Cell Therapy Systems (CTS) Sendai virus reprogramming kit for xeno-free generation of iPSC

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ABSTRACT

For the past decade, the induced pluripotent stem cell (iPSC) field has grown immensely, starting with basic research, and now moving more toward translational and clinical applications. As iPSC move towards the clinic, it is vital that the cells used are of high quality; free of reprogramming transgenes, free of adventitious agents, genetically stable, and functionally pluripotent. Traditional reprogramming workflows typically include one or more components of animal origin, such as fetal bovine serum (FBS) or bovine serum albumin (BSA), which can be sources of adventitious agents. This means that iPSC that are generated in a xeno-free, clinically relevant workflow will be much more readily applicable to clinical uses.
Here, we show the generation of high quality iPSC with a Sendai reprogramming kit manufactured under a certified ISO 9001 Quality Management System, which is free of animal origin components. The kit was used to reprogram both blood-derived cells and skin fibroblasts, in conjunction with xeno-free cell culture and reprogramming workflows, starting from initial cell isolation, all the way through to iPSC expansion and banking. The iPSC generated with this method were shown to have a normal karyotype, were free of Sendai viral vectors, expressed the standard self-renewal markers, and demonstrated functional pluripotency for all three germ layers. The ability to create high quality iPSC in clinically relevant, xeno-free workflows, will allow researchers to more smoothly transition cells to clinical applications.

INTRODUCTION

For iPSC to be used as starting material for clinical applications, it is important that the iPSC are created using clinically relevant workflows. This includes removing animal origin components, and using reagents which are manufactured reliably. In order to achieve this, the CTS™ CytoTune™-iPS 2.1 Reprogramming Kit was developed by applying three major changes to the existing CytoTune-iPS 2.0 Sendai Reprogramming Kit:
1.) Removal of animal-origin components (e.g. BSA) from the viral dilution buffer.
2.) Replacing the C-Myc transgene with L-Myc, which is shown to be a less oncogenic member of the same gene family. (1)
3.) Production of the viral vectors under a certified ISO 9001 Quality Management System.
Given these changes, the new reprogramming kit was used to reprogram both fibroblasts and blood-derived cells in xeno-free workflows, and both reprogramming efficiency and quality of the resulting iPSC clones were examined.

MATERIALS AND METHODS

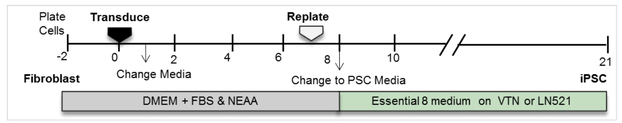
Fibroblast Reprogramming
Fibroblasts were cultured in either standard serum-based fibroblast medium (DMEM, 10% FBS, and 1% Non-essential amino acids), or xeno-free fibroblast medium, and transduced with the CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit. Virus was removed after 24 hours, and cells were harvested and plated onto rh-Laminin521 (LN521) at 7 days after transduction. At 8 days after transduction, medium was changed to Essential 8™ Medium, and cells were fed daily. At 21 days after transduction, cells were either isolated for clonal expansion, or reprogramming efficiency was determined by using the Vector® Red Alkaline Phosphatase stain.

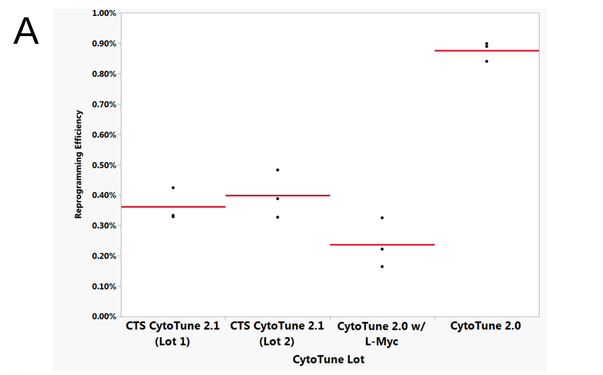
CD34+ Cell Reprogramming
CD34+ Cells were cultured in CTS™ OpTMizer™ T Cell Expansion SFM, plus SCF (100ng/ml), FLT3 (100ng/ml), IL-3 (20 ng/ml), IL-6 (20ng/ml) for 3 days after thaw. Cells were then transduced with CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit. Virus was removed after 24 hours, and cells were plated onto Vitronectin (VTN) or rh-Laminin521 (LN521) at 3 days after transduction. At 7 days after transduction, medium was changed to Essential 8 Medium, and cells were fed daily. At 16-21 days after transduction, cells were either isolated for clonal expansion, or reprogramming efficiency was determined by using the Vector Red Alkaline Phosphatase stain.

