Theranostic effect of serial MEMRI on the hESC induced teratoma

J. Chung[1], R. Dash[1], K. Kee[2], J. Barral[3], I. Weissmann[4], D. Nishimura[3], R. Robbins[5], R. Reijo Pera[2], and P. C. Yang[1]

[1]Cardiovascular medicine, Stanford University, Stanford, CA, United States, [2]Stem cell biology and regenerative medicine, Stanford University, Stanford, CA, United States, [3]Electrical engineering, Stanford University, Stanford, CA, United States, [4]Pathology, Stanford University, Stanford, CA, United States, [5]Cardiothoracic surgery, Stanford University, Stanford, CA, United States

Background: Human embryonic stem cells (hESCs) have shown the pluripotency to differentiate into any type of human cells. Several animal studies have reported significant potential to restore or regenerate damaged organs by hESC therapy. Although hESCs hold tremendous therapeutic potential, unwanted teratoma formation has deterred clinical translation. Teratoma formation may do more harm than benefit. In order to address this critical issue, sensitive *in vivo* detection of early hESC-induced teratoma formation by manganese enhanced MRI (MEMRI) has been demonstrated. Manganese (Mn^{2+}) enters metabolically active cells through a voltage-gated calcium channel and, subsequently, induces T_1 shortening effect. In this study, we hypothesized that a serial *in vivo* MEMRI will have theranostic effect on the teratoma cells. *In vivo* MEMRI will detect and selectively eliminate the hESC-induced teratoma cells due to the intracellular accumulation of Mn^{2+} by the metabolically active teratoma cells relative to the normal surrounding cells.
Methods: The therapeutic dosage of $MnCl_2$ was optimized to minimize any systemic toxicity with serial intraperitoneal administration in severe combined immunedeficient (SCID) mice (n=20). $1X10^6$ of firefly luciferase transduced hESCs (hESC-Luc) were transplanted into SCID mouse hindlimbs (n=12). Chemo group (n=6) was injected with 500 µl of 5 mM $MnCl_2$ intraperitoneally (IP) every other day. Control group (n=6) was given $MnCl_2$ only prior to MEMRI. Longitudinal *in vivo* evaluation by MEMRI and bioluminescence imaging (BLI) was performed on post-transplant week 2, 4, 6 and 8. For MEMRI, all the mice were injected with 250 µl of 5 mM $MnCl_2$ IP 15 minutes prior to imaging. Under general anesthesia, both hindlimbs were scanned using Signa 3.0 T Excite HD scanner (GE Healthcare system, Milwaukee, WI) with a customized small surface coil using T1 weighted spin echo inversion recovery (SE IR) sequence: TR 600 ms, TE 5 ms, TI 120ms, NEX 1, FOV 3, matrix 128X128. MEMRI data was analyzed for CNR and 3 dimensional volume using image J (NIH, Bethesda, MD). Concurrent BLI was performed using IVIS spectrum (Caliper, Mt. view, CA) and averaged luciferase activity was measured using Living image 2.5 (Caliper, Mt. view, CA). At post-transplant week 6 and 8, 3 mice in each group were sacrificed for histology. Teratoma samples were routinely processed for H&E staining.
Results: All mice tolerated Mn^{2+} chemotherapy. None of the mice showed any signs of distress during the follow up period. The chemo group showed significant reduction in the volume of teratoma compared with the control group (9.4±4.2 vs. 16.8±1 at 6 weeks, 64±49 vs. 123±70 at 8 weeks, $p<0.05$, Fig. 1A, B). BLI demonstrated significantly decreased luciferase activity in the chemo group compared with the control group at each time point ($p<0.05$, Fig. 2A, B). H&E staining showed significantly increased dead cells within the teratoma in the chemo group compared with the control group (Fig. 2C, D).
Conclusion: Systemic administration of $MnCl_2$ enabled simultaneous monitoring and selective elimination of hESC induced teratoma cells by higher intracellular accumulation of Mn^{2+}. This is the first study to demonstrate MEMRI has a theranostic effect in both detecting and eliminating early teratoma formation.

