An End-to-End Solution to Accelerate CAR-T Cell Development from Concept to Clinic

CAR-T cell therapies have demonstrated tremendous success in relapsed or refractory haematological malignancies. Now therapeutics companies and academics alike are racing to break through the barrier of solid tumours, taking imaginative approaches to overcome the numerous obstacles in the way of turning a hugely variable starting material into an armed and precisely targeted weapon against cancer. But building a biologic is a team effort, so while therapeutics companies focus on perfecting their scientific approach, partnering with a contract testing development and manufacturing organisation (CTDMO) can help streamline their preclinical and manufacturing processes, and accelerate their journey to the clinic.

Leveraging the Power of the Immune System to Treat Advanced Cancers

TiCARos Therapeutics has leveraged the expertise of their team of immunologists from Seoul National University, College of Medicine to develop a promising pipeline of next generation immunotherapies for intractable haematologic malignancies and advanced solid tumours. TiCARos' mission is to create medication based on science. TiCARos was founded by two leading T-cell immunologists, who have built a team with a deep passion for innovative science and immunology. Their strength is in leading-edge approaches to new CAR-T cell therapies. Since its foundation, TiCARos has established active collaborations with the renowned institutions Seoul National University College of Medicine and National Cancer Center, with which their founders have strong ties.

TiCARos' cutting-edge technology platforms include CLIP CAR Technology, a strategy to stabilise the connection between CAR-T cells and antigen presenting cells, and CONVERTER CAR Technology, a strategy converting an inhibitory signal of CTLA-4 to an activating signal of CD28 in T cells by using CTC28 chimera, which potentiates CAR-T activity without a corresponding increase in side effects.

Accelerating the Pre-Clinical Discovery Phase

To accelerate the development of their CAR-T pipeline, TiCARos partnered with OXGENE, a WuXi Advanced Therapies company, for their expertise in construct design and engineering, and viral vector production.

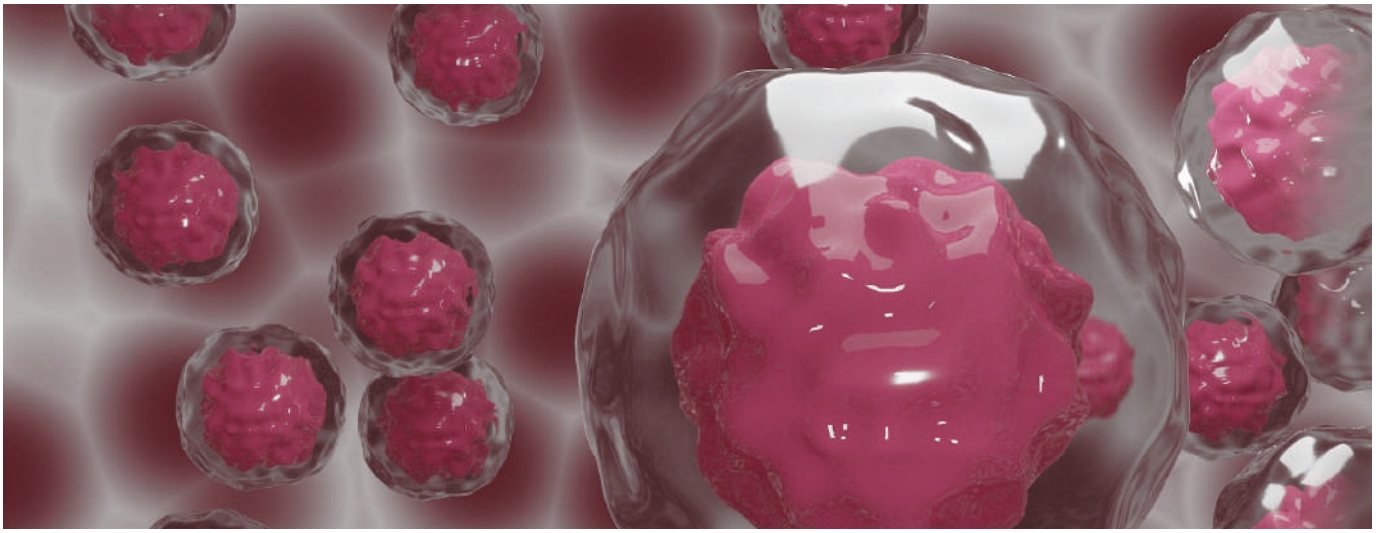
DNA Engineering and Small-Scale Lentiviral Production

In the first step of the project, OXGENE cloned four TiCARos constructs into OXGENE's proprietary XLenti™ backbone. OXGENE's 3rd generation, self-inactivating (SIN) XLenti™ packaging plasmids are optimised for increased translation efficiency and have low homology between expression cassettes for an improved safety profile. This plasmid system outperforms other commercially available equivalents, and in this project, our team of expert molecular biologists' custom engineered this system to optimise expression of TiCARos' genes of interest.

In the second phase of the project, OXGENE generated concentrated and purified lentiviral vectors containing TiCARos' custom constructs at shake flask scale, achieving good yields in each case.

