions focusing on the new discoveries of stem cells in regenerative medicine, manufacturing innovations in clinical trials and bioprocessing methods in cell line development.

Through this year's conference I hope to see the latest developments in the field of cell and gene therapies that offer innovative therapies for treating patients, particularly where other traditional therapeutics have been ineffective.

Dr Vasiliki Kalodimou will be presenting on Day Two of the 6th Annual Stem Cell & Regenerative Medicine Congress with her talk *'Cellular Standard & Accreditation For The Cellular And Regenerative Medicine Labs'*

STEM CELL SECRETOME AS AN ALTERNATIVE TO THERAPEUTIC TRANSPLANTATION OF ADULT STEM CELL

DARIUS WIDERA

Dr Darius Widera,
Associate Professor in Stem Cell Biology and Regenerative Medicine, University of Reading

Dr Widera is leading an interdisciplinary research group at the University of Reading (UK). His lab is mainly interested in adult stem cells and their secretome, inflammatory signaling cascades in health and disease, and novel methods for clinical grade 3D-cultivation of human stem cells.

Despite promising pre-clinical data and multiple clinical trials, there is very little evidence for the ability of adult stem cells including mesenchymal stromal cells (MSCs) and neural crest-derived stem cells (NCSCs) to engraft, survive, and differentiate *in vivo*. This apparent discrepancy suggests that mechanisms other than engraftment and differentiation towards lost cell types might be responsible for the therapeutic effects of adult stem cell transplantation. Indeed, multiple recent studies indicate that transplanted adult stem cells survey the microenvironment after transplantation and modulate the regeneration of the host via paracrine bystander effects with soluble factors and extracellular vesicles (EVs) as vectors.

More specifically, if transplanted into a side of injury or degeneration, MSCs and NCSCs sense the injury signals, release trophic and immunomodulatory factors and may serve as site-regulated 'drugstores' *in vivo*. This behaviour can be explored industrially, where cultivated adult stem cells can be used as 'biofactories' to produce paracrine factors that can be used as a potential substitute for cell transplantation.

In a series of two consecutive case studies, we have explored the potential of stem cell-derived secretome to modulate muscle regeneration in animal models. Surprisingly, despite the very distinct profiles of the soluble and EV-embedded secretome components, secretome harvested from different stem cell populations can mediate very similar, regeneration modulating effects. Moreover, we able to show that the soluble and EV-related components of the cargo act in synergy but affect different aspects and stages of the regeneration process.

In summary, tissue regeneration mediated by MSCs and NCSCs does not seem to depend on their differentiation capacity but rather on the release of soluble factors and extracellular vesicles, which together facilitate the endogenous regeneration process. This represents an exciting clinical and commercial avenue. The use of the secretome to substitute stem cell transplantation has a significantly lower risk profile and is more cost-efficient. However, adult stem cells-secretome is a biological with a poorly defined composition making regulatory affairs and its commercial exploitation challenging. Thus, there is a high need for appropriate quality assurance procedures and standardisation of the production process.

Dr Darius Widera will be presenting on Day One of the 6th Annual Stem Cell & Regenerative Medicine Congress with his talk *'Cellular Standard & Accreditation For The Cellular And Regenerative Medicine Labs'*

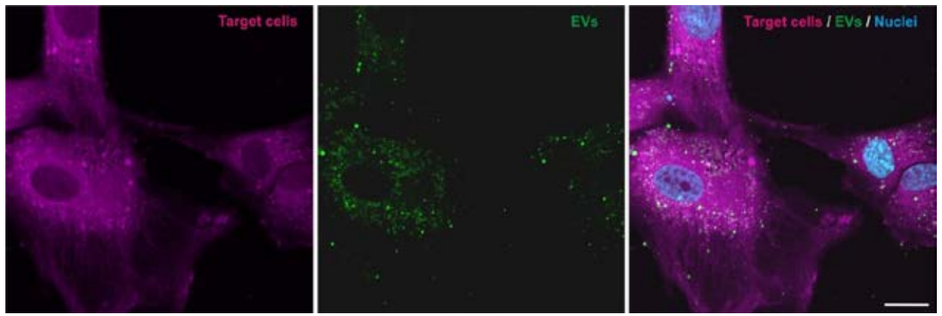


Figure 1. Target cells (magenta) taking up stem cell-derived extracellular vesicles (EVs, green). Modified from Mitchell et al.,Stem Cell Res Ther. 2019 Apr 5;10(1):116.

