Efficacy and Immunogenicity of Induced Pluripotent Stem Cell (iPSC) Vaccine is Independent of iPSC Viability in a Prophylactic Mouse Model of Breast Cancer

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Abstract 414

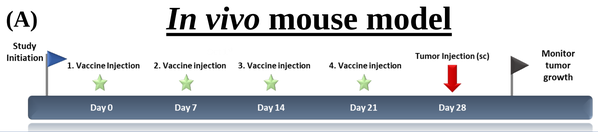
Introduction

- Extensive data on gene expression, metabolic state and glycosylation of cancer cells suggest that cancer represents a reversion of adult cells to an embryonic state and that induced pluripotent stem cells (iPSC) phenocopy this state. In contrast to cancer cells, iPSC have never undergone immunoediting and therefore present hundreds of oncofetal antigens in their native conformations.
- Previous studies have demonstrated the efficacy of vaccination with live (freshly harvested) syngeneic iPSC in a variety of cancer models.
- In this study, we administered vaccines comprising live or dead iPSC either in PBS or in the cryopreservative CryoStor CS10 together with the Toll-like receptor (TLR) 9 agonist CpG1826 (CpG) as an adjuvant and compared their immunogenicity and preclinical efficacy in a prophylactic mouse model of breast cancer.

Methods

- FVB mice (female, 6-8 weeks old) received a course of 4 weekly s.c. injections with (i) live, freshly harvested syngeneic iPSC in PBS; (ii) live, cryopreserved iPSC in CryoStor (CS); (iii) dead iPSC in PBS; and (iv) dead iPSC in CS (each admixed with 1 nmol CpG) or (v) CpG alone as control. All iPSC were irradiated with 60 Gy and the dose per injection was 10^7 cells. Cell death was induced by three freeze-thaw cycles between -195 °C and 37 °C and confirmed by Trypan Blue staining.
- One week after the 4th treatment, serum was obtained for IgG binding studies and 5×10^5 DB7 syngeneic breast cancer cells were injected into the lower right flank s.c. Tumor size was measured as $V=L*W^2$ and monitored for 24 days post tumor cell injection.

(A)



Results (*in vivo*)

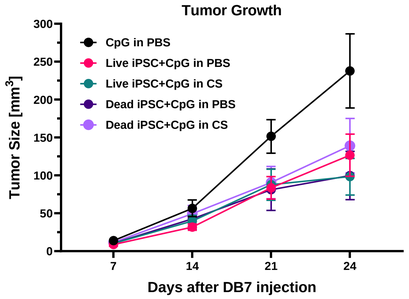


Figure 2: Live and dead iPSC vaccines either in PBS or in CryoStor CS10 (CS) equally delay tumor growth in breast cancer model. All four different iPSC+CpG vaccines significantly reduced tumor growth compared to the treatment with the adjuvant CpG alone (all P<0.03, 2-way ANOVA with Tukey's multiple comparisons test). No statistically significant difference was observed between live and dead iPSC or between iPSC applied in PBS and CryoStor (all P>0.74). n=10, mean with SEM.

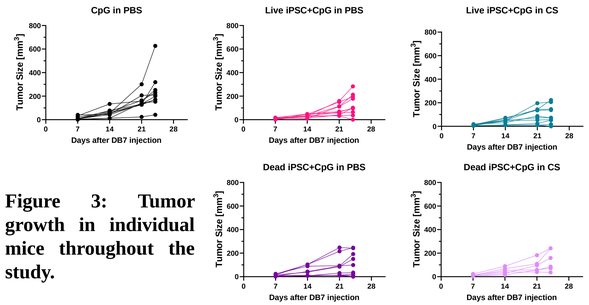


Figure 3: Tumor growth in individual mice throughout the study.

(B)

Group	FVB Mice [n]	Treatment	Dosage	Day 0	Day 7	Day 14	Day 21	Day 28	Endpoint
v	10	CpG	1 nmol CpG	CpG	CpG	CpG	CpG	Serum: 100 µL Tumor Injection: 5×10^5 DB7 cells s.c.	Tumor Growth until Day 24 after Tumor Cell Injection
i	10	Live iPSC+CpG in PBS	10^7 iPSC + 1 nmol CpG	Vac	Vac	Vac	Vac		
ii	10	Live iPSC+CpG in CryoStor	10^7 iPSC + 1 nmol CpG	Vac	Vac	Vac	Vac		
iii	10	Dead iPSC+CpG in PBS	10^7 iPSC + 1 nmol CpG	Vac	Vac	Vac	Vac		
iv	10 (6)	Dead iPSC+CpG in CryoStor	10^7 iPSC + 1 nmol CpG	Vac	Vac	Vac	Vac		

Figure 1: Overall schematic and timeline of the murine breast cancer study, *in vivo*. (A) Timeline of vaccinations for the mouse model. **(B)** Treatment groups studied in this mouse model.