T-Cell Reprogramming
CD3+ cells were purified from whole blood using the Dynabeads™ FlowComp™ Human CD3 Kit, according to manufacturer instructions. Purified CD3+ cells were cultured with Dynabeads™ Human T-Expander CD3/CD28 and 100U/mL recombinant human IL-2 CTS OpTMizer T Cell Expansion SFM for seven days. Cells were then reprogrammed with a protocol modified from Seki et al.(2). After 48 hours, cells were plated onto rhLaminin-521 in Essential 8 Medium. At 18 days after transduction, cells were analyzed for AP activity.

RESULTS

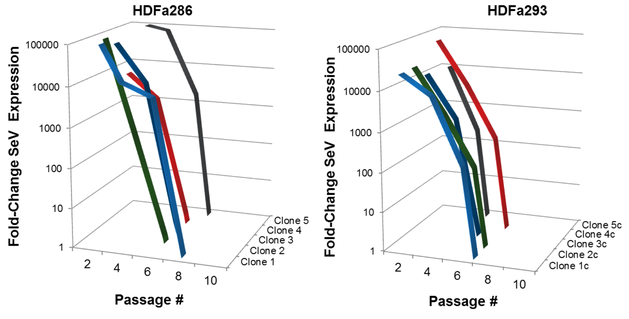
Figure 1. CTS CytoTune 2.1 successfully reprograms human dermal fibroblasts





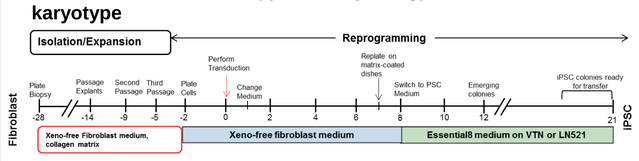
Three different lots (donors) of HDFa were reprogrammed in standard FBS-containing media conditions **(A,B)**, and xeno-free media conditions **(C,D)** with either CytoTune 2.0, CTS CytoTune 2.1, or CytoTune 2.0 where the c-Myc was replaced with L-Myc, according to the schematic. At 21 days after transduction, cells were stained for AP, and reprogramming efficiency was calculated as the number of AP positive colonies, relative to the number of cells plated on Day 7 **(A,C)**. Representative whole-well images of AP stained iPSC colonies **(B,D)**.

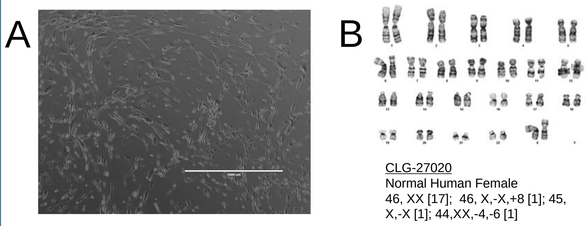
Figure 2. CTS CytoTune 2.1 vectors clears from iPSC clones by Passage 8



The rate of SeV dilution was measured in 5 different iPSC clones derived from each of two different HDFa donors. iPSC clones were passaged for 10 passages and RNA extracts were collected and analyzed at every other passage. SeV expression was measured by qPCR with a TaqMan assay. Fold change in SeV expression was calculated as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct$ (iPSC) – ΔCt (Untransduced Fibroblasts), and $\Delta Ct = Ct$ (SeV) – Ct (ActB).

Figure 3. Human Dermal Fibroblasts isolated and expanded in xeno-free medium have typical morphology and a normal karyotype

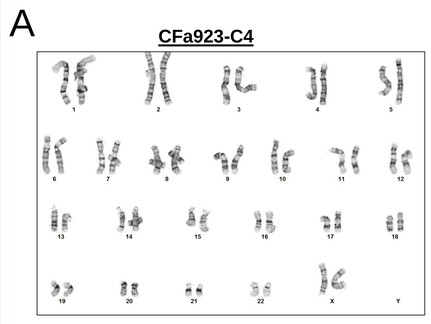




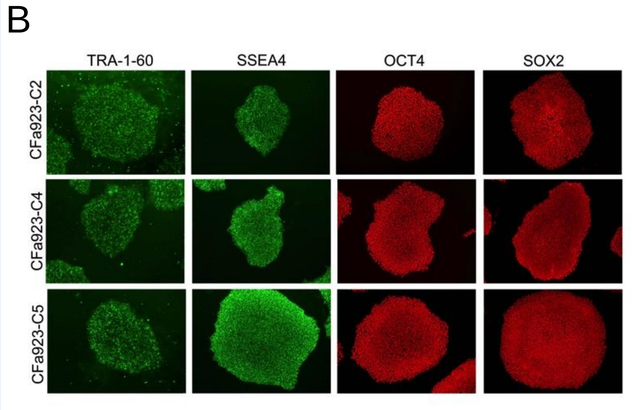
CLG-27020
Normal Human Female
46, XX [17]; 46, X,-X,+8 [1]; 45, X,-X [1]; 44,XX,-4,-6 [1]

