SCIB2 targets NY-ESO-1 epitopes to induce potent anti-tumour immunity which is enhanced by Treg depletion or checkpoint blockade

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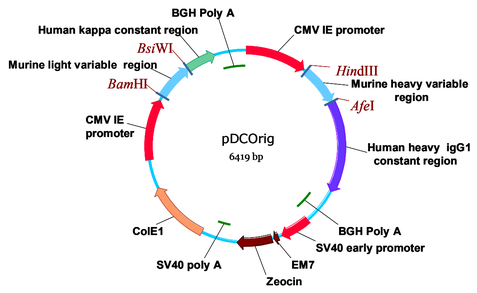
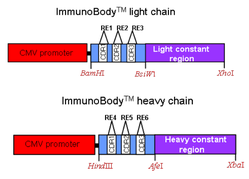
Introduction

Immunobody

ImmunoBody expression vector system





- CDR regions have been replaced with unique restriction sites (RE1-6) within single chain vectors. These are used to open up the DNA.
- Complimentary primers encoding the epitope are annealed and inserted in exchange of a CDR.
- Variable heavy and light regions containing epitopes are transferred into the double expression vector pDCOrig in frame with their human IgG1 or kappa constant regions.
- ImmunoBody® vector targets the high affinity FcγR1 receptor (CD64) on activated dendritic cells via huIgG1.

SCIB2

We have shown that immunobody® DNA incorporating CTL and helper epitopes stimulate high avidity responses and inhibit tumour growth. In this study, four NY-ESO-1 regions, which encoded multiple T cell epitopes and cover HLA type of 82-90% patients, were incorporated into CDR region of immunobody® (SCIB2) (Table 1). We then assessed the T cell responses and anti-tumour activity generated by SCIB2.

Table1 NY-ESO-1 CD8 and CD4 epitopes Inserted into the ImmunoBody™ vector

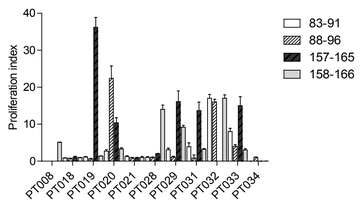
Site	CO-ORDINATES	SEQUENCE	HLA RESTRICTION	HLA Frequency (%)
H1	158-166	LLMWITQCF	A24	41
H2	157- 165	SLLMWITQC	A2	42
H3	83-111	PESRLLEFYLAMPFATPMEAELARRS LAQ	A1/ B44 / Cw3/ Cw6/ B35 / B51/ DR1/4/7/9/ DP4	2/20/28/19/14/8/ 12/41/11/16/21
L1	119-143	PGVLLKEFTVSGNILTIRLTAADHR	DR52b/1/4/7	50/12/41/12

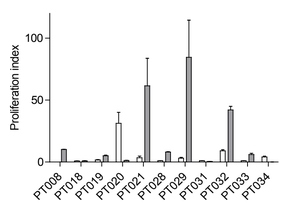
Checkpoint inhibitors

Checkpoint inhibitors are promising, with 20-30% of patients responding to Ipilumamab (CTLA-4 blockade) and to PD1/PDL-1 blockade. However, not all melanoma patients respond and response in other cancers are limited. It is suggested that checkpoint inhibitors can unleash T cells to mutated neo-epitopes that stimulate pre-existing high avidity memory responses to viral antigens. Here, we utilised combination of a vaccine that stimulates high avidity CD8 and CD4 responses with checkpoint inhibitors to maximise the anti tumour effect.

Results

1. Melanoma patients demonstrate NY-ESO-1 specific responses



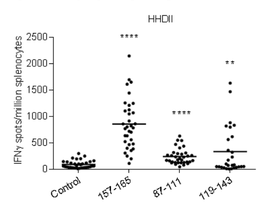


Proliferation assays showed a number of donors had pre existing NYESO-1 specific responses.

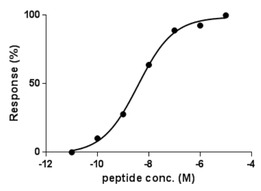
2. SCIB2 DNA stimulates strong CD8 and CD4 antigen specific responses in HHDII transgenic mice

- HHDII Mice received SCIB2 DNA bullets via genegun immunisation on day 1, 8 and 15. Splenocytes were analysed on day 22 by IFNγ elispot.
- SCIB2 stimulates high frequency CD8 and CD4 responses and high avidity CD8 responses.
- SCIB2 stimulates significant amount of granzyme B to NY-ESO-1 CD8 and CD4 epitopes. CD4 responses could be blocked by MHCII Ab.