Results (*ex vivo*)

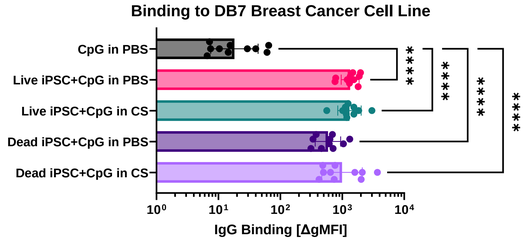


Figure 4: Live and dead iPSC vaccines either in PBS or in CryoStor CS10 (CS) are inducing an IgG immune response against the breast cancer cell line DB7. Serum (1:50 dilution) from one week after the fourth treatment, but before tumor cell injection was incubated with DB7 cells and binding measured with mouse IgG-Fcγ-specific antiserum by flow cytometry. All four iPSC vaccines induced highly significant larger (~100-fold) serum IgG binding to DB7 compared to the adjuvant CpG alone. No biologically relevant differences between the four different iPSC vaccine groups were detectable (maximum fold difference of 2.3). One-way ANOVA with Dunnett's multiple comparison test, n=10, mean with SD, ****, P<0.0001.

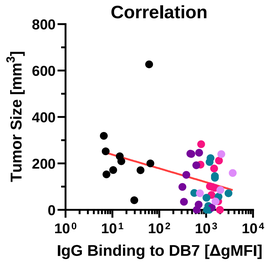


Figure 5: Inverse correlation between IgG binding to DB7 and tumor size. Statistical analysis revealed a Pearson r of -0.41 and a P value of 0.005.

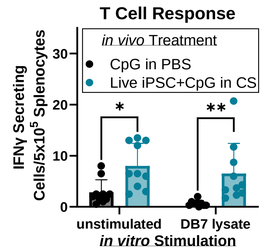


Figure 6: T cell response after vaccination with cryopreserved live iPSC. Increased IFNγ secretion was observed by splenocytes from iPSC treated animals directly ex vivo (unstimulated) and after stimulation with DB7 lysate in comparison to CpG only treated animals.

Mixed-effects analysis with Šídák's multiple comparisons test, *, P<0.05, **, P<0.01, n=10, mean with SD.

Immunogenicity Study

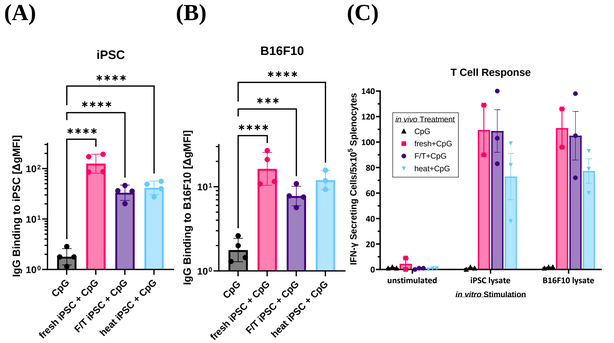


Figure 7: Live and dead iPSC vaccines are immunogenic in mice. In an independent study C57BL/6 mice were four times treated either with CpG (1 nmol) alone or with 10^7 iPSC in combination with CpG. C57BL/6 iPSC were harvested fresh, killed by three freeze/thaw (F/T) cycles or exposure to heat. One week after the fourth treatment mice were euthanized, and sera and spleens harvested. **(A)** Binding of serum IgG to fresh iPSC. **(B)** Binding of serum IgG to syngeneic melanoma cell line B16F10. All three iPSC vaccines induced significantly more IgG binding to iPSC and B16F10 than injection of CpG alone. One-way ANOVA with Dunnett's multiple comparison test, n=4, mean with SD, ***, P<0.001, ****, P<0.0001. **(C)** IFNγ ELISpot analysis revealed that live and dead iPSC vaccines in contrast to CpG induced activation of splenocytes. Mean with SEM, n=2-3.

Conclusions

- Antigen cross-presentation is independent of whether the iPSC are alive or dead.
- Cryopreservation of iPSC and viability have no negative impact on the immunogenicity and efficacy of our iPSC vaccine.
- This simplifies the manufacture, storage and shipment of the vaccine and eases clinical application.

Acknowledgement

The study was approved by Valley Bio Services' Institutional Animal Care and Use Committee; approval number VBS1002.

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