An induced Pluripotent Stem Cell (iPSC) Vaccine is Highly Immunogenic and Reduces Lung Metastases in a Mouse Model of Melanoma

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ABSTRACT

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) model this state. In contrast to cancer cells, iPSC have never undergone immunoediting and therefore present hundreds of oncofetal antigens in their native conformations. In this study, we administered a vaccine comprising syngeneic iPSC together with the Toll-like receptor (TLR) 9 agonist CpG1826 as an adjuvant and assessed its immunogenicity and preclinical efficacy in a mouse model of melanoma lung metastases with and without checkpoint inhibition.

C57BL/6 mice were immunized with $2x10^6$ irradiated (60 Gy) iPSC admixed with 500 pmol CpG, or with PBS or CpG alone as controls. Four immunizations were administered subcutaneously one week apart. Mice were challenged with $1x10^5$ B16F10 murine melanoma cells intravenously one week after the second immunization. After tumor cell injection some groups were also treated with anti-PD-L1 (clone B7-H1, 200 µg, 2x/week, i.p.). All mice were euthanized 19 days after intravenous B16F10 injection and lung metastases were counted in a blinded fashion. Cellular and humoral immune responses were measured by IFN-γ ELISpot, serum IgG binding to iPSC and B16F10 and flow cytometric analysis of splenocytes.

Treatment of mice with anti-PD-L1+CpG, iPSC+CpG and iPSC+CpG+anti-PD-L1 significantly reduced the number of lung metastases in comparison to CpG (One-way ANOVA with Dunnett's multiple comparisons test) (Table below). Immunization with iPSC+CpG was as effective as treatment with anti-PD-L1+CpG. No synergism of iPSC+CpG with anti-PD-L1 was detectable. Only immunization with iPSC+CpG induced a significant increase in IFN-γ spots after *in vitro* challenge with iPSC and B16F10 lysates in comparison to CpG. Comparable results were obtained for serum IgG binding to iPSC and B16F10. Percentage of regulatory T cells in the spleen was significantly reduced in iPSC+CpG and iPSC+CpG+anti-PD-L1 in comparison to CpG. Similar results were obtained in a second independent study.

Irradiated syngeneic iPSC admixed with TLR9 agonist CpG1826 in combination with or without checkpoint blockade induced T cell and antibody responses to iPSC and B16F10 thereby reduced the number of melanoma lung metastases in mice. These results warrant further investigation of autologous iPSC vaccines in clinical trials.

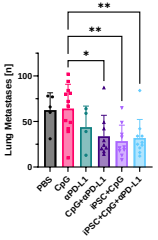
Treatment	PBS	CpG	αPD-L1	CpG+αPD-L1	iPSC+CpG	iPSC+CpG+αPD-L1
Median	66.0	69.5	49.0	24.0	21.5	26.0
Mean	62.4	64.3	43.8	33.7	28.4	31.9
SEM	8.5	7.7	11.6	7.7	5.6	6.4
N	5	12	4	9	10	10

STUDY DESIGN

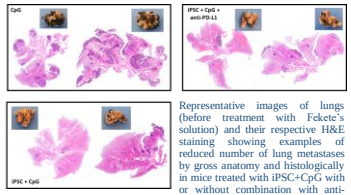
B16F10 iv Lung Metastasis Mouse Model

Group	# of mice	Treatment Groups	Dose	Tumor Cell
1	n=5	PBS (sc)	control	B16F10 Melanoma 100,000 cells iv injection
2	n=12	CpG (sc)	500 pmol	
3	n=5	anti-PD-L1 (ip)	200 µg 2x/wk.	
4	n=10	CpG + anti-PD-L1	500 pmol / 200 µg 2x/wk.	
5	n=10	iPSC + CpG (sc)	$2x10^6$ cells / 500 pmol	
6	n=10	iPSC + CpG + anti-PD-L1	$2x10^6$ cells / 500 pmol / 200 µg 2x/wk.	

LUNG METASTASIS

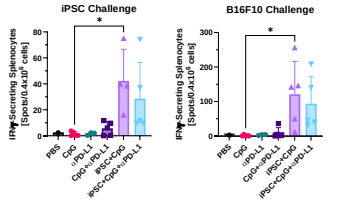


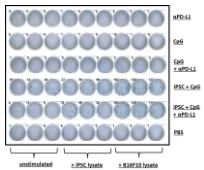
Mice were sacrificed on day 19 after tumor injection and lungs, spleens and serum obtained. Lungs were treated with Fekete's solution and lung metastases counted in blinded fashion. Treatment with CpG+anti-PD-L1, iPSC+CpG and iPSC+CpG+anti-PD-L1 reduced number of lung metastases significantly by around 50% in comparison to treatment with CpG alone. In addition to the total number of metastases, also the size of metastases was assessed (<1, 1-2 and >2 mm). The largest effect was found in metastases smaller than 1 mm (data not shown). No additive or synergistic effect by combining iPSC+CpG with anti-PD-L1 was observed. One-way ANOVA with Dunnett's multiple comparison test (*, p<0.05; **, p <0.01).



