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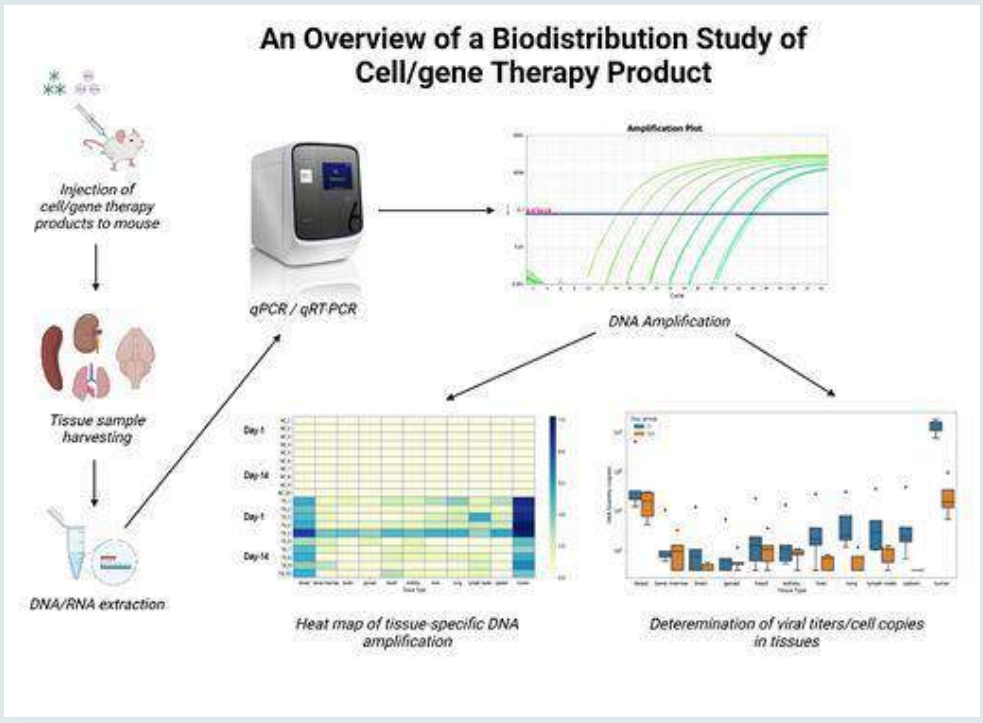

Figure 1. Overview of the biodistribution study of cell/gene therapy (CGT) product. The CGT product is injected into mice; tissue samples are harvested and DNA is isolated from all samples. DNA is then quantified and used as a template in quantitative polymerase chain reaction (qPCR) to determine viral titers/cell copies in each tissue sample (bottom right). DNA is also used as PCR template to amplify tissue-specific gene/vector expression. All PCR products are then combined and sequenced in a single reaction. Sequencing data are then used, in conjunction with viral titers/cell copy number to compute biodistribution for each sample.

Introduction

Cell and Gene therapy (CGT) technologies are the emerging therapies with the greatest potential to impact health care. The field of CGT is currently in an exciting era of expansion, with a large number of candidate therapies advancing into clinical trials and many more progressing through preclinical pipelines. The CGT products are reviewed by the Center for Biologics Evaluation and Research (CBER), FDA for their safety and efficacy before they can be studied in humans. The CGT programs are exceedingly

complex to navigate through preclinical development as each program is unique and poses significant safety challenges for developers.

A major study in the preclinical development of CGT agents is to delineate an in vivo toxicity, sometimes shedding, in vivo biodistribution profile following the administration of viral/vector or cellular agents to animals. Biodistribution studies, required by regulatory authorities, are critical for evaluating the preclinical safety of virus/cell agents such as CAR T-cell therapies, T-cell receptor therapies, NK cell therapies, tumor-infiltrating lymphocytes (TILs), marrow-derived lymphocytes (MILs), and stem cells (ESCs, HSCs, iPSCs, NSCs, ntESCs, MSCs, and NSCs), and gene therapy molecules such as adeno-associated virus (AAV)-mediated therapies, CRISPR/Cas9, TALEN, siRNAs, and antisense oligonucleotides. Table-1 lists some of the currently approved CGT agents in the US. With the rise in the number of early-stage cell and gene therapy products, preclinical development strategies that support clinical translation are needed. Accordingly, there is an increasing need to know and understand biodistribution in the product value proposition.

