SCIB1 DNA vaccination synergises with PD-1 blockade to induce efficient tumor therapy of poorly immunogenic tumours

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Introduction

SCIB1 DNA vaccine

SCIB1, is a immunobody® DNA plasmid encoding a human IgG1 antibody that encodes melanoma-specific T cell epitopes from TRP2 and gp100. A clinical trial is currently running to determine safety, immunogenicity and tumour responses in melanoma patients.

- Only 5/16 patients have shown a recurrence of disease at 4, 14, 17, 18 and 30 months.
- All other patients have been disease-free for between 27 and 46 months since study entry.
- Median survival for stage III patients (n=9) is 34 months and 31 months for Stage IV patients (n=7).

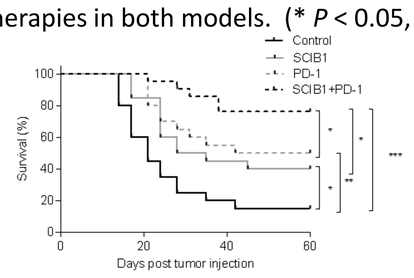
Checkpoint inhibitors

- Anti-PD-1 Ab enhances T cells activity and anti tumour immunity.
- PD-L1 expressed by tumours can limit the efficacy of T cell responses which can be recovered by blocking.
- Checkpoint inhibitor is importantly related to the type of mutation which can give rise to neoepitopes that can stimulate pre-existing memory responses to viral antigens.
- In this study, we look at the anti tumour effect of combining SCIB1 DNA vaccine with anti PD-1 Ab.

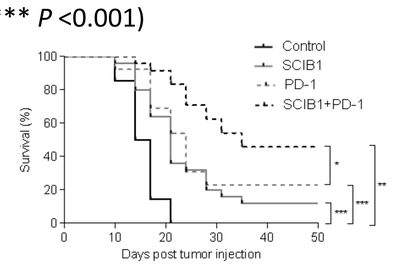
Results

1. Combined SCIB1 and PD-1 blockade generates strong anti tumour effect

- In both low and high tumour load model, SCIB1 and PD-1 Ab alone shows strong anti tumour effect. The combination vaccine further enhances long term survival compared to single therapies in both models. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)



B16DR4 tumour ($2.5x10^4$) was established subcutaneously in HLA-DR4 mice (day 1). SCIB1 bullets were given by genegun i.d. on day 4, 8 and 11. Anti PD-1 Ab (250µg) was given by i.p. immunisation on day 4 and 11.

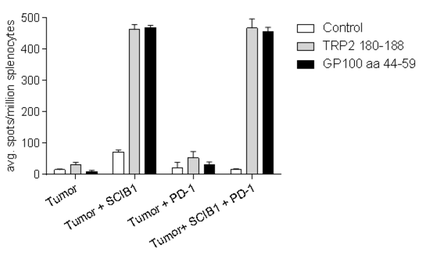


In higher tumour dose model ($1.5x10^5$ B16DR4), SCIB1 bullets were given by genegun i.d. on day 4, 8 and 11. Anti PD-1 Ab (250µg) was given by i.p. immunisation on day 4 and 11.

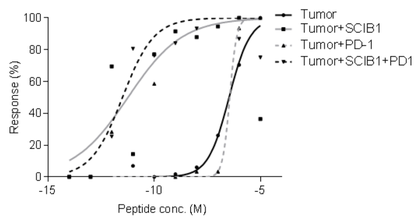
2. Tumour and PD-1 blockade does not influence SCIB1 induced responses

- SCIB1 DNA enhances frequency and avidity of TRP2 CD8 and frequency of gp100 CD4 responses ($P<0.05$), addition of PD-1 does not further enhance the responses ($P>0.05$).

