

August 9, 2022

Company Name: HEALIOS K.K.  
Representative: Hardy TS Kagimoto, Chairman & CEO  
(TSE Growth Code: 4593)  
Contact: Richard Kincaid, Executive Officer CFO  
(TEL: 03-5962-9440)

## Healios' eNK Cells

HEALIOS K.K. (“Healios”) is developing next-generation immuno-oncology therapies, with a focus on solid tumors, using NK cells<sup>\*1</sup> derived from allogeneic iPSCs (Development Code: HLCN061 “eNK cells”) whose specific functions have been enhanced with gene editing technology. Healios hereby summarizes its vision for the eNK program and platform, the eNK efforts it has made to date, and the future research and development plans it will execute with the aim of starting clinical trials in 2024.

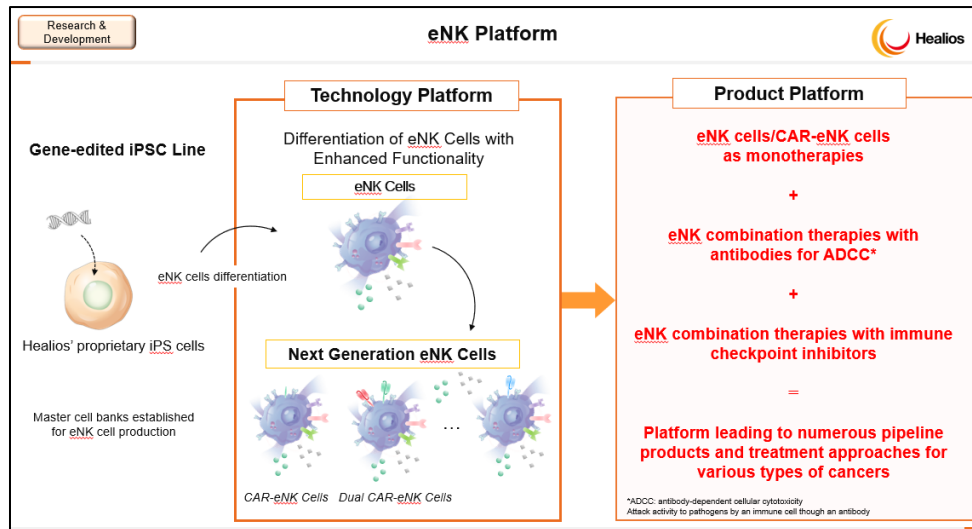
### 1. eNK Program Vision

Healios is striving to become a global biopharma company by creating, developing and commercializing cutting edge cell therapy technologies. Healios believes that iPSC-based immuno-oncology therapies will change the future of cancer treatment, and we are actively working on the development of new eNK cell-based therapies for solid tumors. Rooted in the successes we are experiencing with our eNK platform, Healios is seeking to contribute to the eradication of solid tumors and other cancers by leveraging our unparalleled iPSC expertise and augmenting the innate cancer killing ability of NK cells.

Healios will utilize its strengths, including research, development, and manufacturing capabilities related to iPSCs, gene-editing technology and know-how, and experience in conducting clinical trials, to develop our eNK business. In parallel to driving forward our R&D activities, we are also pursuing partnerships with pharmaceutical companies in Japan and overseas, to leverage resources and create technological synergies, with the goal of efficiently and effectively maximizing the potential of the eNK program and platform. We will accelerate our research and development, aiming to deliver new immuno-oncology therapies using eNK cells to patients as soon as possible.

Healios anticipates that its eNK cells are a relevant therapy to numerous cancer types. To address different cancers, Healios' eNK cells may be used as a monotherapy, in conjunction with CAR constructs for further targeting in certain cancer types, or as combination therapies with existing approved drugs. As such, while eNK cells are a product, they are also a platform with a very large addressable market for which we will seek to produce a number of products

over time to treat a variety of different cancers. We will do so both on our own but also with pharmaceutical companies that we engage with as a result of our partnering activities.



## 2. Efforts to Date

Even though the advent of molecular targeted drugs and cancer immunotherapies has improved treatment outcomes for some cancer patients, the efficacy of existing treatments for solid tumors remains poor. Solid cancers are the leading cause of death among Japanese and one of the leading causes of death worldwide. In 2019, with the goal of delivering new therapeutic drugs to patients suffering from cancer, we began research on eNK cells using allogeneic iPSCs, leveraging the iPSC technology and expertise we built up to that point since our founding in 2011. We [added eNK cells to our pipeline in 2020](#) and today it is our lead asset in the iPSC regenerative medicine field.