No	Trade Name	Type	Indications	Year
1	ZOLGENSMA	Gene Therapy	Spinal Muscular Atrophy	2021
2	ABCEMA	CAR T-cell Therapy	Multiple Myeloma	2021
3	BREYANZI	CAR T-cell Therapy	Relapsed or Refractory Large B-cell Lymphoma	2021
4	MACI	Cell Therapy	Single or Multiple symptomatic, full thickness cartilage defects of knees	2019
5	KYMRIAH	CAR T-cell Therpay	B-cell precursor acute lymphoblastic leukemia (ALL) and B-cell lymphoma	2017
6	LUXUTRNA	AAV2-based Gene Therapy	Inherited form of retinal dystrophy	2017
7	CLEVECORD	Stemcells	Disorders affecting the hematopoietic system	2016
8	IMLYGIC	Oncolytic viral Therapy	Lesions in patients with melanoma recurrent after initial surgery	2015
9	GINTUIT	Cell Therapy	Mucogingival conditions	201
10	laViv(R)	Cell Therapy	Moderate to sever nasolabial fold wrinkles	2011

Table-1 List of some of the approved CGT agents in the US

Biodistribution studies are intended to assess the distribution, persistence, and clearance of a vector/virus or a cellular agent in vivo from the administration site to targeted and non-targeted tissues and biological fluids. Regulatory guidelines describing general principles for assessing preclinical and clinical studies of cell and gene therapy products have been developed by the FDA in the last 15 years. The current widely used FDA-recommended assay method for biodistribution studies is a quantitative polymerase chain reaction (qPCR) assay that can measure the quantity of vector/virus DNA and the number of cellular agents present in tissue samples and biological fluids. Determination of the biodistribution profile of the vector and subsequent expression of the transgene product is essential for the interpretation of any potential therapeutic effects observed in subsequent proof-of-concept studies. Figure 1 illustrates a typical workflow of a biodistribution study for a CGT agent.

Noble Life Sciences offers full services for biodistribution studies of vector/virus or cell agents. We have seasoned experience with the absolute qPCR quantification that can determine the vector/viral copy number per microgram of genomic DNA (see Figure 2 for an example of the standard curve used in copy number quantification) and quantify the cell number of human cellular agents distributed to animal organs (see Figure 3 for an example of the standard curve used in human T-cell quantification). In addition to the absolute qPCR quantification services, we also offer quantitative reverse transcription PCR (RT-qPCR) services for transgene expression profiling in tissues/biological fluids. Biodistribution studies performed in Noble Life Sciences fully comply with GLP guidelines.

Our Molecular Biology facility is equipped with the QuantStudio™ 6 Flex qPCR instrument with capabilities for 96-well and 384-well qPCR studies. As QuantStudio™ 6 Flex can detect various widely used dyes (up to 10), we can perform multiple-color qPCR (e.g., dual- or triple-color qPCR). Noble Life Sciences has a reputable scientific team to run the Molecular Biology facility to ensure the successful and timely completion of preclinical biodistribution studies with a large number of samples.

Our biodistribution services include:

DNA isolation of tissue/biological fluids and qPCR analysis

We are capable of isolating DNA from various biological specimens, including any types of animal tissues, shedding specimens (e.g., injection site, saliva, urine, feces), and blood. A large number of samples can be processed for DNA isolation in our Molecular Biology Facility with a fast turnaround time

RNA isolation of tissue and RT-qPCR analysis

In addition to DNA isolation services, we also provide RNA isolation services from various animal tissue types and RT-qPCR services for transgene expression analysis in biodistribution studies.

qPCR Assay Development and Validation

We offer services to develop and validate qPCR assays using custom-designed primers and probes to ensure their sensitivity and target-specificity in the analysis of the presence/absence of the therapeutic vector/virus or cell therapy agents. We guarantee that the qPCR assays established in Noble Life Sciences meet the FDA's requirement on qPCR sensitivity (≤ 50 copies/microgram of host DNA). In addition, we also evaluate the recovery rate and PCR inhibition effect for the DNA extraction method.