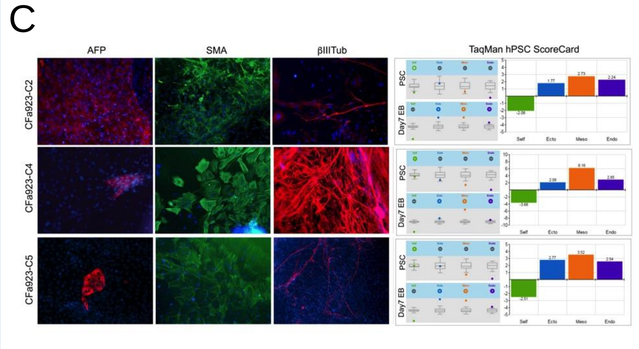
Human dermal fibroblasts (HDF) were isolated and expanded in completely xeno-free fibroblast medium. A skin-punch biopsy was plated onto a collagen matrix and cultured in xeno-free fibroblast medium. Explants were allowed to grow from the biopsy for about two weeks, and then cells were passaged with TrypLE Select. Subsequently, cells were passaged every 4-5 days. HDFa displayed typical fibroblast morphology **(A)**, and had a normal karyotype **(B)**.

Figure 4. iPSCs derived in a completely xeno-free workflow have a normal karyotype, expression of self-renewal markers, and display functional pluripotency



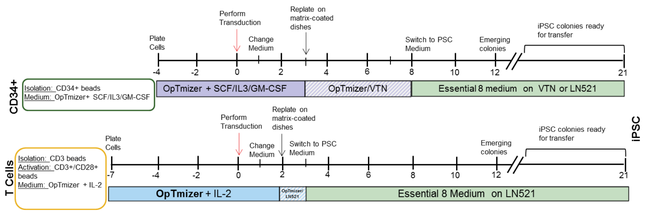
CLG-27021: **NORMAL FEMALE**
46, XX [19]
47,XX,+mar [1]

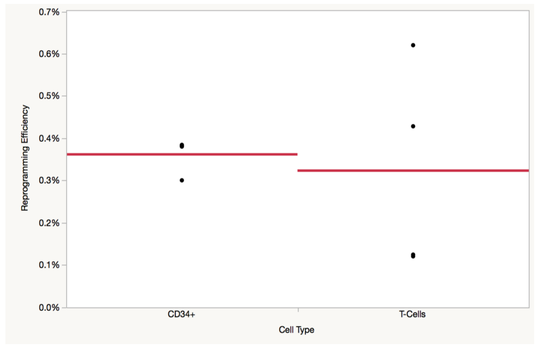




iPSC clones reprogrammed with CTS CytoTune 2.1 and derived in completely xeno-free conditions were characterized. Clones displayed a normal karyotype **(A)**, expressed the self-renewal markers Tra-1-60, SSEA4, Oct4, and Sox2 **(B)**; and displayed functional pluripotency; as assessed by embryoid body formation followed by antibody staining (at day 21 of differentiation) for markers representative of the three germ layers, and gene expression (at day 7 of differentiation) measured by TaqMan™ hPSC ScoreCard™ **(C)**.

Figure 5. CTS CytoTune 2.1 successfully reprograms blood-derived cells





Two different types of blood-derived cells (CD34+ Cells and T-Cells) were reprogrammed with CTS CytoTune 2.1, according to the schematic above. At 16 days after transduction, cells were stained for AP, and reprogramming efficiency was calculated as the number of AP positive colonies, relative to the number of cells replated.

CONCLUSIONS

CTS CytoTune 2.1 can successfully reprogram HDF and blood derived cells (CD34+ and T-Cells); though efficiency is about 2 fold lower than reprogramming with CytoTune 2.0.

The reduction in reprogramming efficiency is likely due to replacement of C-Myc with L-Myc, and not due to the xeno-free formulation.

Ten CTS CytoTune 2.1-derived iPSC clones from two different donors showed no detectable virus by Passage 8.

Human dermal fibroblasts derived and expanded in completely xeno-free conditions showed a normal karyotype.

iPSC generated with CTS CytoTune 2.1 in completely xeno-free conditions - from fibroblast derivation through iPSC expansion - displayed all of the standard characteristics of iPSC (i.e. normal karyotype, self-renewal marker expression, functional pluripotency).

REFERENCES

1.Nakagawa M, Takizawa N, Narita M, Ichisaka T, Yamanaka S (2010). Promotion of direct reprogramming by transformation-deficient Myc. Proc Natl Acad Sci U S A. 107(32):14152-7.

2. Seki T, Yuasa S, Fukuda K (2012) Generation of induced pluripotent stem cells from a small amount of human peripheral blood using a combination of activated T cells and Sendai virus. Nat Protoc;7(4):718-28.

TRADEMARKS/LICENSING