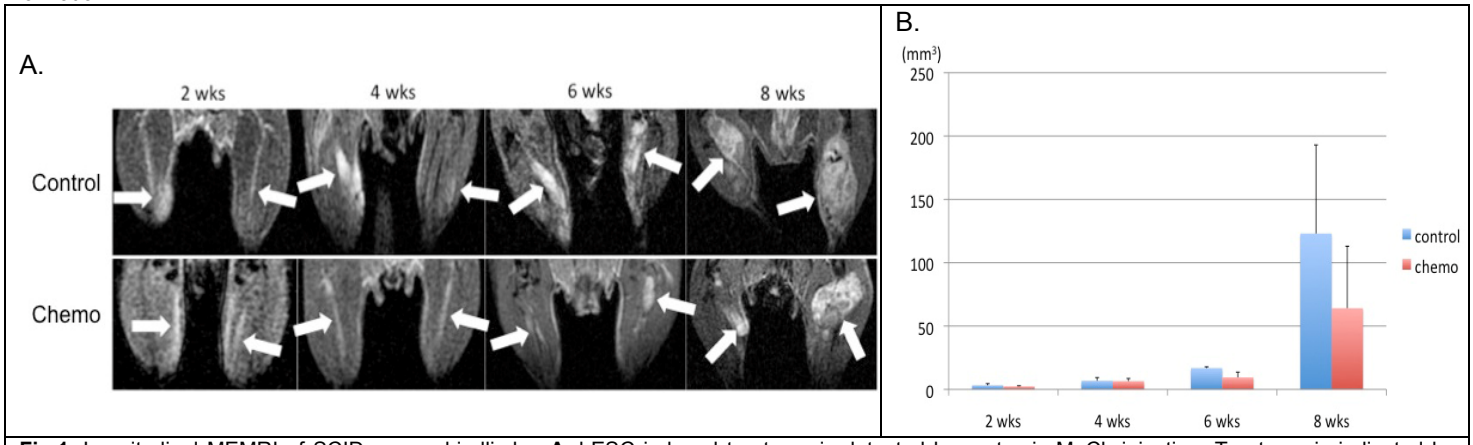

Fig.1. Longitudinal MEMRI of SCID mouse hindlimbs. **A.** hESC induced teratoma is detected by systemic $MnCl_2$ injection. Teratoma is indicated by white arrows. **B.** 3 dimensional volume obtained from MEMRI shows significant reduction in the volume of teratom in chemo group compared with the control group in 6 and 8 weeks ($p<0.05$).

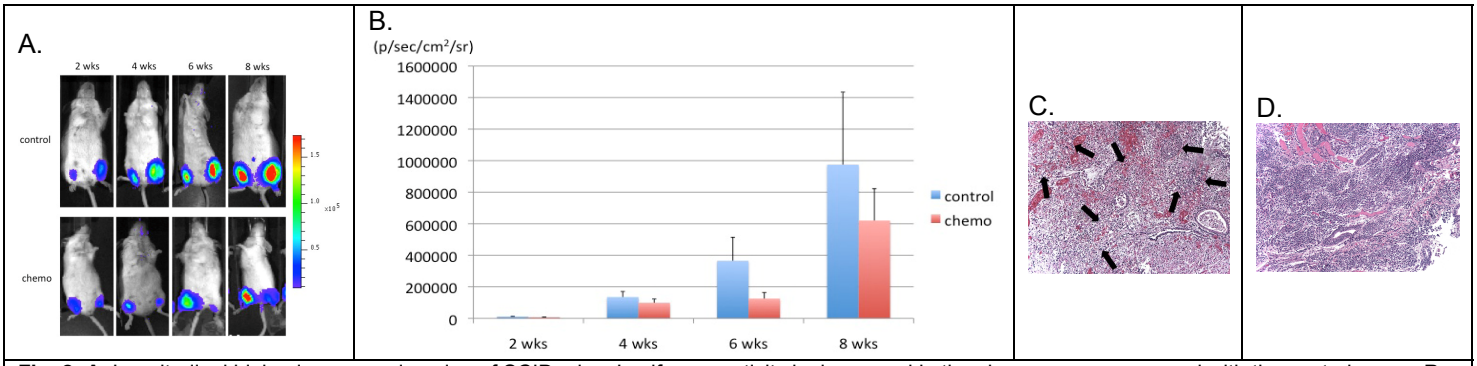

Fig. 2. A. Longitudinal bioluminescence imaging of SCID mice. Luciferase activity is decreased in the chemo group compared with the control group. **B.** Averaged luciferase activity is significantly reduced in the chemo group compared with the control group at each time point ($p<0.05$). **C.** H&E staining of the chemo group shows dead cells noted as dark and small nuclei are diffusely scattered with focal interstitial hemorrhages indicated by black arrows. **D.** H&E staining of the control group does not show dead cells but viable teratoma cells.