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Company Name: HEALIOS K.K.
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(TSE Growth Code: 4593)

Academic Paper on eNK Cells in "Stem Cell Research and Therapy"

Human iPSC-derived NK cells armed with CCL19, CCR2B, high-affinity CD16, IL-15, and NKG2D complex enhance anti-solid tumor activity

HEALIOS K.K. ("Healios") is 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 revealing the anti-tumor activity of human iPSC-derived NK cells (Healios eNK cells) expressing CCL19, CCR2B, high-affinity CD16, IL-15, and NKG2D complexes by gene editing on solid tumors. This work has been published in the peer-reviewed journal "Stem Cell Research & Therapy".

In peer-reviewed papers, scientific research has shown that the six transgenes not only enhance killing activity, but also perform important functions such as recruiting patient immune cells and enhancing invasive properties of solid tumors. The eNK cells are expected to be a promising therapeutic agent for solid tumors.

Title: Human iPSC-derived NK cells armed with CCL19, CCR2B, high-affinity CD16, IL-15, and NKG2D complex enhance anti-solid tumor activity
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Abstract:
Studies of chimeric antigen receptor (CAR-T) and natural killer (NK) cells have shown promising results in treating hematological malignancies. However, there are still obstacles to effectively treating solid tumors. These include the challenges of CAR-T cell homing and infiltration, the presence of immunosuppressive microenvironments, and the potential for antigen escape in solid tumors.

We introduced the six genes, CCL19, CCR2B, FCGR3A (CD16), IL-15, KLRK1 (NKG2D), and HCST (DAP10), into NK cells. We evaluate the antitumor function, including killing activity, antibody-dependent cytotoxicity, migration ability, and recruitment of dendritic cells. In addition, in vivo antitumor activity was determined by using an orthotopic lung cancer mouse model.

Healios eNK cells showed improvement in viability without additional cytokine supplement in vitro and in vivo. Overexpression of NKG2D complex and high-affinity CD16 enhanced the antitumor function of the eNK cells against cancer cell spheroids. Forced expression of CCR2B enhanced eNK cell tumor infiltration against cancer cell spheroids. Forced expression of CCL19 endowed the eNK cells with the ability to recruit dendritic cells. We found that the eNK cells injure cancer cells, specifically observing that eNK cells were able to lyse HLA-E-expressing cancer cell spheroids, but not normal human cells. Moreover, eNK cells demonstrated superior anti-tumor activity in an orthotropic lung cancer mouse model. These proof-of-concept studies demonstrate the promise of our eNK cells as a novel adoptive cell therapy product for the treatment of solid tumors.

* eNK cells
(Development code: HLCN061)
Healios' eNK cells are an iPSC-derived NK cell therapy with several functional enhancements achieved through gene-editing 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 has succeeded in developing eNK cells through its own research and has 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. Through its collaboration with Akatsuki Therapeutics, Healios is 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.

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 MultiStem® (HLCM051), a proprietary cell product comprised of multipotent adult progenitor cells ("MAPCs") derived from the bone marrow of healthy adult donors. MultiStem 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 MultiStem 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-editing. These cells have demonstrated robust anti-tumor efficacy in animal models, benefit from a scalable 3D bioreactor manufacturing process, and are currently being prepared for initial human testing in collaboration with Akatsuki Therapeutics. The company 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

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