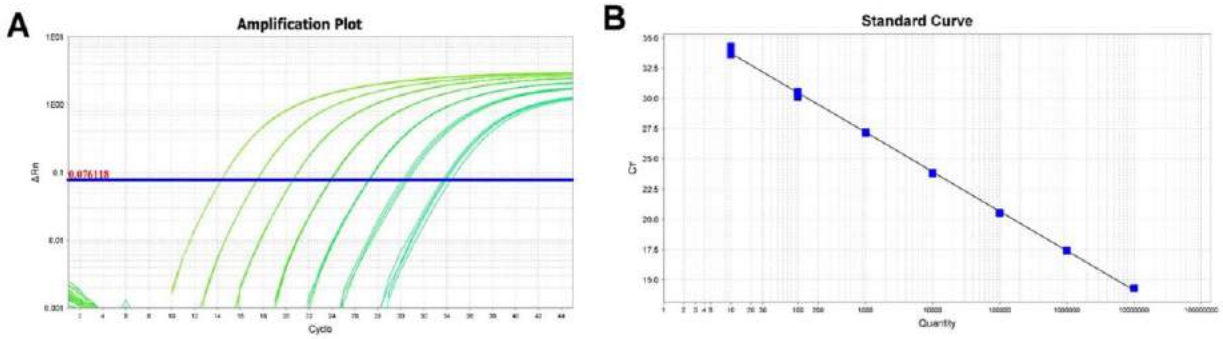

Figure 2. The standard curve for the copy number quantification of target DNA. (A) The amplification plot of serially diluted DNA standards. The linearized plasmid DNA standards were serially diluted with mouse genomic DNA starting from ten million copies to 10 copies (serial 10-fold dilutions) to make DNA standards for qPCR. The prepared DNA standards were subjected to qPCR with target-specific primers and the FAM-labeled probe using the QuantStudio™ 6 Flex qPCR instrument. **(B)** The standard curve was generated from qPCR of serially diluted DNA standards. The standard curve was plotted according to the expected copy numbers and measured Ct values of serially diluted DNA standards.

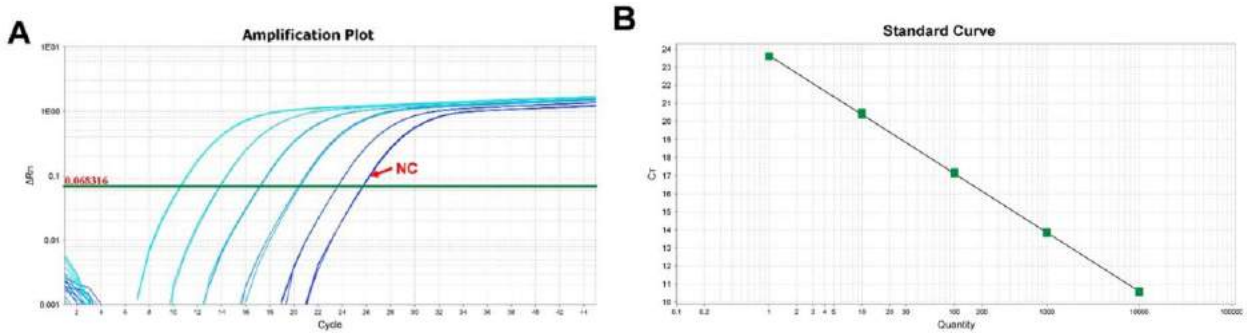

Figure 3. The standard curve for the quantification of human cell DNA. (A) The amplification plot of serially diluted human T-cell DNA standards. The T-cell DNA was serially diluted with mouse genomic DNA (200 ng/µl) starting from 10,000 pg to 1 pg (serial 10-fold dilutions) to make DNA standards for qPCR. The input amount of mouse DNA for T-cell DNA standards was 1 µg. The qPCR of T-cell DNA standards was performed with human-specific Alu primers and probe (FAM-labeled) using the QuantStudio™ 6 Flex qPCR instrument. One microgram of mouse DNA was used as a negative control (NC) in the qPCR assay. The Ct difference between 1 pg of T-cell DNA and NC is about 2 Ct. The amplification curves of DNA standards were plotted based on ΔRn vs. Cycle number. (B) The standard curve was generated from qPCR of serially diluted T-cell DNA standards. The standard curve was plotted according to the expected DNA amounts (pg) and measured Ct values of serially diluted T-cell DNA standards. Based on 6 pg DNA equivalent to one human cell, the qPCR assay can sensitively quantify 1 pg of human DNA (equivalent to 0.1667 of human T-cell) within 1 µg of mouse genomic DNA.