別紙２

## Results report

１．Title of Research and Development： Directing Cellular Identity to Move Towards Progenitor Cell Therapies

２．Principal Investigator：Yasuhiro Yamada（Professor, Center for iPS Cell Research and Application (CiRA), Kyoto University）

３．Counterpart Principal investigator：Andras Nagy（Senior Investigator, Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital（Canada））

４．Results of Research and Development:

The goal of the collaborative research is to characterize the epigenetic states accompanying cell identity changes, or Assisted Cell state Alterations (ACsA) during reprogramming and differentiation. The knowledge gained from this joint effort will hold significant value for improving ACsA techniques and control over cell-state changes to multiple fate destinations, which in turn will result in cell products better suited for disease modeling and translation to regenerative medicine applications. Furthermore, we believe the efforts of this project will better bridge our extensive insights gained from mouse models toward better understanding human conditions, many currently regarded as incurable. The foundation of this proposal lies in the enormous dataset that has been assembled under “Project Grandiose,” (PG) which is built on use of the *piggyBac* reprogramming transposon developed by the Nagy group to generate transgenic mouse iPSC lines that is now utilized worldwide. Using parallel molecular analyses including genome wide mapping of chromatin modifications, CpG methylation, and small (miRNA) and large transcriptome, and proteome analysis, we characterized the AScA that arise during reprogramming. The data were analyzed to specifically define the molecular differences between two alternative pluripotent states (reprogramming factor expression dependent F-class and factor independent ESC-like iPS cells) (*Nature* 2014, *Nature* 2014, *Nature Comm* 2014, *Nature Comm* 2014). In addition, the *in vivo* reprogrammed mouse model developed by the Yamada lab (*Cell* 2014) provides a tremendous complement to the *in vitro piggyBac* method.

The Yamada and Shinkai groups characterized the epigenetic marks associated with the tendency for some cell-states to gain a cancer cell identity resulting from transient expression of reprogramming factors in the reprogrammable mouse model. The Shinkai group performed genome-wide histone post-translational modifications during the reprogramming process of the *in vivo* models. The Woltjen group determined how the stoichiometry of Klf4, one of reprogramming factors, drives heterogeneity and alternate reprogramming outcomes (cell states). Variations in Klf4 protein levels can change reprogramming kinetics, success rates, and the identities of “partially” reprogrammed cells (*Stem Cell Reports* 2015). The Nagy, Woltjen and Yamada research teams have completed microarray analyses of their respective cells associated with reprogramming – the Nagy team’s fibroblast-derived F-class cells, the Woltjen group’s intermediate cells during *in vitro* reprogramming from MEFs and Yamada group’s kidney cell-derived cancer-like cells. Progress on reducing the required cell number for ChiP analysis by the Shinkai group now positions us to be able to address the role of specific posttranslational modification of histone tail residues in these cell types as well. Combining the microarray and epigenetic data of respective cells that are associated with reprogramming should contribute to better understanding of epigenetic changes during cell fate alteration. We hope to find commonality within observed epigenetic changes during the respective ACsA processes, as well as epigenetic signatures consistently associated with tumor development.