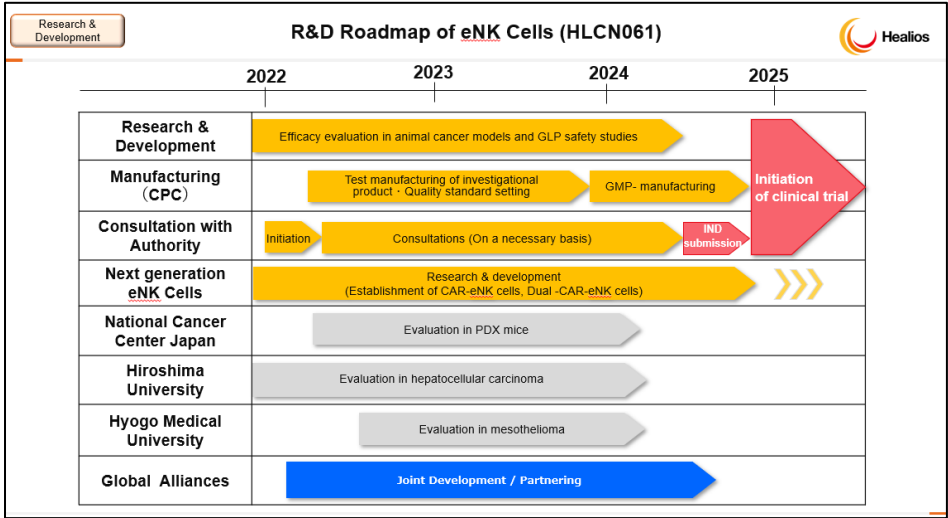
Healios has succeeded in developing eNK cells through its own research. Healios began its research efforts by establishing a fundamental NK cell platform by generating un-edited iPSC derived NK cells that demonstrated robust cytotoxicity towards cancer. Healios built upon this strong foundation by then establishing its eNK cells and confirming their even greater anti-tumor effects in mice engrafted with human lung cancer cells and human liver cancer cells, thereby establishing “animal POC” for the efficacy of eNK cells ([please see page 30 of the FY2022 Q1 Financial Results](#)). We are conducting joint research with the National Cancer Center Japan, Hiroshima University, and the Hyogo Medical University, to further evaluate the effect of eNK cells in various human solid cancers. In the cell therapy industry, manufacturing is of paramount importance to the ultimate success of a therapeutic program, and leveraging our deep experience in the production of cell products, we have established a proprietary automated, 3D perfusion bioreactor based manufacturing system that enables efficient and stable mass production of eNK cells. Healios has [initiated the operations of its in-house Cell Processing Center \(CPC\)](#), a GMP manufacturing facility based on these technologies. The manufacturing process and facility positions us to efficiently supply global

clinical trials using eNK cells. In preparation for the start of these clinical trials, we have [conducted a regenerative medicine product quality consultation](#)\*2 (face-to-face advice) with the Pharmaceuticals and Medical Devices Agency (PMDA).

**3. Research and Development Plans**

Healios will continue its research and development activities with the aim of starting clinical trials in 2024 and further expanding and deepening our research and development efforts. To promote research and development of next-generation immuno-oncology cell therapy using eNK cells, as well as global clinical trials, we will seek to engage in collaborations and alliances with partners, including domestic and foreign pharmaceutical companies, in addition to our own development. In the development of next-generation cancer immunotherapy, which is driving the growth of the pharmaceutical market, alliances between biotech ventures and major pharmaceutical companies are being formed at various stages of research and development to promote them on a global scale. Not only in terms of funding, but also by leveraging the clinical know-how of the development partner, we will aim to bring eNK cell based therapies to patients at an earlier stage.

As described above, Healios’ eNK cells represent a broad platform opportunity, that includes their use across a range of cancers as a monotherapy, as CAR-eNK products, such as the dual CAR-eNK that we are developing as a priority at Healios, as well as in combination with existing cancer drugs. We anticipate expanding our internally developed eNK based pipeline over time. We will also aim to form alliances in various ways to support this pipeline and to address different cancer types, countries and regions beyond the scope of our internal areas of focus.



Note: This plan is a target at this time and is subject to revision depending on future conditions.

**Attached: "Future research and development plans for eNK cells"**

**\*1 Natural killer (NK) cells**

Natural killer (NK) cells are a subset of lymphocytes, a type of white blood cell. NK cells play a central role in a cell mediated defense system that human bodies naturally have, and attack cancer cells and virus-infected cells. The expected efficacy of treatments using NK cells includes life extension, promotion of healing, relief of symptoms, and improvement of quality of life.