Representative images of lungs (before treatment with Fekete's solution) and their respective H&E staining showing examples of reduced number of lung metastases by gross anatomy and histologically in mice treated with iPSC+CpG with or without combination with anti-PD-L1.

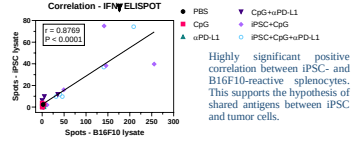
CELLULAR IMMUNE RESPONSE





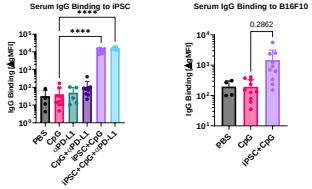
Splenocytes (400,000) were stimulated *in vitro* for 20 h with medium, iPSC and B16F10 lysates (35 µg). Only *in vivo* treatment with iPSC+CpG without or with anti-PD-L1 lead to *in vitro* induction of IFN-γ spots. The *in vivo* treatment with iPSC+CpG was significant. One-way ANOVA with Dunnett's multiple comparison test (*, p<0.05).

Representative IFN-γ Elispots are shown on the left.

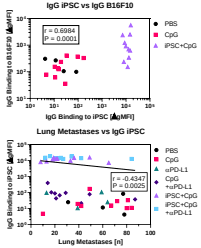


Highly significant positive correlation between iPSC- and B16F10-reactive splenocytes. This supports the hypothesis of shared antigens between iPSC and tumor cells.

HUMORAL IMMUNE RESPONSE



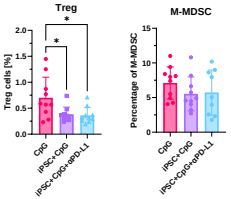
Serum (1:50 dilution) was incubated with iPSC and B16F10 and binding measured with IgG-specific antiserum by flow cytometry. Only treatments containing iPSC induced serum IgG specific for target cells. Binding to iPSC was highly significant. Binding to B16F10 was increased, but not statistically significant in this particular experiment. One-way ANOVA with Dunnett's multiple comparison test (****, p<0.0001).



Serum IgG binding to iPSC and B16F10 is highly correlated. Again, this supports the hypothesis of shared antigens between iPSC and tumor cells.

The number of lung metastases negatively correlated with serum IgG binding to iPSC. This suggests that induced iPSC and B16F10 specific IgG antibodies might be functionally relevant.

IMMUNOSUPPRESSIVE CELLS



Splenocytes were analyzed for frequency of regulatory T cells (as defined by CD3+, CD4+, CD25+ and FoxP3+ cells) and monocyte-myeloid derived suppressor cells (as defined as CD11b+, Ly6G-, Ly6C+ and F4/80 high).

The frequency of Treg was significantly reduced after iPSC+CpG treatment with or without combination with anti-PD-L1. Like Treg there was a reduction (albeit not statistically significant) of M-MDSC after iPSC+CpG treatment with or without combination anti-PD-L1.

The importance of the reduction of the frequency of these suppressor cell populations in the spleen for the reduction of lung metastases requires further investigation.

CONCLUSIONS

A mouse model of lung metastasis of melanoma with low sensitivity to checkpoint inhibition was chosen to investigate the efficacy of a syngeneic iPSC vaccine and its immunogenicity.

1. Anti-tumor efficacy:

Significant reduction of lung metastasis was observed after treatment with CpG+anti-PD-L1, iPSC+CpG and iPSC+CPG+anti-PD-L1.

2. Cellular immune response:

Significant increase of IFNγ-secreting splenocytes after *in vitro* challenge with iPSC and B16F10 lysates was shown after treatment with iPSC+CpG, but not with CpG+anti-PD-L1. Strong correlation between iPSC and B16F10 reactive splenocytes.

3. Humoral immune response:

Increase of serum IgG binding to iPSC (significant) and B16F10 after treatment with iPSC+CpG independent of anti-PD-L1. Significant correlation of IgG binding to iPSC and B16F10 and significant negative correlation between number of lung metastases and IgG binding to iPSC.

4. Immunosuppressive cells:

Reduction of the frequency of regulatory T cells and M-MDSC in the spleen after iPSC+CpG treatment independent of anti-PD-L1.

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