A STAY AT THE STEM CELL HOTEL
DAVIDE DANOVI

Davide Danovi,
Director, HipSci Cell Phenotyping Programme, Centre for Stem Cells and Regenerative Medicine, King's College London

Dr. Davide Danovi is leading the Cell Phenotyping Unit at King's College London in the framework of the HipSci project, funded by the Wellcome Trust and MRC. Prior to this, he was principal scientist at Progenitor Labs, a novel biotechnology company founded to isolate drugs for regenerative medicine using innovative stem cell technologies. Before, he worked with Prof. Austin Smith and Dr. Steve Pollard at the University of Cambridge and at University College London where he developed a live image based chemical screening platform to isolate compounds active on human neural stem cells from normal or brain tumour samples. He holds an MD from University of Milan and a PhD in Molecular Oncology from the European Institute of Oncology where he demonstrated the causative role of the HdmX protein in human cancer.

All cells in our body came from one original cell – a fertilised egg. A blood cell and a nerve may look very different, yet they actually share the same DNA information. Special characteristics are obtained by 'highlighting' parts of their genomes to be made more active, and others idle. Nonetheless, some of our cells can make exact copies of themselves (self-renewal) or become specialised, performing a specific function (differentiation). These are stem cells, the cells we study at the Centre for Stem Cells and Regenerative Medicine at King's College London. Self-renewal and differentiation are thus the two defining features of stem cells. Stem cells can do both – we joke that it's a bit like managing a family and a career.

Understanding our cells

In order to understand cells' behaviour, scientists have been taking pictures of them under microscopes for a long time. In recent times, computers have joined forces with microscopes, helping us to make sense of imaging data. This means we can now try to unravel the mysteries of the shapes of our cells. How a cell looks, moves and divides – how is this shaped by signals it received from its culture conditions? How much of it is down to the genetic background of the volunteer who donated it? Now imagine a dedicated team of experts in stem cell biology, image analysis and artificial microenvironments, working with other scientists to try and answer these questions.

Well, you've just pictured the 'Stem Cell Hotel', a project supported through our NIHR Guy's and St Thomas' Biomedical Research Centre.

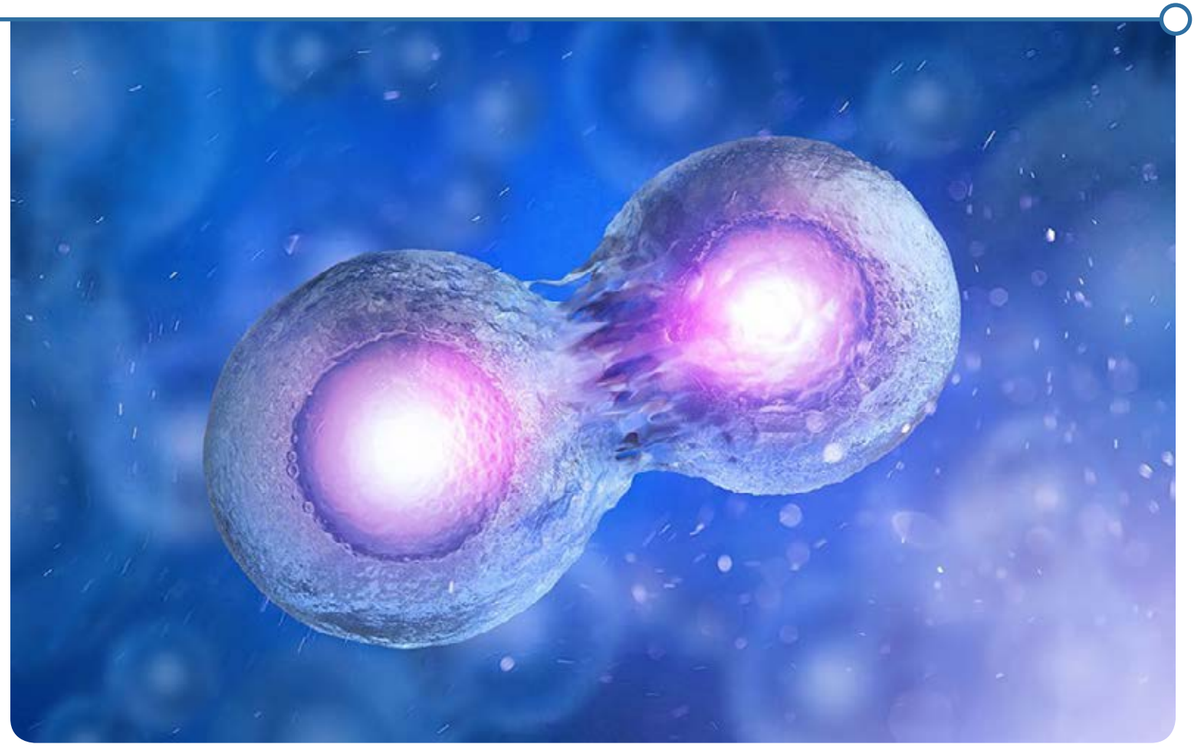
In our laboratories high up in Guy's Tower, next to the Shard, we help visiting scientists find out more about their experiments, hosting both the users and the cells they carry. We make sure the cells are welcome, fed and imaged, as important guests. The scientists who brought them to us can access our devices, deploy tailored characterisation methods, develop assays and perform experiments. We can help them phenotype the cells – and predict their characteristics from their genetics. The assistance we provide ranges from a single room for one – simple acquisition of microscopy images, to the presidential suite – data analysis and integration to scientific collaborations such as co-development of software with technology providers. Like any hotel, our cost models vary accordingly.