**\*2 Regenerative medicine product quality consultation**

Guidance and advice are provided as a specialized consultation on matters related to the quality of processed cells, etc. and regenerative medicine products, such as specifications, test methods, stability, and manufacturing methods.

**About Pharmaceuticals and Medical Devices Agency (PMDA):**

PMDA is Japanese regulatory agency, working together with Ministry of Health, Labour and Welfare. Its obligation is to protect the public health by assuring safety, efficacy and quality of pharmaceuticals and medical devices. It conducts scientific reviews of marketing authorization application of pharmaceuticals and medical devices, monitoring of their post-marketing safety. It is also responsible for providing relief compensation for sufferers from adverse drug reaction and infections by pharmaceuticals or biological products. In response to applications from sponsors, etc., we provide guidance and advice on clinical trials for pharmaceuticals, medical devices, regenerative medicine products, etc., as well as on clinical trials for reevaluation and reassessment.

<https://www.pmda.go.jp/english/review-services/consultations/0002.html>

**About Healios' eNK cells:**

Healios eNK cells are a gene edited iPSC-NK cell therapy with several functional enhancements achieved through gene-editing including enhanced cytotoxicity towards cancer, improved capability to migrate 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 that eNK cells have anti-tumor effects in mice engrafted with human lung cancer cells and human liver cancer cells. In joint research with [the National Cancer Center Japan](#) (“the NCCJ”) we are evaluating the antitumor effects of eNK cells in a PDX mouse disease model created using the NCCJ’s JPDX samples. Furthermore, Healios is conducting joint research on cancer immunotherapies using eNK cells for hepatocellular carcinoma with [Hiroshima University](#) and for mesothelioma with [Hyogo Medical University](#). Healios is continuing with in vitro and animal testing of its eNK cell therapy in preparation for its first clinical trials. In addition, we are working on the development of a dual CAR-eNK cell product, in which chimeric antigen receptors (CAR) that specifically recognize cancer antigens are introduced into the eNK, with the aim of expanding the application of eNK cells to other solid tumors.

**About Healios:**

Healios 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, where it has established a proprietary, gene-edited “universal donor” induced pluripotent stem cell (iPSC) line to develop next generation regenerative treatments in immunooncology, ophthalmology, liver diseases, and other areas of severe unmet medical need. Healios’ lead iPSC-derived cell therapy candidate, HLCN061, is a next generation NK cell treatment for solid tumors that has been functionally enhanced through gene editing. Its near-term pipeline includes the somatic stem cell product HLCM051, which has been evaluated in Japan in Phase 2/3 and Phase 2 trials in ischemic stroke and acute respiratory distress syndrome (ARDS), respectively. 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>



# (Supplementary Explanatory Materials) Research and Development Plans for eNK Cells



Company

Healios K.K. (TSE Growth: 4593)

Date

August 9, 2022

***Contribute to the eradication of solid tumors and other cancers by leveraging Healios' iPS cell expertise and augmenting the innate cancer killing ability of NK cells***

## Research & Development

- **Advanced technology at Healios' Kobe Research Institute**
  - In-house implementation from gene editing through to process development
- **Establishment of data for conducting clinical trials**
  - Generation and accumulation of efficacy and safety data

## Manufacturing

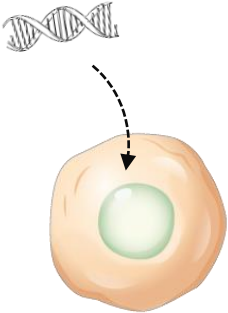
- **Manufacturing Capabilities**
  - In-house production of clinical product in proprietary 3D system

## Alliances & Collaborations

- **Joint Development / Partnering**
  - Maximize the potential of the eNK cell program and platform

