No tumorigenicity of allogeneic induced pluripotent stem cells in major histocompatibility complex-matched cynomolgus macaques

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**Supplemental Figure 1: Immunohistochemistry of iPSCs.**

Four iPSCs were stained with antibodies specific for TRA-1-60, SSEA4, OCT3/4, and NANOG. The magnification of all figures was ×40. *: passage number

**Supplemental Figure 2: RT-PCR of iPSCs**

The numbers at the top of panels shows cells of which mRNA were collected. The correspondence between numbers and cell names is as follows; (1) monkey ESCs p27, (2) CMF1/1-1 p35, (3) CMF1/1-2 p21, (4) CMT1/1-4 p23, and (5) CMT1/1-6 p20.

**Supplemental Figure 3: Characteristics of iMSC derived from CMF1/1-1 cells.**

(A) Schema for mesenchymal stem cell-like cells (iMSC) induction. iMSCs were derived from iPSCs during hematopoietic differentiation. iMSC progenitor cells were included in the CD45−CD34− fraction (circled). (B) Stereoscopic image of iMSCs. Cuboidal adherent cells can be seen. The magnification was ×40. (C) Flow cytometric analysis of iMSCs. The cells were positive for CD105, CD73, CD44, CD90, and CD29, being consistent with the definition given by the Mesenchymal and Tissue Stem Cell Committee of ISCT. (D) iMSCs differentiated into chondrocytes (Alcian Blue), osteoblasts (Alkaline phosphatase), and adipocytes (Oil-red O). (E) TGF-β in the culture supernatant of iMSCs. iMSCs produced TGF-β. ND: not detected.

**Supplemental Figure 4: Immune cells infiltrating the teratoma derived from CMF1/1-1 cells in autologous transplantation #733 and NOD-SCID mice.**

(A, B) The excised teratoma derived from CMF1/1-1 in autologous transplantation of #733 was cut into 2 pieces. One piece of teratoma was minced and passed through a cell strainer to remove debris for flow cytometric assay (A). The other piece was fixed with formalin for histological analysis (B). (A) Flow cytometric analysis of infiltrating lymphocytes. Whole cells were stained with antibodies specific to indicated molecules and analyzed after gating on a lymphocyte fraction. (B) Immunohistochemistry of the excised teratoma. Infiltrating lymphocytes were found. The magnifications of sections stained for CD3 (T cells), CD4 (helper T cells), CD8 (killer T cells), CD20 (B cells), CD68 (macrophages), and Granzyme B (NK cells, allow heads) were ×200, ×200, ×200, ×200, ×400, and ×400, respectively. (C) The excised teratoma derived from CMF1/1-1 in NOD-SCID mice. The upper and middle
panels respectively show HE staining section in low magnification (×40) and high magnification (×400). The lower panel showed immunohistochemistry of the excised teratoma. Allow heads show Granzyme B positive cells (NK cells). The magnification was ×400.

Supplemental Figure 5: Flow cytometric analysis of IgG specificity against MHC-matched 733F PBMCs and MHC-matched embryonal carcinoma cells

Monkey embryonal carcinoma were derived from CMF1/1-1 and expressing embryonic antigens such as SSEA4 and OCT3/4. Cells were incubated with the collected plasmas. Fluorescein conjugated anti-monkey IgG was used for secondary antibody to detect the specific IgG attaching on the cell surface. Filled histograms indicate cells stained with plasmas collected before transplantation as control, lines indicate cells stained with plasmas collected at 4 weeks after transplantation. *: passage number.

Supplemental Figure 6: Flow cytometric analysis of iPSCs.

(A, B) Flow cytometric analysis of the expression of MHC class I (A) and CD47 (B) on the surface of iPSCs. Lines indicate cells stained with antibodies against indicated molecules. Filled histograms indicate negative controls stained with isotype-matched control antibodies. (C) iPSCs were stained by lectins, MALII and SNA, which specifically recognize α2-3 and α2-6 sialic acids, respectively. Filled histograms indicate PBS control. Lines indicate cells stained with lectins. Hatched lines indicate cells stained with lectins after sialidase treatment. Lower intensity of the lectin staining after sialidase treatment showed that the lectins certainly recognized sialic acid on the surface of iPSCs. *: passage number.