Legends Supplemental Figures

Supplemental Figure 1. Human iPS cells derivation and characterization. A) Morphology of human keratinocytes grown in serum-free medium. (B) Typical example of a human iPS line derived after 7 days from the mechanical picking. (C) iPS colonies (panel B) show strong alkaline phosphatase activity typical of human iPS cells. (D) Typical Embryoid Bodies (EBs) derived from human iPS cells cultured in suspension in absence of FGF. E-F) Immunofluorescence of the pluripotent transcription factors OCT4 and NANOG and of pluripotent surface antigens TRA-1-60, SSEA3, SSEA4, and TRA-1-81. G) Immunofluorescence experiments using IgG Isotype control for mouse, rabbit and rat antibodies reveals very low fluorescence background. H-J) iPS cells were specifically directed to differentiate in vitro into the three germ layers derivatives: (H) mesoderm (actinin-positive cardiac myocytes, red), (I) ectoderm (TuJ1-positive neuronal cells, green), and (J) endoderm (α-fetoprotein-positive, green). K-M) In vivo differentiation into all three germ layers through teratoma formation. (K) Histology clearly identified rivulets of muscle cells (mesoderm), (L) neuro-epithelium rosettes (ectoderm) and (M) respiratory epithelium structures (endoderm). N) Expression levels of endogenous pluripotent genes quantified by qRT-PCR and plotted relative to GAPDH expression. Error bars represent standard deviation.

Supplemental Figure 2. A) Laser Scanning Microscopy (LSC) showing the presence of human CD45+ blood cells (green) and CD34+CD45+ blood stem/progenitors cells (red and green) indicated by white arrows (right panels, 40x objective, scale bar 20 µm), within the context of an entire teratoma section (left panel, 10x objective, scale bar 500 µm). Panels 2 and 3 show the presence of CD34−CD45+ at teratoma’s borders. B) iPS and cord blood cells were stained with
human CD34 and CD45 antibodies to assess expression of hematopoietic markers at a pluripotent stage. Isotype antibodies were used as control for gates setting. C) Example of teratomas harvested after 7 and 9 weeks after iPS cells injection into immunodeficient mice (upper panels). Example of teratomas generated by iPS cells injection and co-injection of iPS cells with OP9, OP9W3a and OP9D stroma cells, respectively (lower panels).

Supplemental Figure 3. A) Teratomas and human cord blood were stained with human and murine CD45 antibodies to assess antibody specificity. B) Total number of teratomas analyzed and of teratomas which did not give rise to human CD45⁺ cells in each category. C) Murine bone marrow of control mice and murine bone marrow of mouse carrying teratomas were stained with human and murine CD45 antibodies to assess antibody specificity. Right panel shows murine bone marrow of mouse carrying teratomas depleted of murine cells. D) Representative flow cytometry plots of the human CD34⁺, CD19⁺, CD15⁺, and CD3⁺ in bone marrow and spleen of mice carrying teratomas obtained by co-injection of iPS and OP9W3a cells. Bone marrow of non-injected NSG was used as negative control and to set up the gates. The population was analyzed after depletion of murine CD45⁺ cells.

Supplemental Figure 4. A) CFU assay for human CD45⁺CD34⁺ cells derived from teratoma and for CD45⁺CD34⁺ isolated from human cord blood. B) Representative flow cytometry plots of the Glycophorin A⁺ CD45⁻ erythroid population isolated from NSG murine bone marrow following transplantation with CD34⁺CD45⁺ cells isolated from teratoma and from cord blood.
C) Schematic model for NSG-Ter immunization protocol used and immunoglobulin evaluation through ELISA assay.

**Supplemental Methods**

**In vitro differentiation**

To allow spontaneous endoderm formation after 3–4 days embryoid bodies were transferred onto 0.2% gelatin-coated glass six well plates and cultured in differentiation medium (DMEM supplemented with 20% fetal bovine serum, 2 mM l-glutamine, 0.1 mM 2-mercaptoethanol, nonessential amino acids, and penicillin-streptomycin, all from Invitrogen) for 2–3 weeks. For mesoderm/cardiomyocyte differentiation, iPS cells were maintained on gelatin-coated plates in differentiated medium supplemented with 100 μM ascorbic acid (Sigma). For ectoderm differentiation, EBs were cultured on laminin (Stemgent) coated six well plates. Briefly, after 4 days in EB medium as a floating culture, the cells were plated on laminin (Stemgent) coated plates in N2B27 medium (Invitrogen), Sonic Hedgehog (0.1 μg/μl), and FGF8 (100 ng/ml) (both from Peprotech), and maintained for 3–5 weeks in the absence of FGF2.