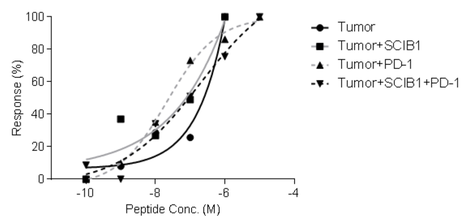
IFNγ frequency responses to TRP2



IFNγ avidity responses to TRP2



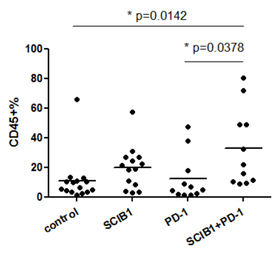
IFNγ avidity responses to gp100DR4



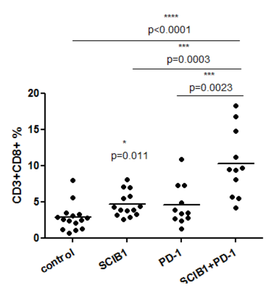
HLA-DR4 mice were challenged with B16DR4 tumour ($2.5x10^4$) on day 1. On day 4, 8 and 11, mice were given SCIB1 bullets via genegun. On day 4 and 11, mice receive anti PD-1 Ab (250µg) via i.p. immunisation. Splenocytes were analysed at day 18 for peptide specific IFNγ responses by Elispot assay.

3. Combined SCIB1 DNA and PD-1 blockade increases CD8 and CD4 T cells within the tumour microenvironment

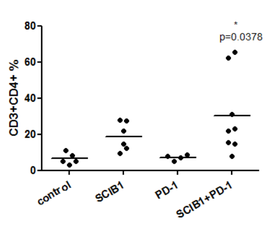
- When tumours reached 10mmx10mm, they were dissected and stained for T cell markers and analysed by flow cytometry.
- Combination vaccine increases CD8+ and CD4+ T cell infiltration into tumour environment.
- Combination vaccine shows increased proliferating CD8+ T cells within tumour environment.



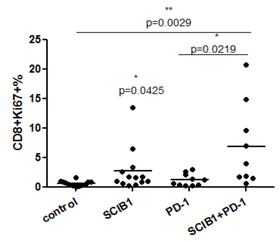
Combination of PD-1 blockade and SCIB1 DNA increases CD45+ cell infiltration into tumour



Combination vaccine increases CD8+ cell infiltration into tumour



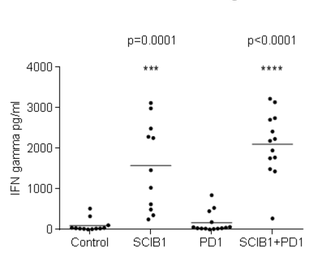
Combination vaccine increases CD4+cell infiltration into tumour



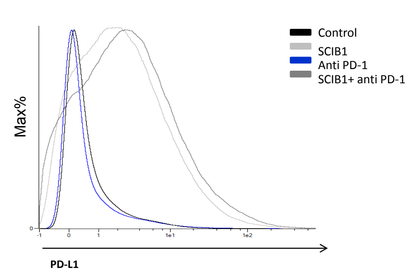
Combination vaccine increases CD8+ cell proliferation within tumour

4. SCIB1 and combination vaccine increased inflammatory cytokine production and PDL expression within tumour microenvironment

- Tumour infiltrating lymphocytes (TIL) were isolated and in vitro stimulated with anti-CD3 antibody overnight. IFNɣ release was measured by ELISA.
- TILs from both SCIB1 and SCIB1+PD-1 vaccinated mice show enhanced IFNɣ release.
- Both SCIB1 and SCIB1+PD-1 enhance PD-L1 expression on tumour which highlights the importance of combining anti PD-1 Ab with vaccine.



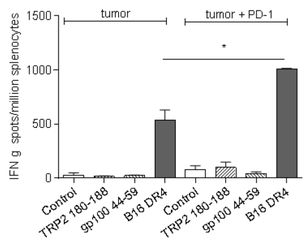
IFNɣ ELISA showing SCIB1 and SCIB1+PD-1 vaccinated mice with enhanced IFNɣ release from TILs.