This innovative, collaborative phenotyping space combines elements of a research facility, and of a research and development incubator. While serving internal and external scientists, it is able to accommodate contract-research activities where intellectual property (IP) remains with the user, unless when significant input from our scientists is required.

The Stem Cell Hotel has been an enabling feature of the Centre for Stem Cells and Regenerative Medicine that has grown together with it as their history intertwined. Professor Fiona Watt, Director of the Centre for Stem Cells and Regenerative Medicine commented: "Under Davide Danovi's leadership, the Stem Cell Hotel has grown from concept to reality – it is exciting to see how many collaborations are already underway, or in the planning phase."

The Centre was inaugurated in December 2015 and the Stem Cell Hotel has moved on a long way since, and is now fully operational. Created within the framework of the Human Induced Pluripotent Stem Cell Initiative (HIPSCI), it leverages expertise from several leading edge research projects which are ongoing at the Centre, on different cell types.

From a personal perspective, I am privileged to be leading a team of scientists with expertise in high content and high throughput analysis and data integration. One of the most exciting things about this work is the collaboration and the impact it has on a diverse multidisciplinary community of scientists. We have genuinely become a meeting point for university and biotechnology companies. We host scientists



from a range of different disciplines and backgrounds including clinical, translational and industrial who all have their own take on stem cells. It's illuminating to hear different perspectives, and this allows us, like the cells we study, to be flexible in what we do.

Turning back the clock

Imagine bringing one specialised cell back into an embryonic-like stage so that it can again become any type of cell. This Nobel-prize winning technology is referred to as reprogramming cells into 'induced pluripotent stem cells'.

One recent example of the power of this approach is our study detailing the characterisation of a panel of 110 human induced pluripotent stem cell lines from 75 individual volunteers. We looked for cells behaving in an unusual way and were able to associate these unusual behaviours with single changes in a nucleotide, a letter of their DNA code. Data, including raw images from this study have been published to open access repositories, granting the wider scientific community access and the ability to replicate the analysis. Scientists can now access specific cell lines with particular genomic characteristic or even a particular shape. This open science project is at the cross-road between databases with information about biological models (genes, proteins, etc) and biobanks of human samples. The impact comes here from the ability to define criteria to 'benchmark' or quality control cells based on these analysis methods.

We have also recently published another study as a collaboration between our team of scientists at the Stem Cell Hotel and experts on endothelial cells. Endothelial cells are the cells that line the interior of our blood vessels and the vessels in our lymphatic system. Characterising these cells, and understanding how they differ when derived from different sources could help us find new ways to obtain the right vessels for tissue engineering for specific organs.

In parallel, we compared two cell types: endothelial cells forming colonies derived from induced pluripotent stem cells (iPSC-ECFC) alongside primary human umbilical vein endothelial cells (HUVEC). We have derived information on the cells' shape and form, and how they interact with each other in order to capture differences between cell populations. This approach exemplifies an important yet simple strategy to benchmark endothelial cells in different conditions, and to assist in development of new tools for biological research and regenerative medicine applications.

