Journal Club: Current Topics in Applied Immunology

Ex vivo modelling of PD-1/PD-L1 immune checkpoint blockade under acute, chronic, and exhaustion-like conditions of T-cell stimulation

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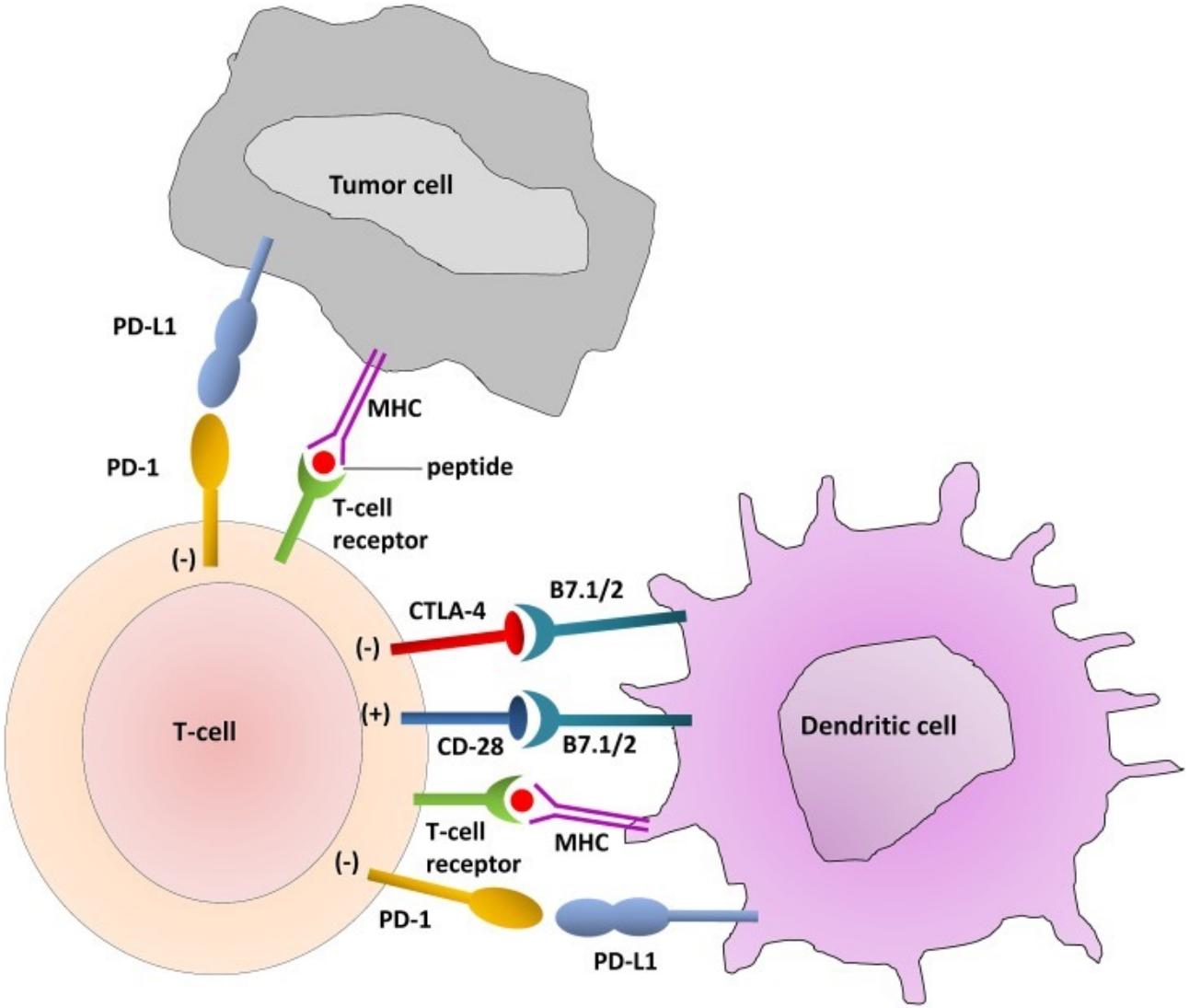
Comparative Medicine

Background



Zaravinos A, 2014, Oncotarget

Aim of the study

To study the impact of checkpoint inhibitors e.g., anti-PD-1 antibody (pembrolizumab) on the activation of human T cells through assessment:

- **The release of pro-inflammatory IFNγ**
- **The release of anti-inflammatory IL-10**

Materials and Methods

Generation of DCs: isolated monocytes from PBMCs were cultured in complete RPMI + IL-4 + GM-CSF for 6 days

Generation of mature DC (mDCs): DCs were cultured for further 2 days with IL-1β + IL-6 + TNFα + PGE2.