PDL expression on B16DR4 tumour

5. Anti-PD-1 Ab alone enhances tumour recognition

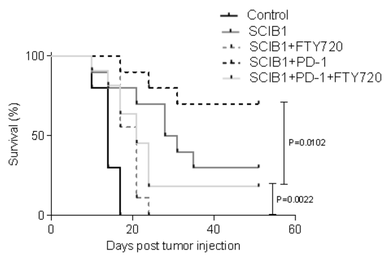
- Anti PD-1 Ab enhances *in vitro* tumour recognition of B16DR4 tumour cells but does not enhance TRP2 and gp100 specific responses which suggests that PD-1 blockade can enhance responses to other tumour specific antigens.



HLA-DR4 mice were given B16DR4 tumour ($2.5x10^4$) on day 1. Anti PD-1 Ab (250 µg) was given i.p. on day 4 and 11. Splenocytes were analyzed at day 18 by IFNɣ elispot.

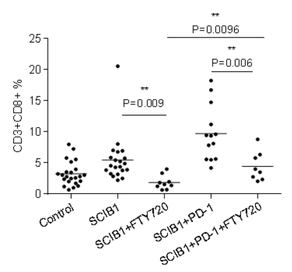
6. Anti tumour effect of SCIB1 DNA combined with PD-1 blockade is dependant on T cell migration however PD-1 blockade has effect on T cells within tumour environment

- FTY720, an immunomodulating drug that prevent lymphocytes migration, effectively inhibited survival of SCIB1 or SCIB1+PD-1 treated groups (P= 0.0094; P= 0.01).
- However, this inhibition is not completely effective as FTY720 treated SCIB1 or SCIB1+PD-1 groups still show survival advantage over the control (P=0.0006; P=0.0022).

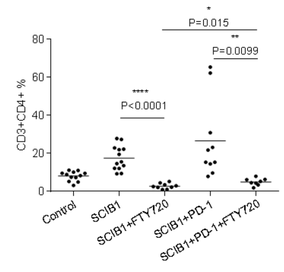


HLA-DR4 mice were challenged with B16DR4 cells on day 1 and then immunised with SCIB1 bullets via genegun on day 4, 8 and 11. Anti PD-1 Ab (250µg) was administered i.p. on day 4 and 11. 200µg FTY720 were administrated 4 hours before immunisation on day 4, 8 and 11.

- FTY720 completely blocks CD8 and CD4 T cell infiltration into tumours in SCIB1 or SCIB1 plus PD-1 vaccinated mice.
- There is a significant difference in CD8 and CD4 tumour infiltration between FTY720 treated SCIB1 and SCIB1 plus PD-1 vaccinated mice (P=0.0093; P=0.0154). This suggests that PD-1 can partially reverse the inhibitory effect of FTY720.



CD8 TIL analysis



CD4 TIL analysis

Conclusions

- Combination of SCIB1 DNA and PD-1 blockade results in an additive anti-tumor effect with 85% long term survival in a pre-clinic animal model.
- This is associated with increased CD8+ and CD4+ T cell infiltrate and increased number of functional T cells capable of secreting IFN-γ at the tumor site.
- We observed that vaccination with SCIB1 or SCIB1 plus PD-1 blockade both upregulate PD-L1 levels in the tumor microenvironment which may suppress T cell mediated anti tumour responses which highlight the importance of combining anti-PD-1 Ab with vaccine.
- Addition of PD-1 blockade does not have an effect on SCIB1 induced responses but enhances *in vitro* tumour recognition which indicates that PD-1 might amplify other tumour antigen specific T cell responses. These will be assessed in future studies.
- T cell migration was required for SCIB1 DNA to generate efficient anti tumor response. However, blockade of T cell migration has less effect when SCIB1 is combined with PD-1 blockade which suggests that PD-1 blockade plays a role within the tumor environment.