Born from boldly translational research embracing the spirit of open innovation, the Stem Cell Hotel grants access to state-of-the-art technology for stem cell biology, high content analysis and artificial microenvironments. Its highly collaborative environment serves communities centred around academic, clinical, and commercial research.

Dr Davide Danovi will be presenting on Day One of the 6th Annual Stem Cell & Regenerative Medicine Congress with his talk *'Collaborative Phenotyping: HipSci And The Stem Cell Hotel'*

Q&A WITH BRAINSTORM CELL THERAPEUTICS
RALPH KERN

Ralph Kern, Managing Director, MHSc

Dr. Kern brings significant industry and neurodegenerative disease experience to BrainStorm. His biotech experience includes senior medical roles at Genzyme, Novartis, and Biogen. At Novartis he was Vice President and Head of the Neuroscience Medical Team, leading the global launch of Gilenya® in relapsing remitting multiple sclerosis (rrMS). At Biogen he was Senior Vice President and Head of the Worldwide Medical Organization. His team launched Zinbryta® in rrMS and Spinraza® in spinal muscular atrophy (SMA), and developed the medical and scientific strategy for MS, SMA, and Alzheimer's disease.

Dr. Kern completed his neurology training at McGill University, practiced neuromuscular neurology at Mount Sinai Hospital in Toronto, and was head of the Postgraduate Academic Neurology Program at the University of Toronto. He completed further postgraduate training in Health Administration at the Institute for Health Policy Management and Evaluation at the University of Toronto. Dr. Kern received his MD degree from Queen's University, is board-certified in neurology and neuromuscular disease, and is a member of the Royal College of Physicians and Surgeons of Canada.

What Is Brainstorm Cell Therapeutics and what is the Company's Mission?

We are a small group of 33 dedicated employees in New York and Israel who have become a leading developer of adult stem cell therapies for neurodegenerative diseases. Our investigational candidate NurOwn® is currently in Phase 3 clinical trials for Amyotrophic Lateral Sclerosis (ALS) and Phase 2 for Progressive Multiple Sclerosis (MS).

What is NurOwn?

NurOwn® is Brainstorm's investigational therapy of Autologous MSC-NTF cells that are produced from the patient's own bone marrow-derived MSCs that have been differentiated in culture. The resulting MSC-NTF cells can effectively deliver multiple Neurotrophic Factors (NTFs) and immunomodulatory cytokines directly to the site of damage to elicit a desired biological effect and ultimately slow or stabilize disease progression. In the NurOwn manufacturing process, the patient's own MSCs are harvested and differentiated to secrete higher levels of NTFs using a proprietary technology and are not genetically modified. The differentiated MSCs, known as MSC-NTF cells, are then harvested and prepared for injection into the patient, directly into the cerebrospinal fluid by standard lumbar puncture.

Why is BrainStorm focused on ALS?

Currently there is an enormous unmet need in ALS. Approximately 30,000 individuals in the U.S. alone, have been diagnosed with ALS, with an additional 6,000 new patients diagnosed annually. At the same time, there are only two approved treatments, both of which have modest effects on the natural history of the disease.

What is the current status of your ALS Clinical Trials?

We are conducting a Phase 3 pivotal trial investigating repeat-administration of NurOwn® in ALS at six clinical sites of excellence in the U.S. Our clinical sites include include Massachusetts General Hospital, The Mayo Clinic, and The University of Massachusetts Medical School among others. The study will enroll 200 ALS patients, randomized 1:1 to receive NurOwn® or placebo, and will evaluate the ALS functional rating scale (ALSFRS-R) responder analysis as a primary efficacy outcome measure. The trial is nearing the end of patient recruitment. To date, we have enrolled 180 patients (90 enrollment). Based on our current projections, we plan to enroll a total of 200 patients by the end of September or, depending on the screen-failure rate, by the second week of October. Topline study results are expected approximately 12 months after the last patient is enrolled.

How are the protocol and operational design of BrainStorm's Phase 3 study uniquely adapted for ALS?

From a protocol standpoint, our trial includes a three-month run-in period, where we harvest the patient's bone marrow cells and manufacture their first treatment from those cells. During that time, we also evaluate the patient's rate of decline for our inclusion criteria. We know that the rate of decline in ALS can vary by patient, and we learned from our Phase 2 trial that individuals who progress more rapidly may experience a better result from this treatment. These rapid progressors, who compose about half of the total ALS patient population, typically are known to have higher levels of inflammation compared to other patients. This is important, as NurOwn® treatment is expected to reduce neuroinflammation in addition to promoting repair and support for dying and diseased neurons through secreted NTFs.