**Accelerate activities in the above three areas**

## Gene-edited iPSC Line



Healios' proprietary iPS cells

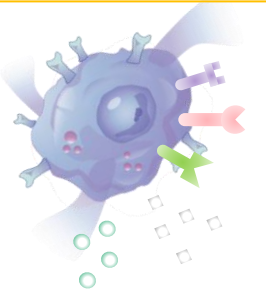
Master cell banks established for eNK cell production

eNK cells differentiation

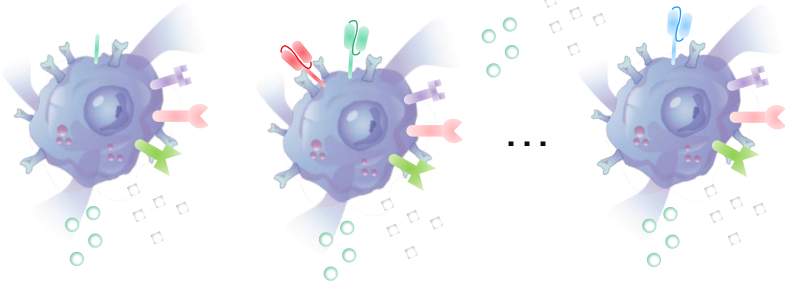
### Technology Platform

Differentiation of eNK Cells with Enhanced Functionality

eNK Cells



### Next Generation eNK Cells



CAR-eNK Cells Dual CAR-eNK Cells

### Product Platform

**eNK cells/CAR-eNK cells as monotherapies**

+

**eNK combination therapies with antibodies for ADCC\***

+

**eNK combination therapies with immune checkpoint inhibitors**

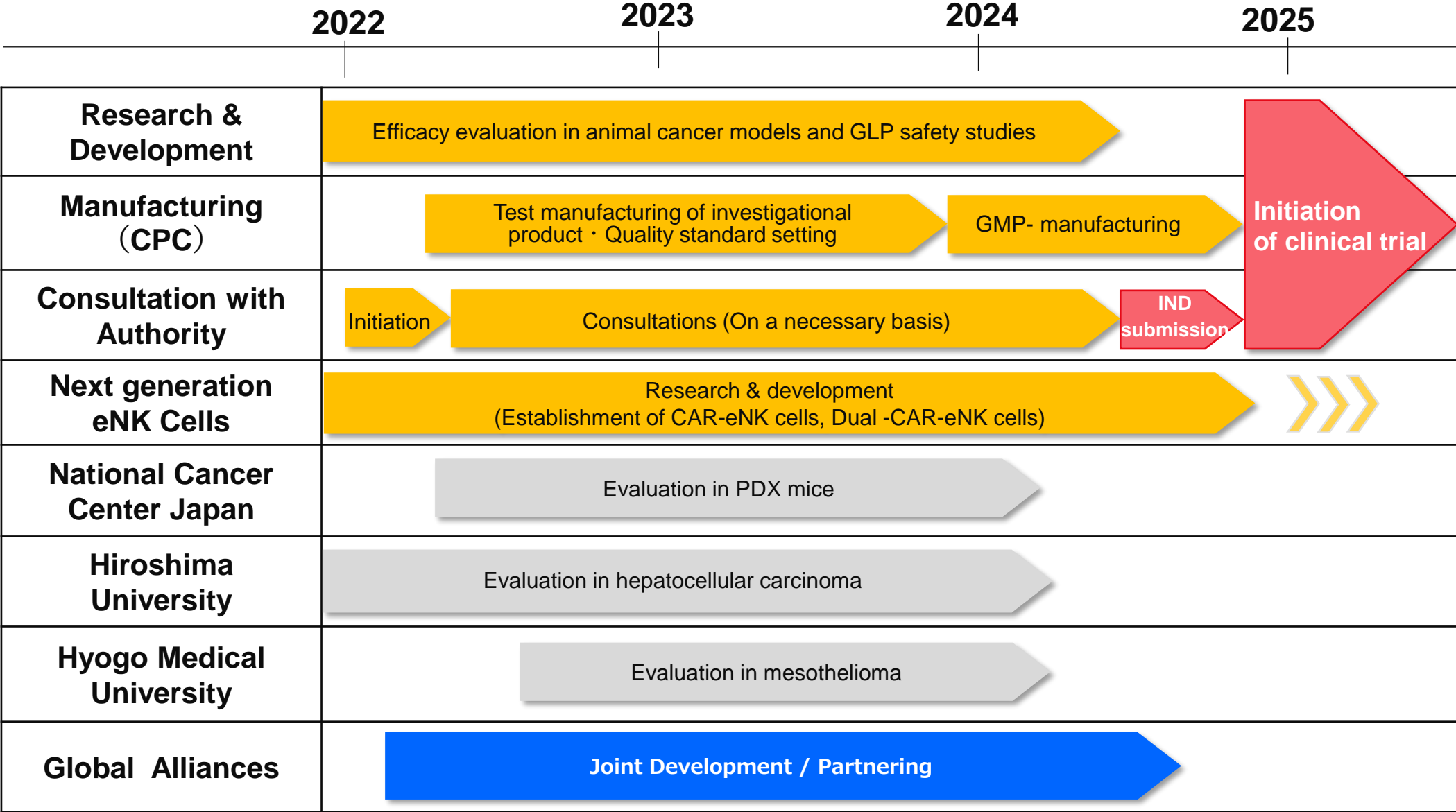
=

**Platform leading to numerous pipeline products and treatment approaches for various types of cancers**

\*ADCC: antibody-dependent cellular cytotoxicity  
Attack activity to pathogens by an immune cell through an antibody



