December 2, 2025

Press Release

C4U Corporation
Center for iPS Cell Research and Application, Kyoto University

Announcement of New Collaborative Research Between C4U and CiRA

C4U Corporation (“C4U”) and the Hotta Laboratory (Associate Professor Akitsu Hotta) at the Center for iPS Cell Research and Application (“CiRA”), Kyoto University are pleased to announce that we have entered into a new collaborative research agreement for the purpose of the research and development of therapeutic methods for Duchenne Muscular Dystrophy (DMD) utilizing CRISPR-Cas3 genome editing technology.
C4U and Hotta Lab at CiRA have been conducting collaborative research utilizing CRISPR-Cas3 technology in iPS cells since January 2020. This new collaborative research will pursue further application and development, focusing on specific disease treatments.

The potential application of CRISPR-Cas3 technology for treating Duchenne Muscular Dystrophy was reported in *Nature Communications* in 2019, based on research findings by Dr. Tomoji Mashimo (at that time: the University of Osaka, currently: the University of Tokyo), Dr. Junji Takeda of the University of Osaka, and Dr. Akitsu Hotta of CiRA (Morisaka et al., *Nat Commun.* 2019 doi: 10.1038/s41467-019-13226-x). Furthermore, CiRA Hotta Laboratory published a new gene mutation repair method for DMD using CRISPR-Cas3 in *Stem Cell Reports* in 2023 (Kita Y et al., *Stem Cell Reports* 2023 doi: 10.1016/j.stemcr.2023.07.007). Additionally, the T-CiRA joint research program between CiRA Hotta Laboratory and Takeda Pharmaceutical Company Limited ("Takeda") has been conducting research and development on delivery technology to efficiently transport genome editing enzymes to skeletal muscle over a 10-year period since 2016 (Kenjo E et al., *Nat Commun*. 2021 doi: 10.1038/s41467-021-26714-w).
Concurrently, C4U and Takeda conducted collaborative research from 2023 to 2024, and these results, along with others, will be leveraged to the upcoming collaborative research.

Regarding the commencement of this collaborative research, Dr. Akitsu Hotta of CiRA stated the following:
"I am delighted that we have signed a new collaborative research agreement with C4U aimed at developing a treatment for Muscular Dystrophy. Duchenne Muscular Dystrophy is a severe, life-threatening disease for which there is no curative treatment, and patients and their families desperately desire the development of therapeutic drugs. By combining our patient-derived iPSC evaluation system and skeletal muscle delivery technologies with C4U’s CRISPR-Cas3 platform, we anticipate creating new synergies that will accelerate therapeutic innovation."

Akimitsu Hirai, President and CEO of C4U, commented as below:
"I am pleased and proud as we move forward with the research and development of a therapeutic method for Duchenne Muscular Dystrophy through this collaborative research with CiRA Hotta Laboratory. We will utilize our past achievements to advance this research with CiRA Hotta Laboratory, aiming to realize a new curative treatment using genome editing technology. Since Muscular Dystrophy

is a rare disease, we are also considering future alliances with pharmaceutical companies that have an interest in this disease and this area."

About C4U

C4U is a privately held biotech company based in Osaka, Japan, and is focused on the development of safe and efficient gene therapies utilizing its proprietary next generation CRISPR-Cas3 gene editing platform. In comparison to the CRISPR-Cas9 platform, CRISPR-Cas3 presents the distinct benefits of: 1) no off-target by the higher selectivity of deletion site (improved safety); 2) efficient knockouts by the larger deletion of gene sequences; and 3) an entirely independent patent portfolio. C4U has been granted a worldwide exclusive license to CRISPR-Cas3 by the University of Osaka for use in eukaryotic cells thus simplifying sublicensing transactions which is in sharp contrast to the complex and heavily litigated CRISPR-Cas9 patent landscape.
URL: https://www.crispr4u.jp/en/

Glossary of Terms

Genome Editing Technology: A technique that introduces artificially designed DNA cleavage enzymes into cells to selectively cleave and modify localized parts of the genome.

CRISPR-Cas3: Similar to CRISPR-Cas9, it cleaves double-stranded DNA. It is considered a safer genome editing tool because its crRNA (guide) recognition sequence is longer (27-base guide sequence), resulting in high specificity for recognizing genomic sequences and a low risk of inducing off-target mutations (mutations at unintended sites). It is also proficient at causing large deletions, allowing for loss of gene function or the removal of large regions containing disease-causing genetic mutations, in addition to gene modification.

CRISPR-Cas9: A type of widely used genome editing technology. Cas9 binds with a guide RNA, and selectively cleaves DNA complementary to a part of the guide RNA (20-base guide sequence). By changing the guide sequence, it can selectively cleave DNA with various base sequences. It was developed by multiple researchers in the US and Europe and has a complex background of numerous intertwined patents, with patent litigation currently ongoing in the US and other countries.

Duchenne Muscular Dystrophy (DMD): A serious type of muscular dystrophy, designated as one of Japan's intractable diseases. Muscular dystrophy is primarily characterized by the progressive atrophy of skeletal muscles. In the Duchenne type, mutations in the dystrophin gene prevent the body from producing the dystrophin protein, leading to a gradual decline in muscle strength. Symptoms typically begin in infancy (around 3 to 5 years old), and the disease is mainly observed in boys.

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