October 22, 2026

Company Name: HEALIOS K.K.
Representative: Hardy TS Kagimoto, Chairman & CEO
(TSE Growth Code: 4593)

Academic Paper on the Development of an effective direct qPCR method for cell therapy product quantification in non-clinical biodistribution studies in "Regenerative Therapy"

HEALIOS K.K. ("Healios") and Akatsuki Therapeutics, Inc. ("Akatsuki") are involved in the research of new therapeutic products using eNK cells*. We are pleased to announce that researchers at Healios have published a recent work the Development of an effective direct qPCR method for cell therapy product quantification in non-clinical biodistribution studies. This work has been published in the peer-reviewed journal "Regenerative Therapy".

Title: Development of an effective direct qPCR method for cell therapy product quantification in non-clinical biodistribution studies
Journal: "Regenerative Therapy"
Manuscript Number: 33 (2026) 101189
Authors: Yoichi Naritomi, Yuka Sato, Kumiko Goto, Yuriko Takeno, Hironobu Kimura, Kouichi Tamura
Affiliation: Kobe Research Institute, HEALIOS K.K., Kobe, Japan

Abstract:
Evaluating the non-clinical biodistribution (BD) of cell therapy products (CTPs) is essential to predicting their efficacy and safety in clinical trials. Although BD is typically quantified by qPCR following DNA extraction, this process is labor-intensive and time-consuming, and subject to variability in extraction yield, which complicates normalization to organ weight or blood volume. In contrast, direct qPCR enables the quantification of lysed samples without DNA extraction. Here, we systematically developed an effective direct qPCR method applicable to BD studies of CTPs.

We used HLCN061, a genetically modified natural killer (NK) cell derived from human induced pluripotent stem (iPS) cells. Mouse organs and blood containing HLCN061 cells were lysed, diluted, and directly subjected to real-time PCR using a human-specific Alu primer set. PCR reactions employed an inhibitor-tolerant master mix. Assay performance was evaluated by linearity (R^2), amplification efficiency, relative error (RE), coefficient of variation (CV), and lower limit of quantification (LLOQ). The validated method was then applied to evaluate the BD of HLCN061 cells over 24 h following intravenous administration to mice.

Across all tissues and blood samples, R^2 exceeded 0.99, PCR amplification efficiency ranged from 90% to 110%, and RE was within ±25%. CV values were within 25% except for one case. LLOQ was 0.25 cells/mg weight for the liver, lungs, kidneys, and heart; 1 cell/mg weight for the spleen and ovaries; and 0.3 cells/μL for blood. Weight-normalized concentrations were highest in the lung, with the most rapid clearance from blood.

This direct qPCR method is effective and suitable for non-clinical BD assessment of CTPs.

* eNK cells
Development code: AKT-01/HLCN061 (engineered NK cells)
eNK cells are an iPSC-derived NK cell therapy with several functional enhancements achieved through gene-modification including enhanced recognition of and cytotoxicity towards cancer, improved persistence, increased capability to migrate to and infiltrate solid tumors, and the ability to recruit host immune cells. Healios and our development partner, Akatsuki Therapeutics, Inc., have succeeded in developing eNK cells through its own research and have confirmed the anti-tumor effect of eNK cells in mice engrafted with human lung cancer cells and human liver cancer cells. In joint research with the National Cancer Center Japan ("the NCCJ") Healios confirmed the antitumor effect of eNK cells in a PDX mouse disease model created using the NCCJ's JPDX samples. Healios is also conducting joint research using eNK cells for hepatocellular carcinoma with Hiroshima University and for mesothelioma with Hyogo Medical University. Akatsuki Therapeutics, Inc. and Healios are collaborating to continuing to advance eNK cells in preparation for its first clinical trials. In addition to advancing eNK cells as a monotherapy and in combination with existing drugs, Healios is developing a dual CAR-eNK cell product, in which chimeric antigen receptors (CARs) that specifically recognize cancer antigens are introduced into the eNK to facilitate enhanced targeting of certain solid cancers. Healios is studying cancer immuno-cell therapy using CAR-eNK cells against brain tumors with Kyushu University.

About Healios:
HEALIOS K.K. is Japan's leading clinical stage biotechnology company harnessing the potential of stem cells for regenerative medicine. It aims to offer new therapies for patients suffering from diseases without effective treatment options. Healios is a pioneer in the development of regenerative medicines in Japan and owns proprietary, global platforms utilizing both somatic stem cells and iPS cells. In the somatic stem cell field, Healios is developing HLCM051(invimestrocel), a proprietary cell product comprised of multipotent adult progenitor cells ("MAPCs") derived from the bone marrow of healthy adult donors. HLCM051 has been shown to exhibit powerful anti-inflammatory and immunomodulatory properties with applicability in a range of disease states, has been tested in hundreds of patients in late stage clinical trials, is manufactured consistently at scale in 3D bioreactors, and has demonstrated both safety and suggested efficacy in hundreds of patients across multiple indications. Healios is seeking to advance HLCM051 on a global basis for ARDS, trauma, and ischemic stroke. In the iPSC regenerative medicine field, Healios has developed HLCN061, a next generation NK cell treatment for solid tumors that has been functionally enhanced through gene-modification. These cells have demonstrated robust anti-tumor efficacy in animal models, benefit from a scalable 3D bioreactor manufacturing process. The development of AKT-01/HLCN061 is currently being led by our development partner, Akatsuki Therapeutics, Inc., in preparation for the initiation of clinical trials. Healios has also established a proprietary, gene-edited "universal donor" induced pluripotent stem cell line to develop next generation regenerative treatments in immuno-oncology, ophthalmology, liver diseases, and other areas of severe unmet medical need. Healios was established in 2011 and has been listed on the Tokyo Stock Exchange since 2015 (TSE Growth: 4593).
https://www.healios.co.jp/en

About Akatsuki:
Akatsuki Therapeutics Inc. (Head office: 8-1 Kakuda-cho, Kita-ku, Osaka, Japan; President: Junya Yamazaki; a Saisei Ventures portfolio company) is developing innovative cellular immunotherapies with the potential to transform the treatment of cancer and other serious diseases. Its lead program harnesses advanced genetic

enhancements, cellular reprogramming, and scalable manufacturing to address the limitations of existing cell therapy approaches. Driven by a mission to create accessible, off-the-shelf solutions, the company aims to deliver life-changing therapies that will improve worldwide patient access and improve the standard of care. At Akatsuki Therapeutics, the company is committed to advancing the next generation of cellular immunotherapies to usher in a new dawn for patients and their families.

Contact:
IR & PR Division
HEALIOS K.K.
E-mail: ir@healios.jp