1st: Co-culture of T cells with allogeneic DCs: T cells were cultured with either iDCs or mDCs in the presence of α-PD-1 (pembrolizumab), IgG4 isotype control, α-CTLA-4 (ipilimumab), or IgG1 isotype control for 4 days.

2nd: EBV peptide stimulation of PBMCs: PBMCs were stimulated with an EBV peptide pool (EBNA-1) in the presence of α-PD-1 or IgG4 isotype control for 7 days (chronic conditions of stimulation).

Materials and Methods

3rd: To assess the effect of pembrolizumab on long-term stimulated CD4+ T cells:

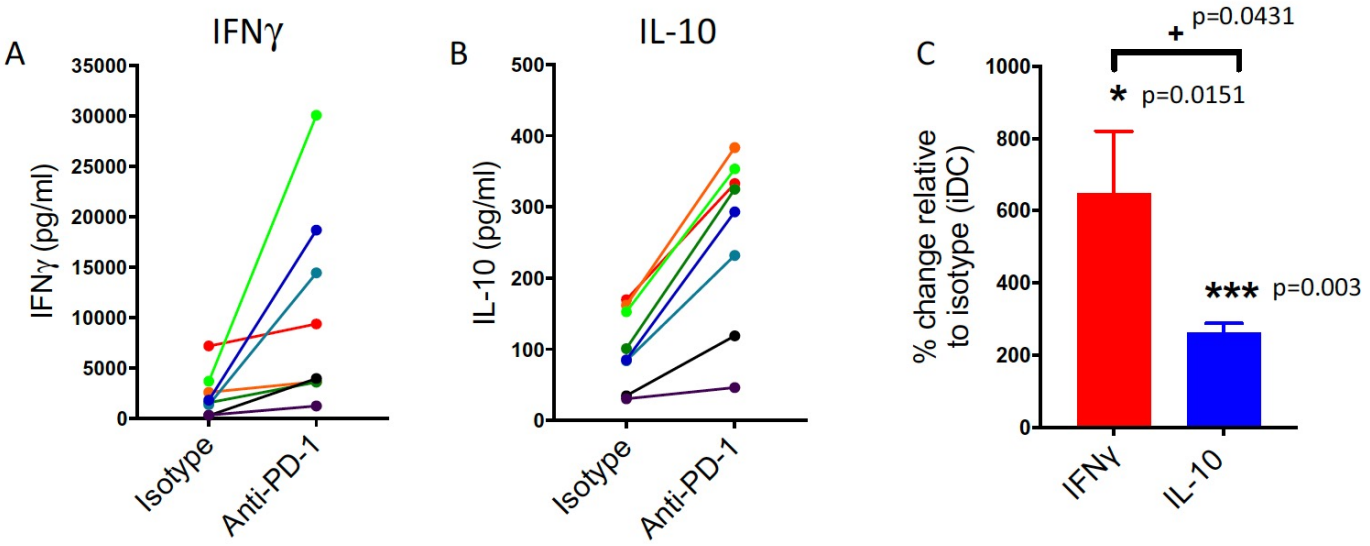
First: CD4+ T cells were stimulated with PHA for 14 days and the expression of exhaustion markers on chronically stimulated T cells were determined.

Second: unstimulated and PHA-stimulated CD4+ T cells were cultured with either iDCs or mDCs in the absence or presence of α-PD-1, IgG4 isotype control, α-CTLA or IgG1 isotype control.