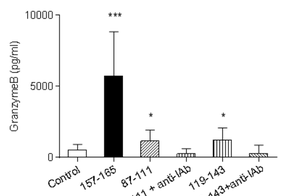
IFNɣ frequency responses to NYESO 157 87-111 and 119-143



Avidity responses to NYESO 157



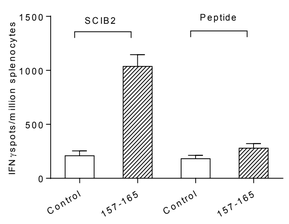
Granzyme B responses to NEYSO 157, 87-111 and 119-143



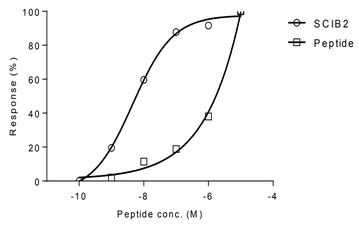
3. SCIB2 DNA stimulates better responses than peptide vaccine

- SCIB2 generates higher CD8 frequency and avidity responses than peptide vaccine ($P<0.05$)
- Only CTL from mice immunised with SCIB2 can recognize naturally processed and presented tumour targets.

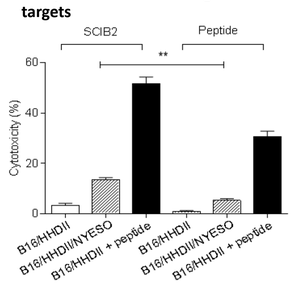
Comparison of IFNɣ frequency responses to NY-ESO-1 157



Comparison of IFNɣ avidity to NY-ESO-1 157

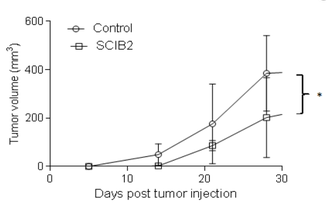


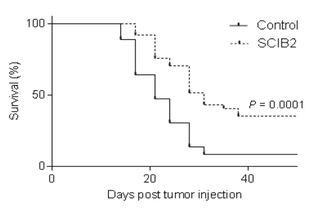
Comparison of CTL killing over tumour targets



4. SCIB2 DNA generates strong anti-tumour immunity

- SCIB2 DNA vaccination significantly delays the tumour growth ($P<0.05$) and 35% of mice remain tumour free with long term survival ($P=0.0001$).



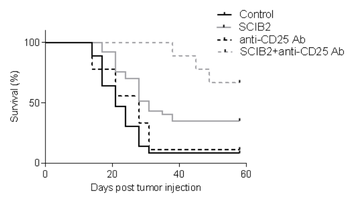


HHDII mice were challenged with $2.5x10^4$ B16/HHDII/NYESO on day 1, then was immunised with SCIB2 bullets by genegun on day 4, 11 and 18.

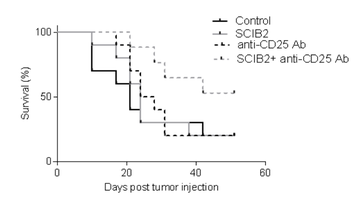
SCIB2 and Treg depletion

5. Treg depletion enhances anti tumour effect of SCIB2

- In lower tumour dose model ($2.5x10^4$ tumour cells), Treg depletion alone is not sufficient for tumour rejection, however, SCIB2 alone demonstrates enhanced survival. In combination with Treg depletion tumour growth is further inhibited with 64% of mice showing long term tumour free survival.
- In higher tumour dose model ($1.5x10^5$ tumour cells), neither Treg depletion nor SCIB2 alone shows any survival advantage. However, SCIB2 shows synergy with Treg depletion with 50% long term survival.



B16/HHDII/NYESO tumour ($2.5x10^4$) was established subcutaneously in HHDII mice (day 1). Anti CD25 Ab (400µg) was administrated i.p concurrent with tumour implant. SCIB2 bullets were given by genegun i.d. on day 4, 11 and 18.

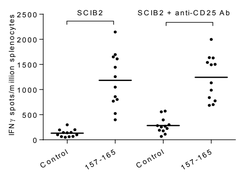


In higher tumour dose model ($1.5x10^5$), anti CD25 Ab (400µg) was administrated i.p concurrent with tumour implant. SCIB2 bullets were given by genegun i.d. on day 4, 11 and 18.

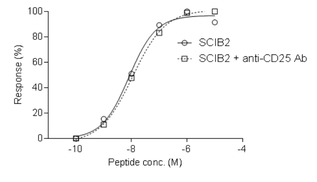
6. Treg depletion has no effect on T cell responses generated by SCIB2

- Similar frequency and avidity induced by SCIB2 was observed in the presence and absence of Treg depletion
- The cytotoxicity by SCIB2 induced CTL is similar in the presence and absence of Treg depletion.