From an operations standpoint, we've simplified the investigator workload dramatically compared to what is common in many stem cell studies. In our study, once the bone marrow is harvested, it is sent to the manufacturing site in a pre-labeled shipping container. All the steps necessary to manufacture the treatment occur at our manufacturing site before being sent back in a pre-labeled syringe. Typically, stem cell studies can be highly complex and require extensive, hours-long, on-site preparation in the cell and biology labs before going through the micro lab. In the case of NurOwn®, this is greatly simplified.

Can you explain the process in more detail?

Once a patient's bone marrow is harvested, it is shipped to the manufacturing site in a shipping container, where all the steps needed to manufacture the finished product occur. The treatment is then packaged into a pre-labeled syringe containing 4MLs of fluid hosting 125 million differentiated mesenchymal stem cells that secrete neurotropic factors. That syringe is shipped directly to the study site, where the investigator is required to verify that the temperature has been maintained during transportation, using a digital thermometer connected to the shipping container. Once verified, the investigator then receives approval from the manufacturing site via a coded email, after which treatment is administered to the patient by a simple lumbar puncture.

To decrease the impact to patients, we have introduced cryopreservation into our technology platform, which allows us to make repeated MSC-NTF cell dosing from a single bone marrow aspirate. The patient experience is limited to a 30-minute bone marrow aspirate and repeated 30-minute treatments once every two months when the cells are re-introduced back as the therapeutic agent.

Over the past 10 years that our technology has been in development, we've built a strong track record in terms of quality control and product manufacturing. Each step of our process is fully validated, from manufacturing, to cryopreservation, to the shipping parameters we have put in place so that the cells are validated for use up to 72 hours after release from the manufacturing site. We are confident we have laid the groundwork for highly successful manufacturing logistics and future commercialization.

What outcomes do you anticipate from this phase 3 trial?

The best outcome we can hope for is drug approval so that ALS patients have an additional option to treat the enormous unmet need. Our primary [endpoint] is to show that a high proportion of treated patients achieve meaningful change in the rate of decline in the ALS Functional Rating Scale. The study design is quite rigorous with a 90% power to demonstrate efficacy in our primary outcome measures. Once enrollment is completed in Q4 2019, completion will take place approximately one year.

You are also conducting clinical trials for progressive MS?

Yes, this past year, we initiated a Phase 2 open-label, multicenter study of repeated intrathecal administration of autologous MSC-NTF cells in participants with progressive Multiple Sclerosis (MS). The Phase 2 study will enroll progressive MS patients [Expanded Disability Status Scale (EDSS) 3.0-6.5] based on 2017 revised McDonald Criteria. MS is a chronic neuroinflammatory and neurodegenerative disorder that affects the brain and spinal cord. MS affects approximately 1 million individuals in the U.S. and 2.5 million individuals worldwide. Approximately half of affected individuals will eventually develop a progressive disease, which may lead to increasing levels of motor, visual, and cognitive functional impairment and disability.

Currently, we are enrolling patients at the Cleveland Clinic, the Stanford School of Medicine and the Keck School of Medicine of the University of Southern California (USC). This phase 2 study will evaluate validated MS efficacy outcome measures and sophisticated CSF and serum biomarkers. We hope that the study will be fully enrolled by the first quarter of 2020 and we should see top line data by the middle of 2020.

How might the BrainStorm platform be applied to other unrelated indications?

Our approach is based upon our hypothesis that in conditions such as ALS, Alzheimer's, Parkinson's and Progressive MS, neurons and other brain tissue go through a period of injury prior to cell death that may be salvageable by modulating the inflammatory environment and by providing cellular support via neurotropic and other repair factors. We have very strong pre-clinical data in Parkinson's, Huntington's Disease, Autism and other brain diseases. We're optimistic that this technology platform can play widely across many brain diseases.

Ralph Kern's colleague, Chaim Lebovits, will be presenting on Day One of the 6th Annual Stem Cell & Regenerative Medicine Congress with his talk *'Stem Cell Therapeutic Approaches For ALS'*

THERE'S A NEW CAR IN TOWN: CAR-EXPRESSING NATURAL KILLER CELLS

DEBBIE KING

Debbie King, Scientific Technical Writer, The Cell Culture Dish, Inc.