Lentiviral Production and Process Development in Stirred Tank Bioreactors

Following a positive outcome from the first project, TiCARos initiated a second project with OXGENE; this time to produce lentivirus containing the two best performing constructs in 1L bioreactors, followed by downstream concentration and purification. As well as measuring final infectious viral titre (through integrated viral copy number assay), OXGENE's analytics team confirmed the total number of viral particles by p24 ELISA and quantity of viral genomes present in each



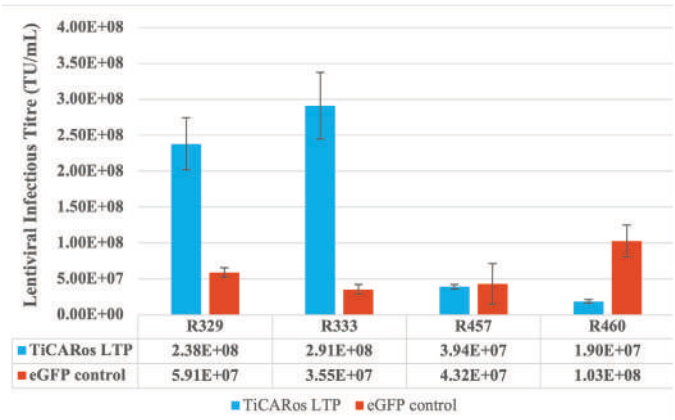


Figure 1 – ?

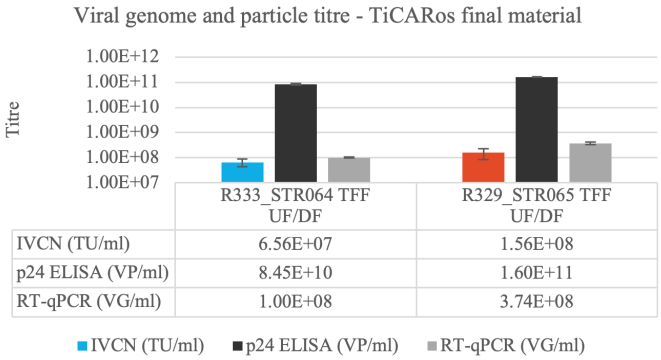


Figure 2 – ?

sample by reverse transcription qPCR. The results were consistent across all assays.

Jae Won Lee, the CEO of TiCARos stated, "We've been extremely pleased with the work conducted by OXGENE to promote the pre-clinical development of our next generation CAR-T cell therapies. Working together with OXGENE in the UK, and later with WuXi Advanced Therapies in China has helped to accelerate the development of these promising clinical candidates."

Producing GMP Grade Material for Clinical Studies

TiCARos chose to work with WuXi Advanced Therapies for GMP plasmid and lentiviral vector manufacture. The process began with full characterisation of TiCARos' plasmids; three lentiviral packaging plasmids and two transfer plasmids (from OXGENE). WuXi Advanced Therapies then screened for the highest yielding clones to establish the research cell bank (RCB), and subsequently the master cell bank (MCB) and working cell bank (WCB) under GMP conditions. After establishment of the bacterial banks, WuXi Advanced Therapies conducted an upstream demo run to confirm and optimise the upstream fermentation process to meet target yield, as well as stability and long-term storage stability studies to ensure long-term plasmid quality. Next, WuXi Advanced Therapies carried out a downstream verification run to confirm and optimise the downstream purification process, before confirming the analytical assays to be used for quality control.

The final step before scheduling GMP plasmid production of the three lentiviral packaging plasmids and one of the transfer plasmids was for WuXi Advanced Therapies to produce process development and template batch record reports for TiCARos. Alongside GMP plasmid manufacture, WuXi Advanced Therapies also ran a full set of analytical and stability studies, as well as providing all the necessary documentation for their Investigational New Drug (IND) filing.

The last part of the project was to produce lentiviral vectors at GMP grade, using the plasmids OXGENE had created and WuXi Advanced Therapies had manufactured. Once again, WuXi Advanced Therapies conducted a demo run first to verify and optimise the upstream process and a verification run to confirm platform suitability and downstream processing conditions, making sure that optimal conditions were locked in for GMP production.

TiCARos is now using the GMP grade material produced at WuXi Advanced Therapies for pre-clinical studies and plans to begin clinical trials and submit an IND application next year. The biotech is committed to developing and commercializing novel CAR-T therapies in the near future for complete remission of both hematologic and solid tumours, and ultimately improve cancer patients' survival. TiCARos is seeking potential global partnerships for its CAR-T development.

OXGENE and WuXi Advanced Therapies: an End-to-End Solution to Accelerate the Development of Cell and Gene Therapies.

In March 2021, OXGENE was acquired by WuXi AppTec to become part of WuXi Advanced Therapies. This means that, like TiCARos, our customers now have access to OXGENE's industry leading technologies and WuXi Advanced Therapies' comprehensive manufacturing and testing capabilities. With sites in the UK, USA and China, OXGENE and WuXi Advanced Therapies' combined capabilities support cell and gene therapy developers across the globe, offering technical support, manufacturing solutions and integrated testing, all the way from early discovery and pre-clinical research to GMP manufacture. Together, WuXi Advanced Therapies and OXGENE can accelerate this transition; aligned systems and processes allow for streamlined internal tech transfer between teams, accelerating development time and reducing the costs involved in bringing a cell or gene therapy to market.

Dr. Sophie Lutter

Dr. Sophie Lutter is Scientific Communications and Marketing Manager at OXGENE, a WuXi Advanced Therapies company. OXGENE provides end-to-end research services to cell and gene therapy companies seeking to discover, develop, manufacture and test innovative drug candidates at scale for global commercialization. OXGENE's proprietary technologies and automation platforms for molecular discovery are seamlessly integrated with a full suite of technologies for cell and gene therapy manufacturing.

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