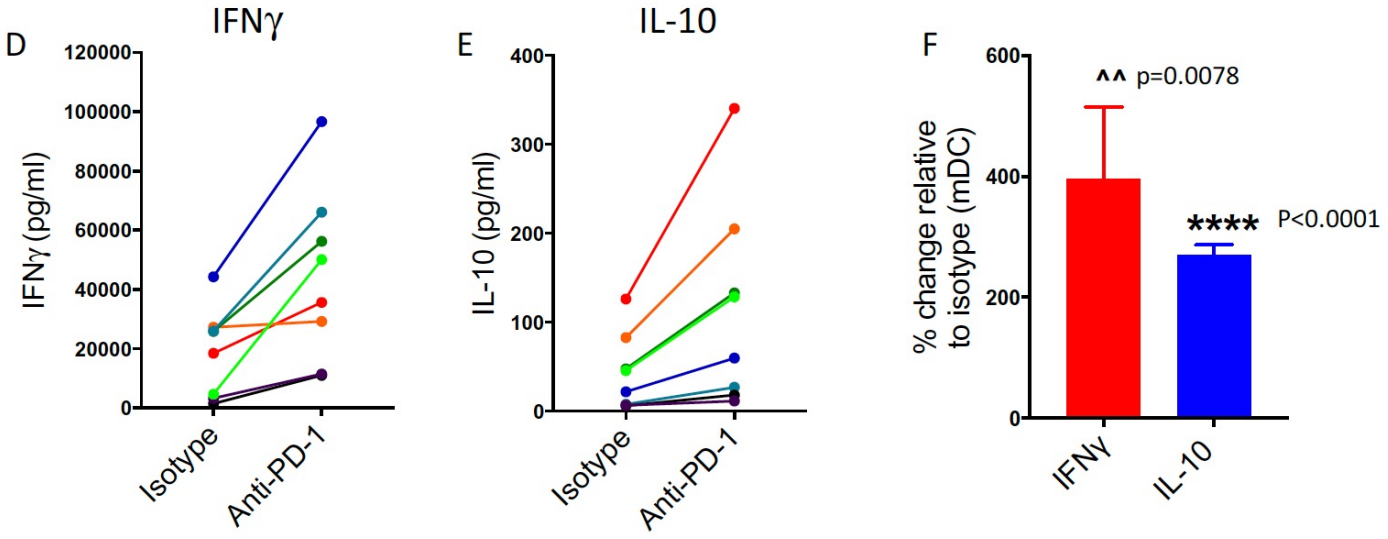
4th: Dissociated tumour cell experiments: Dissociated cells from solid tumours (either lung or colon tumours) were cultured with allogeneic DCs in the presence of α-PD-1 or IgG4 isotype control for 2 days.