# R&D Roadmap of eNK Cells (HLCN061)



Large number of researchers (more than 30 Ph.D.'s) on staff and efficient, in-house implementation of everything from gene editing to process development

### 1. Exploratory Research

- I. Development of iPSC differentiation induction methods
- II. Functional evaluation of iPSC derived cells
- III. Functional evaluation of iPSC derived cells
- IV. Evaluation of gene-edited cells

### 2. QC

- I. Functional evaluation of various cells
- II. Development of evaluation protocols

### 3. Genetic Recombination Experiments

- I. Construction of plasmids
- II. Construction of viral vectors
- III. Creation of transgenic cells



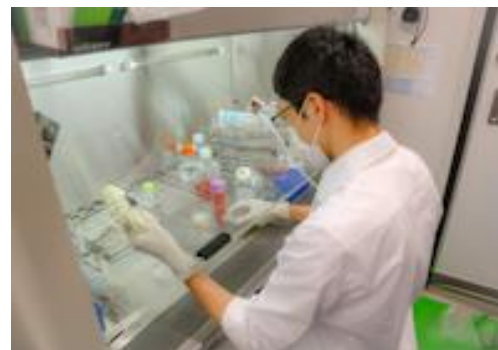
Healios' Kobe Institute Area(Photo by Kobe Urban Promotion Service Co.,Ltd.)

### 4. Experiments on animals

- I. Generation of disease mice models
- II. Evaluation of antitumor effects *in vivo*
- III. Evaluation of immune response *in vivo*
- IV. Evaluation: tissue section and immunostaining