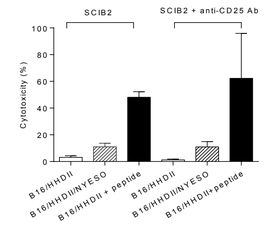
IFNɣ frequency responses to NY-ESO-1 157



IFNɣ avidity responses to NY-ESO-1 157



CTL killing over tumour targets



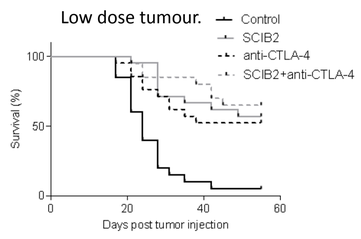
Anti CD25 Ab (400µg) was administrated to HHDII mice on day 1. SCIB2 bullets were given by genegun i.d. on day 4, 11 and 18.

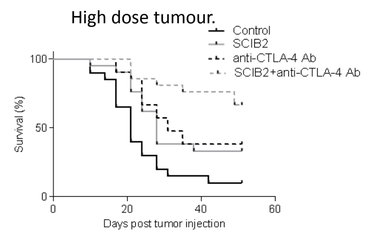
Splenocytes from immunised mice were *in vitro* cultured with NY-ESO-1 157 peptide for 6 days. CTL killing were performed over tumour targets.

SCIB2 and checkpoint inhibitor

7. Anti CTLA-4 antibody treatment enhances the anti tumour effect of SCIB2

- In lower tumour dose model, SCIB2 or anti CTLA-4 alone significantly delays tumour growth and combination vaccine only slightly enhances survival with 65%.
- In high tumour dose model, combination vaccine significantly enhances survival with 67% compared to single therapies.

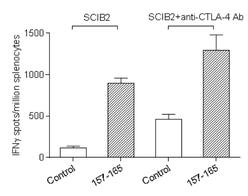




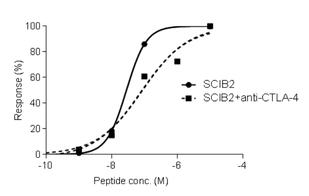
B16/HHDII/NYESO tumour ($2.5x10^4$ or $1.5x10^5$) was established subcutaneously in HHDII mice (day 1). SCIB2 bullets were given by genegun i.d. on day 4, 8 and 11. Anti CTLA4 Ab (200µg) was given by i.p. injection on day 4 and 11.

8. Anti CTLA-4 enhances CTL killing induced by SCIB2

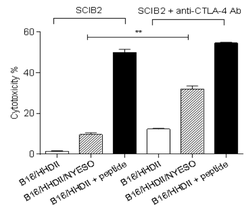
- Anti CTLA-4 does not significantly enhance frequency and avidity of T cell response, However, it significantly enhances tumour killing of target expressing NY-ESO-1 when compared to SCIB2 alone immunised mice.



IFNɣ frequency responses to NYESO 157 were compared from mice immunized with SCIB2 or SCIB2 plus anti CTLA-4 Ab



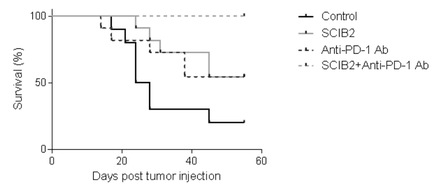
Comparison of IFNɣ avidity responses from mice immunized with SCIB2 or SCIB2 plus anti CTLA-4 Ab



Comparison of CTL killing over tumour targets by SCIB2 or SCIB2 plus anti CTLA-4 immunized mice

9. Anti PD-1 Ab enhances anti tumour effect of SCIB2

- Anti PD-1 Ab and SCIB2 alone delays tumour growth with long term survival of more than 50%, this is further enhanced to 100% of survival when these modalities are combined.



B16/HHDII/NYESO tumour ($5x10^4$) was established subcutaneously in HHDII mice (day 1). SCIB2 bullets were given by genegun i.d. on day 4, 8 and 11. Anti PD-1 Ab (250µg) was given by i.p. injection on day 4 and 11.

Conclusions

- SCIB2 DNA stimulates strong CD8 and CD4 antigen specific responses that produce IFNɣ and granzyme B. These strong immune responses lead to anti tumour immunity.
- SCIB2 DNA generates better immune responses than peptide vaccine which are capable of lysing NYESO-expressing tumour targets *in vitro*.
- Treg depletion has no effect on the SCIB2 induced immune responses whereas CTLA-4 blockade further enhances the cytotoxicity of CD8 cells.
- When SCIB2 is given in combination with Treg depletion, CTLA-4 blockade or PD-1 blockade, long term survival from established tumours is significantly enhanced to 64, 67 and 100% respectively.