Results: α-PD-1 enhanced IFNγ and IL-10 release from purified CD4+ T cells

Cultured with iDCs

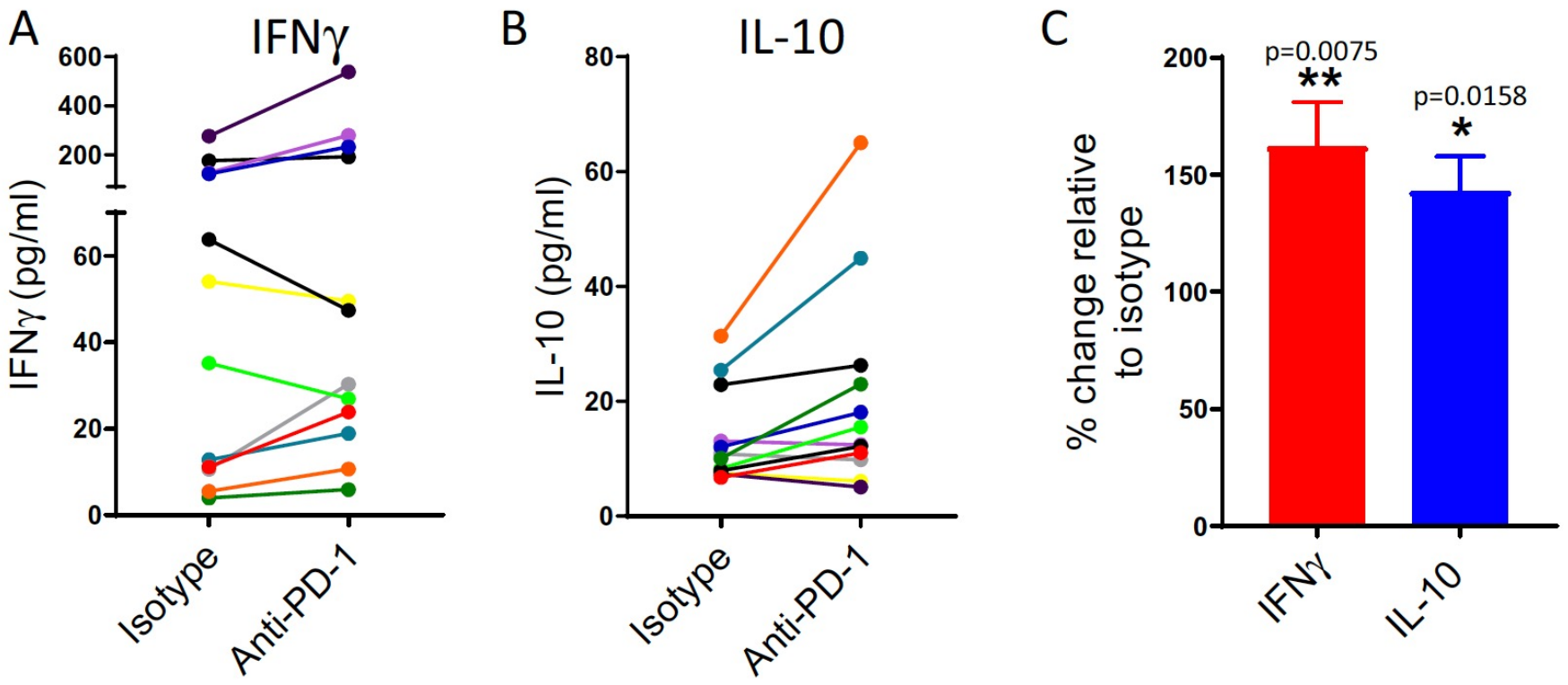


Cultured with mDCs



α-PD-1 increases IFNγ and IL-10 levels in cultures of PBMCs stimulated with EBV peptides

Methods: PBMCs were stimulated with an EBV peptide pool in the presence of α-PD-1 or IgG4 isotype control for 7 days.



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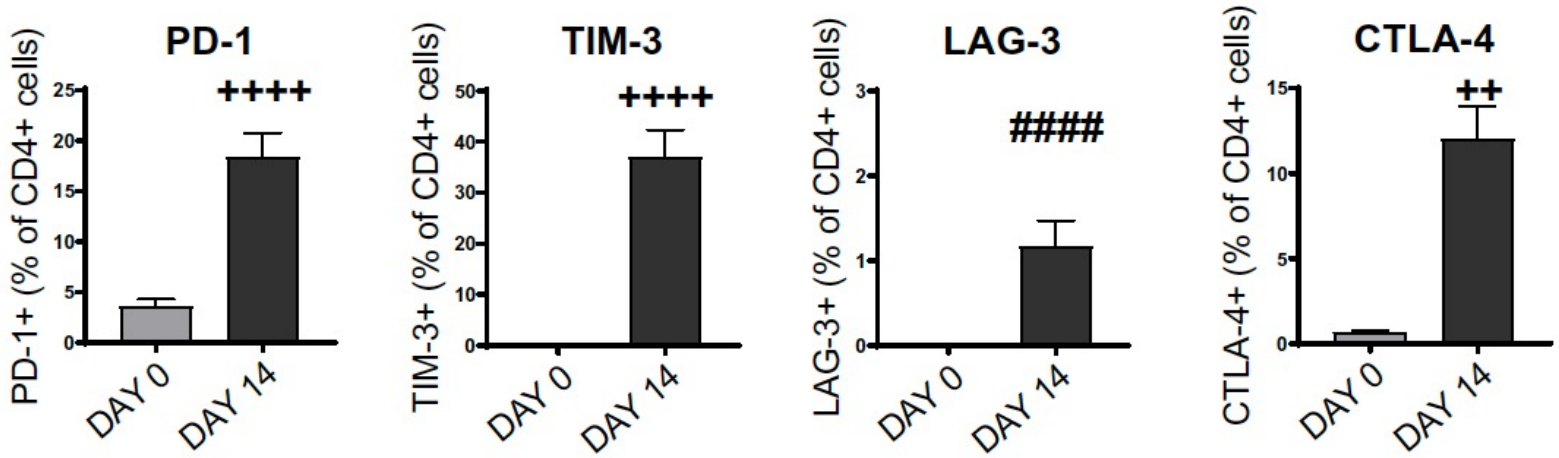
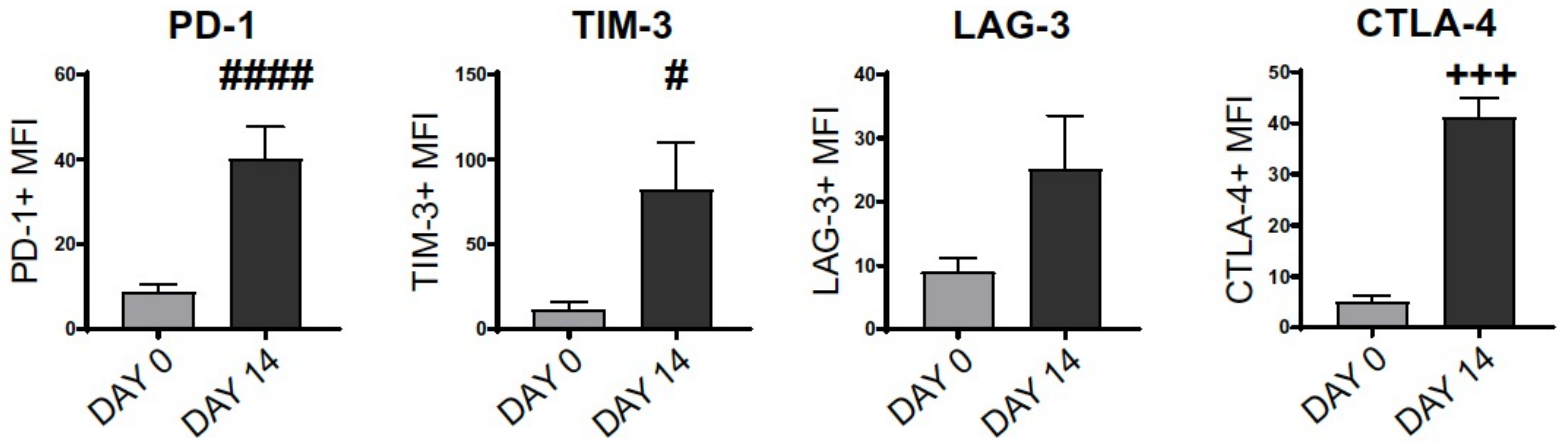
Expression of exhaustion markers on chronically stimulated T cells