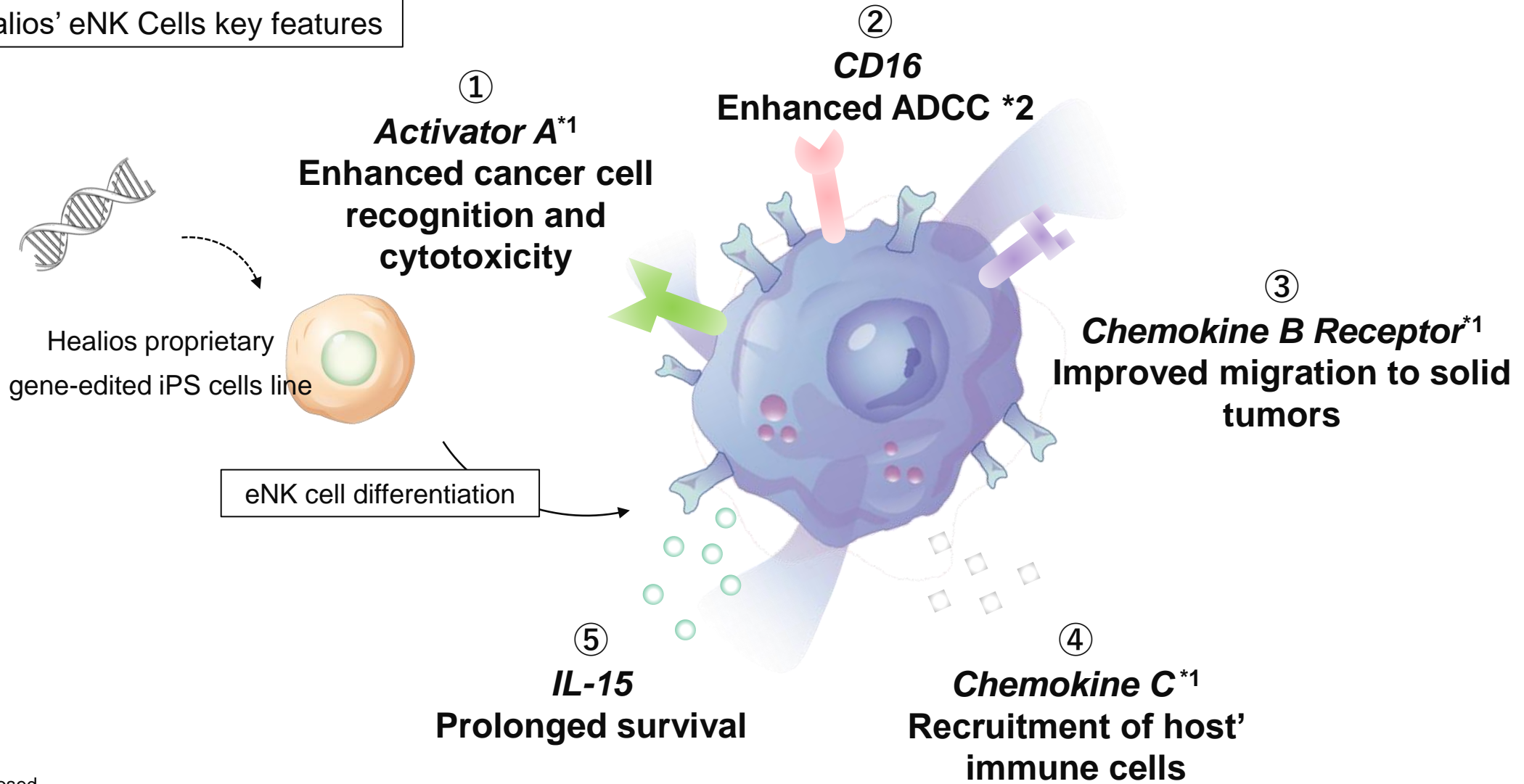
### 5. Process Development Research

- I. Optimization of differentiation
- II. Development of mass production methods
- III. Development of freezing processes
- IV. Analysis of culture media



# eNK Cells Enhanced Not Only with Improved Cytotoxicity and Persistence, but with Greater Migration to Tumors and Recruitment of Host Immune Cells

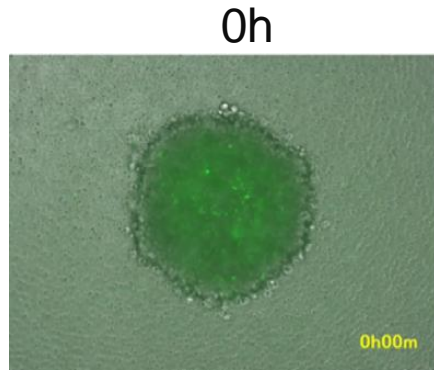
## Healios' eNK Cells key features



\*1 Not disclosed  
\*2 ADCC: antibody-dependent cellular cytotoxicity  
Attack activity to pathogens by an immune cell through an antibody

# In Vitro: Evidence of Anti-tumor Effect as Mono- and Combination Therapy (Lung, A549)

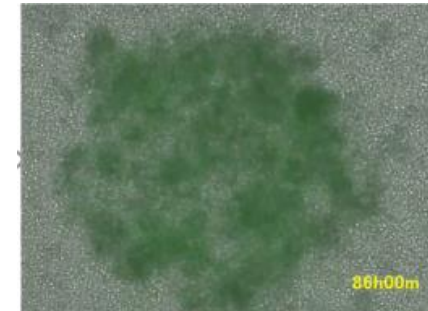
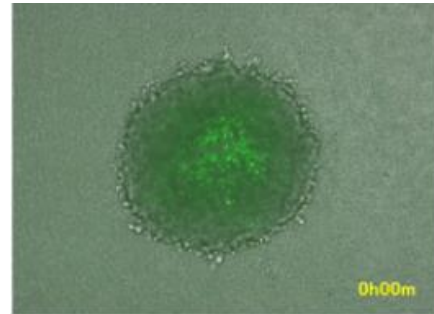
eNK only



Bright green: apoptotic cells

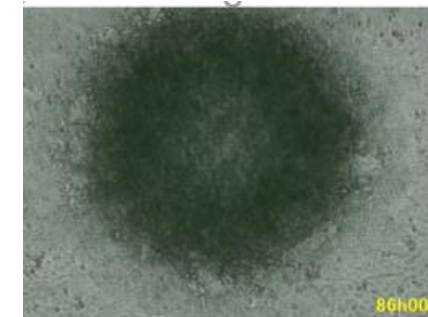
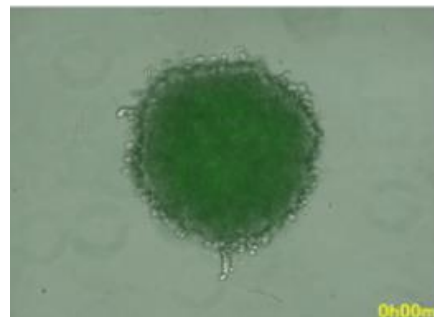
eNK cells have killed the cancer cells.

eNK with anti-EGFR antibody



The lung cancer cells were efficiently killed and the lung cancer cell spheroid was destroyed.