Debbie is a scientific technical writer with The Cell Culture Dish, Inc. specializing in editorial content in the cell culture and gene therapy space.

Debbie previously worked at STEMCELL Technologies, Inc. where she gained experience in Quality Control for primary and stem cell culture media before becoming a Manufacturing Sciences Scientist. She was an integral part of the process development team to commercialize the human embryonic/induced pluripotent stem cell culture media – mTeSR1. She also has experience as a scientific educator in North America and abroad to lecture and provide hands-on training to researchers on how to use various specialized STEMCELL media products. Her practical laboratory experience has been an asset to her as a technical writer.

The cost and safety of CAR-T therapies has prompted researchers to look at other immune cells to engineer with CARs against cancer modalities. Natural killer (NK) cells, with their ability to target cells in an HLA-independent manner and innate tumorigenicity makes them attractive candidates for immunotherapies against lymphoma, leukemia and even solid tumor malignancies. Not only can NK cells be targeted to cancer cells with engineered CARs, they may provide additional cancer-killing advantages over CAR-T cells through their native receptors and tumor surveillance capabilities, which may make them more effective against certain malignancies where CAR-T cells have been less successful, like solid tumors. And, their safety in an allogeneic, "off-the-shelf" format could overcome some of the challenges facing autologous CAR-T cell therapies. While this research is still in its infancy compared to CAR-T cells and optimizing protocols for expansion, genetic manipulation will be paramount to success, CAR-NK cells could represent a new tool in the arsenal in the fight against cancer.

The ability of the body's immune cells to target and eliminate infectious organisms and foreign invaders has been studied for decades. It is no surprise that scientists want to harness the power of these cells in immunotherapy to target unwanted cells in the body. CAR (chimeric antigen receptor)-expressing T cell adoptive cell therapies have generated a lot of excitement and investment in recent years due to the unprecedented clinical results. However, its autologous (patient-specific) nature, complex manufacturing workflows and risk of graft versus host disease (GVHD) has raised concerns over the cost and safety.

Because of this, researchers are looking at other immune cells - the natural killer (NK) cells – to modify with the same cancer-homing CAR receptor. NK cells are attractive because they have potent anti-tumor activity, and their safety in an allogeneic, "off-the-shelf" format could overcome some of the challenges facing autologous CAR-T cell therapies.

What are NK Cells?

Discovered in the early 1970s[1], NK cells are granular lymphocytes and potent cytolytic effectors of the innate immune system. They are the body's first line of defense against infections and tumors, able to mount a rapid immune response against cancer and virus-infected cells without prior sensitization. NK cells induce target cell cytolysis and recruit of other immune cells through the release of cytokines[2]. Their ability to target cells missing the "self" MHC (major histocompatibility complex)-1 markers is unique and allows them to capture harmful cells that lack or downregulate their MHC1 markers, which go undetected by other immune cells (like T cells)[2,3]. The NK cell's specificity for target cells is instead determined by the balance between activating and inhibitory surface receptors.

According to the 'missing self' hypothesis[4], NK cells recognize and eliminate cells that fail to express self-MHC-I molecules, express foreign MHC-1 or "stress ligands" (Figure 1). Cytokines important to NK activation include interleukin-12 (IL-12), IL-15, IL-18, IL-2, and CCL5 (Chemokine (C-C motif) ligand 5)[5].

Clinical Trial #	Target Antigen	Disease	NK Cell Source	Phase	Sponsor
NCT02742727	CD7	Lymphoma Leukemia	NK-92 cell line	I/II	PersonGen BioTherapeutics (Suzhou) Co., Ltd.
NCT02839954	MUC1	Solid tumors	NK-92 cell line	I/II	PersonGen BioTherapeutics (Suzhou) Co., Ltd.
NCT02892695	CD19	Lymphoma Leukemia	NK-92 cell line	I/II	PersonGen BioTherapeutics (Suzhou) Co., Ltd.
NCT02944162	CD33	Acute myeloid leukemia	NK-92 cell line	I/II	PersonGen BioTherapeutics (Suzhou) Co.
NCT03056339	CD19	Lymphoma Leukemia	UCB	I/II	MD Anderson Cancer Center
NCT03383978	HER2	Glioblastoma	NK-92 cell line	I	Johann Wolfgang Goethe University Hospital
NCT03415100	NKH2D ligands	Solid tumors	Autologous or allogeneic NK cells	I	The Third Affiliated Hospital of Guangzhou Medical University
NCT03579927	CD19	Lymphoma Leukemia	UCB	I/II	MD Anderson Cancer Center
NCT03656705	-	Non-small cell lung cancer	Modified NK-92 cell line	I	XinXiang Medical University
NCT03841110	-	Solid tumors	iPSC-derived CAR-NK cells (FT500)	I	Fate Therapeutics

Table 1: List of active clinical trials involving CAR-NK cells worldwide.