**Human specific mitochondrial PCR amplification**

Human CD45+ and CD34+CD45+ cells isolated using a FACSarias II (Becton Dickinson) from mice transplanted with 2000 and 10000 teratoma-HSPCs, respectively. Human CD34+CD45+ cells isolated from mice transplanted with human cord blood-HSPCs were used as positive controls. For the analysis, fragments that include D-loops were chosen in such a way that their length could be easily distinguished by agarose gel electrophoresis. Human primers are
15760F37-CGGAGGACAAACCAGTAAGCTACCTTTTTACCATT and 780R36-GAGCTGCATTGCTGCGTGCTTGATGCTTGTTCCTTT creating a 1589 bp length amplicon. Murine primers are 15197F36-GGCCAAACTAGCCTCCATCTCATACTTCTCAATCATC and 147R38-GGGATTATACACCGGTCTATGGAGGTTTGCATGTGTAA creating a 1250 bp length amplicon. An ExTaq Takara amplification system was used with the following PCR protocol: initial denaturing at 94°C for 1 min, followed by 45 cycles of 94°C for 20 sec and 68°C for 2 min. PCR products were analyzed by 2% agarose gel electrophoresis with ethidium bromide staining. FACS sorted cells were pelleted by spinning and lysed with 2 ul solution containing SDS, EDTA and proteinase K in a humidified chamber at 37°C. Cell lysates were diluted 100 times and subjected to PCR multiple times with murine or human primers in parallel.
Amabile_Supplemental Figure 1

- **Muscle cells**: (Mesoderm)
- **Neuro-epithelium**: (Ectoderm)
- **Respiratory epithelium**: (Endoderm)

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**Images**

- **H**: SMA
- **I**: α-fetoprotein
- **J**: Tuj1
- **K**: Keratinocytes
- **L**: iPS-Ker
- **M**: H1 hES

**Graphs**

- **REX1**, **OCT4**, and **NANOG**
- **CRIPTO**, **SOX2**

- Relative expression values from 0 to 90

**Colors**

- Keratinocytes: blue
- iPS-Ker: green
- H1 hES: orange
Amabile Supplemental Figure 2

A

B

C

Amabile_Supplemental Figure 2
**Amabile_Supplemental Figure 3**

**A**

- **Isotype Control**
  - hCD45: 0%
  - mCD45: 0%

- **Cord Blood**
  - hCD45: 99.99%
  - mCD45: 0.01%

- **Teratoma**
  - hCD45: 0.01%
  - mCD45: 0.01%

**B**

**Total number of teratomas analyzed in each category**

<table>
<thead>
<tr>
<th>Category</th>
<th>No OP9</th>
<th>OP9</th>
<th>OP9W3A</th>
<th>OP9D</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM Control Mice</td>
<td>14</td>
<td>10</td>
<td>14</td>
<td>10</td>
</tr>
</tbody>
</table>

**Number of teratomas which did not give rise to hCD45+ in each category**

<table>
<thead>
<tr>
<th>Category</th>
<th>No OP9</th>
<th>OP9</th>
<th>OP9W3A</th>
<th>OP9D</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM Control Mice</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**C**

- **Isotype Control**
  - hCD45: 0%
  - mCD45: 0%

- **BM Control Mice (depleted of murine cells)**
  - hCD45: 91.7%
  - mCD45: 8.0%

**D**

**Control mice**

- **BM-Ter**
  - hCD45: 0.2
  - hCD34: 0.1

- **Spleen-Ter**
  - hCD45: 0.2
  - hCD34: 0.0

- **SSC-A**
  - hCD15: 11.4
  - hCD19: 5.2
  - hCD3: 0.2

- **Total number of teratomas analyzed in each category**
  - No OP9: 14
  - OP9: 10
  - OP9W3A: 14
  - OP9D: 10

- **Number of teratomas which did not give rise to hCD45+ in each category**
  - No OP9: 3
  - OP9: 3
  - OP9W3A: 1
  - OP9D: 1
Antigen + Freund's Adjuvant

7 days

Antigen

15 days

Sorting

CD45+CD19+

2 days

ELISA assay for total and antigen-specific IgG content

Amabile_Supplemental Figure 4