Anti-EGFR antibody only

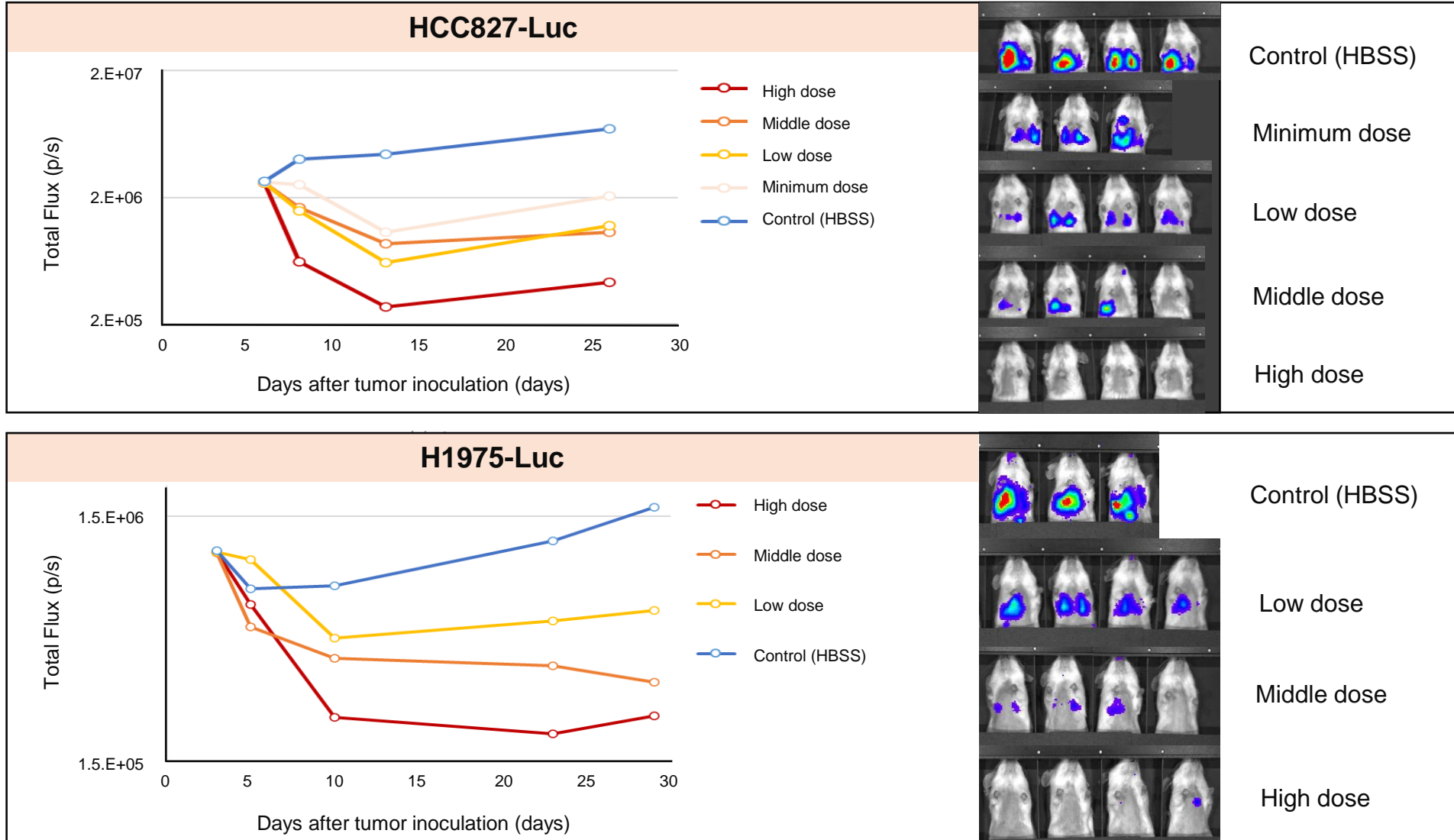


The cancer cells survived and the cancer cell spheroid expanded for 86 hours.