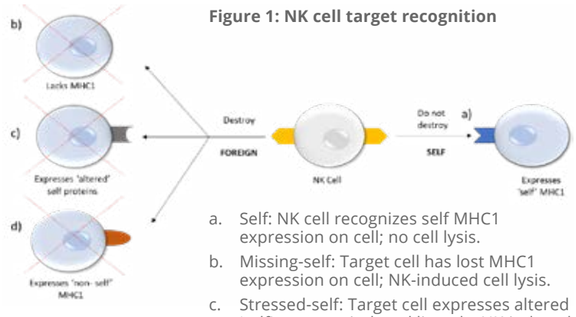


Figure 1: NK cell target recognition

a. Self: NK cell recognizes self MHC1 expression on cell; no cell lysis.
b. Missing-self: Target cell has lost MHC1 expression on cell; NK-induced cell lysis.
c. Stressed-self: Target cell expresses altered 'self' or stress-induced ligands; NK-induced cell lysis.
d. Non-self: Target cell expresses foreign MHC1; NK-induced to cell lysis.

Sources of NK Cells for CAR-NK

Although autologous NK cells can be utilized for adoptive therapy, their *in vivo* efficacy has been limited, even with CAR engineering[6]. Allogeneic NK cells may be more effective because they are not restricted by the patient's HLA expression. Sources include peripheral blood (PB), umbilical CB, bone marrow (BM), human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), or readily available NK cell lines[6]. Each has its own advantages and disadvantages in terms of scalability, transducibility and safety for CAR engineering.

NK cells can be transduced to express chimeric antigen receptors (CARs) for cancer retargeting in much the same way as in T cells. The CAR constructs consist of an extracellular antigen-recognition ScFv connected via a flexible linker to a transmembrane domain and an intracellular signaling/activation CD3ζ domain which activates NK cells.

Clinical Trials

CAR-NK cells have two advantages over CAR-T cells: safety and accessibility. CAR-T cells must be generated from the patient's own T-cells to avoid GVHD. Because of this, manufacturing autologous T cell therapies requires long lead times and are costly because one patient/donor can only generate one dose. Allogeneic CAR-NK cells don't appear to cause GVHD and undesirable side effects are avoided by their short-term persistence and the lack of antigen clonality[7]. This could allow for an "off the shelf" product to greatly decrease time, cost and increase accessibility of CAR-NK therapy. Currently, there are a number of clinical trials underway involving CAR-NK cells for several indications (Table 1).

Final Remarks

While the preclinical and clinical data thus far has been promising, there are still concerns surrounding CAR-NK cells that must be addressed, which is still in its infancy in comparison to CAR-T immunotherapy. For example, identifying the best source of NK cells, improving ex vivo expansion, determining the optimal vector system, and designing CARs that work optimally for NK cells to provide the most potent therapeutic effect while maintain safety will be paramount[6].

It seems unlikely that CAR-NK will replace CAR-T cell therapy in the near future because there are still many unknowns, but they could be a valued addition to the immunotherapy arsenal perhaps acting as a complementary therapeutic option to CAR-T cells. It may be possible that cancer clearance is improved if both CAR-T and CAR-NKs are used in a combinatory approach than with CAR-T alone. There is more work to be done, but this is an exciting new direction for CAR-based immunotherapies.

The full version of this article was published on *Cell Culture Dish*, a blog designed to provide a community for scientists and others involved in biotechnology to share expertise and best practices as well as discuss topics of interest to the community. The blog covers areas important to the application, development and regulatory approval of cell culture processes and products. This includes biomanufacturing, vaccines, cell culture media and equipment, regenerative medicine, cord blood stem cells, cellular therapy, cell-based assays, diagnostic antibodies, life science research and related applications of cell culture.

Follow the link below to read the entire article:

The Cell Culture Dish

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