(Source) in-house data



# In Vivo: Healios' eNK Cells Show Robust Anti-Tumor Activity Against Lung Cancers



(Source) in-house data

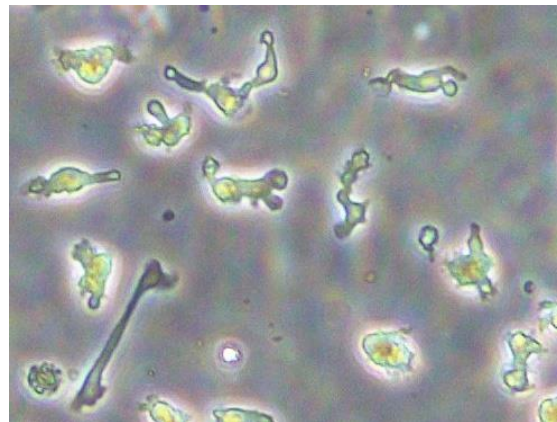
*-GMP facility fully operational and being advanced in preparation for clinical trials  
-In-house manufacturing enables control of the schedule and quality of clinical production*



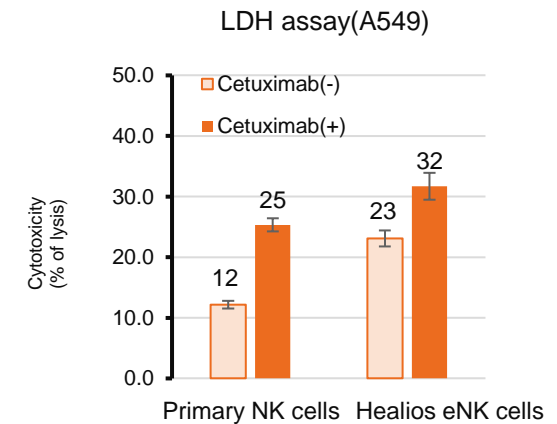
**Fully-closed, feeder free, 3D perfusion bioreactor system**



KCMI; Kobe Center for Medical Innovation  
Photo by; OM Kobe (KCMI management company)



**100 billion eNK cells per batch**



**Cryopreserved samples show high cytotoxicity post thaw**

## *Maximize the potential of the eNK cell program and platform*

We are pursuing partnerships with pharmaceutical companies, to access financial and other resources as well as to leverage technological synergies.

We aim to accelerate our research and development to deliver new immunology therapies using eNK cells to patients as soon as possible.

This material has been prepared solely for the purpose of disclosing relevant information regarding HEALIOS K.K. (“HEALIOS”). This document does not constitute an offer to sell or the solicitation of an offer to buy any security in the United States, Japan or any other jurisdiction.

This presentation contains statements that constitute forward-looking statements, including estimations, forecasts, targets and plans, and such forward-looking statements do not represent any guarantee by management of future performance. [In many cases, but not all, HEALIOS uses such words as “aim,” “anticipate,” “believe,” “continue,” “endeavor,” “estimate,” “expect,” “initiative,” “intend,” “may,” “plan,” “potential,” “probability,” “project,” “risk,” “seek,” “should,” “strive,” “target,” “will” and similar expressions to identify forward-looking statements.] You can also identify forward-looking statements by discussions of strategy, plans or intentions. Any forward-looking statements in this document are based on the current assumptions and beliefs of HEALIOS in light of the information currently available to it, and involve known and unknown risks, uncertainties and other factors. Such risks, uncertainties and other factors may cause HEALIOS’s actual results, performance, achievements or financial position to be materially different from any future results, performance, achievements or financial position expressed or implied by such forward-looking statements.

This presentation is based on the economic, regulatory, market and other conditions as in effect on the date hereof, and HEALIOS does not guarantee that the information contained in this presentation is accurate or complete. It should be understood that subsequent developments may affect the information contained in this presentation, which HEALIOS is not under an obligation, or does not plan, to update, revise or affirm. The information in this presentation is subject to change without prior notice and such information may change materially. Neither this presentation nor any of its contents may be disclosed to or used by any other party for any purpose without the prior written consent of HEALIOS.

The information in connection with or prepared by companies or parties other than HEALIOS is based on publicly available and other information as cited, and HEALIOS does not have independently verified the accuracy and appropriateness of, nor makes any warranties with respect to, such information.

The information about regenerative medicine products (including products currently in development) which is included in this material is not intended to constitute an advertisement or medical advice.





# Healios

< Contact information >

Corporate Communications  
HEALIOS K.K.

Press contact: [pr@healios.jp](mailto:pr@healios.jp)

Investor contact: [ir@healios.jp](mailto:ir@healios.jp)

<https://www.healios.co.jp/contact/>