First: Stimulation of CD4+ T cells with PHA for 14 days.

Percentage:



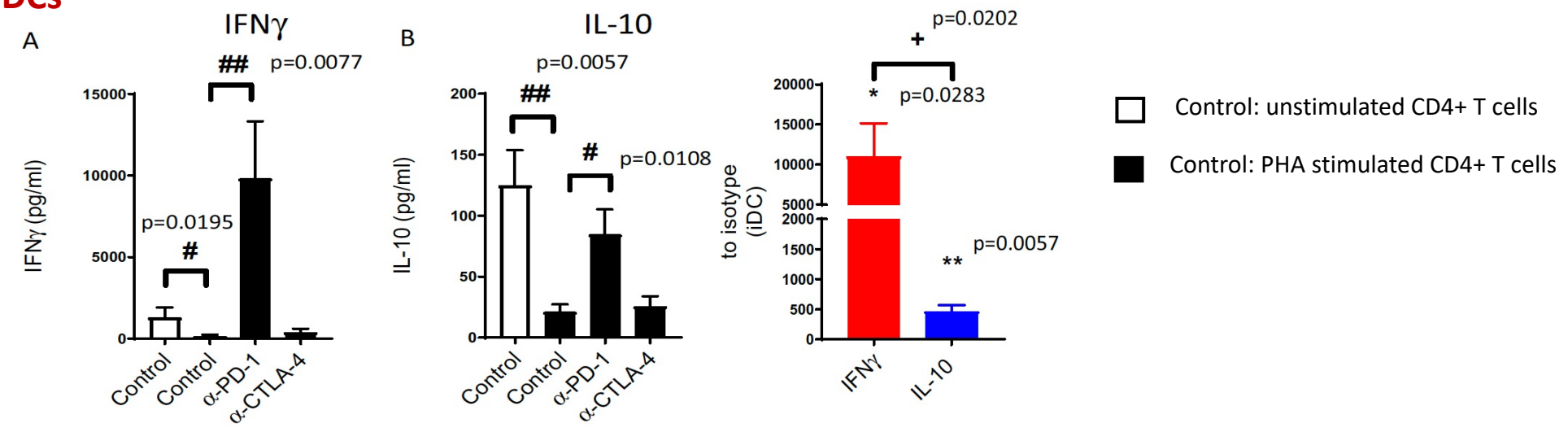
MFI:



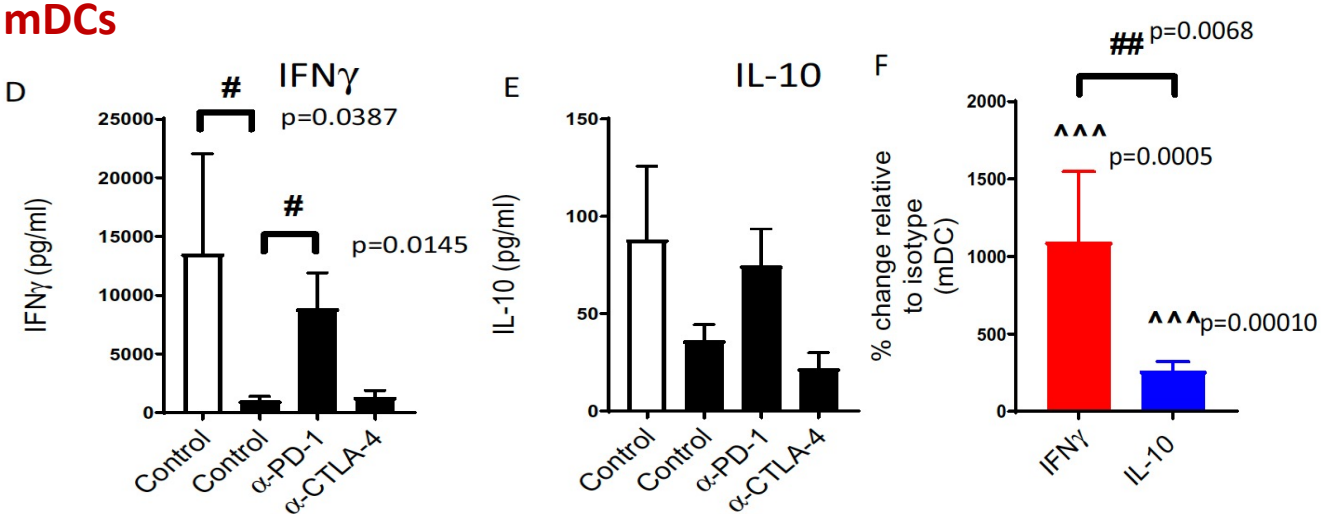
Treatment of chronically activated ‘exhausted’ CD4+ T cells with α-PD-1 greatly enhanced IFNγ release

Second: unstimulated and PHA-stimulated CD4+ T cells were cultured with either iDCs or mDCs in the absence or presence of α-PD-1, IgG4 isotype control, α-CTLA or IgG1 isotype control.

Stimulation with iDCs



Stimulation with mDCs



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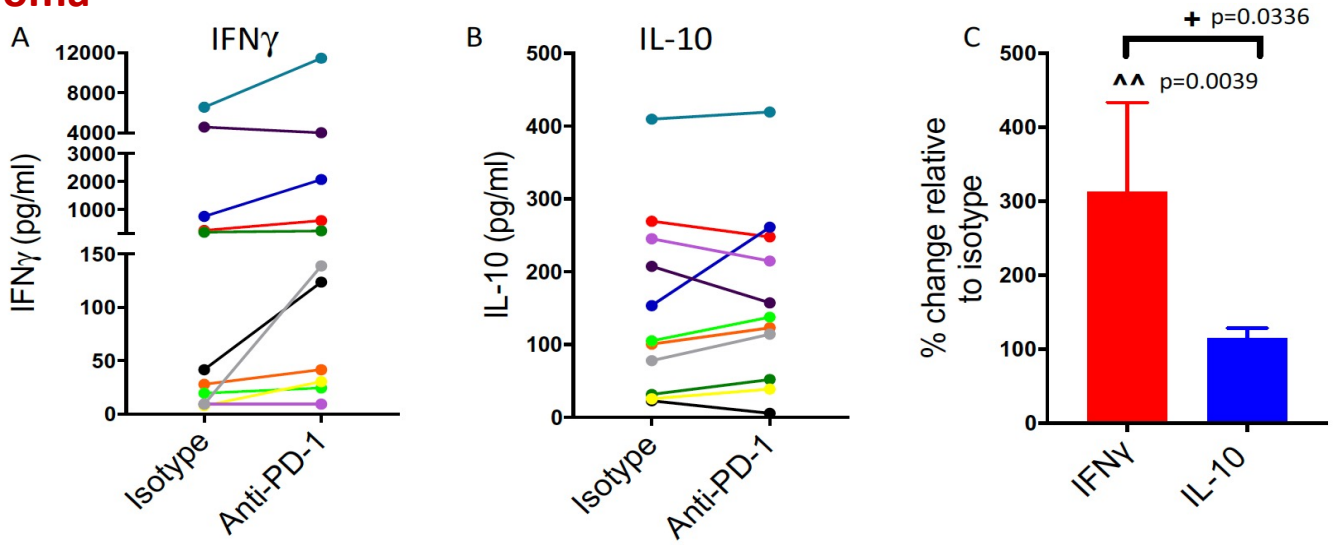
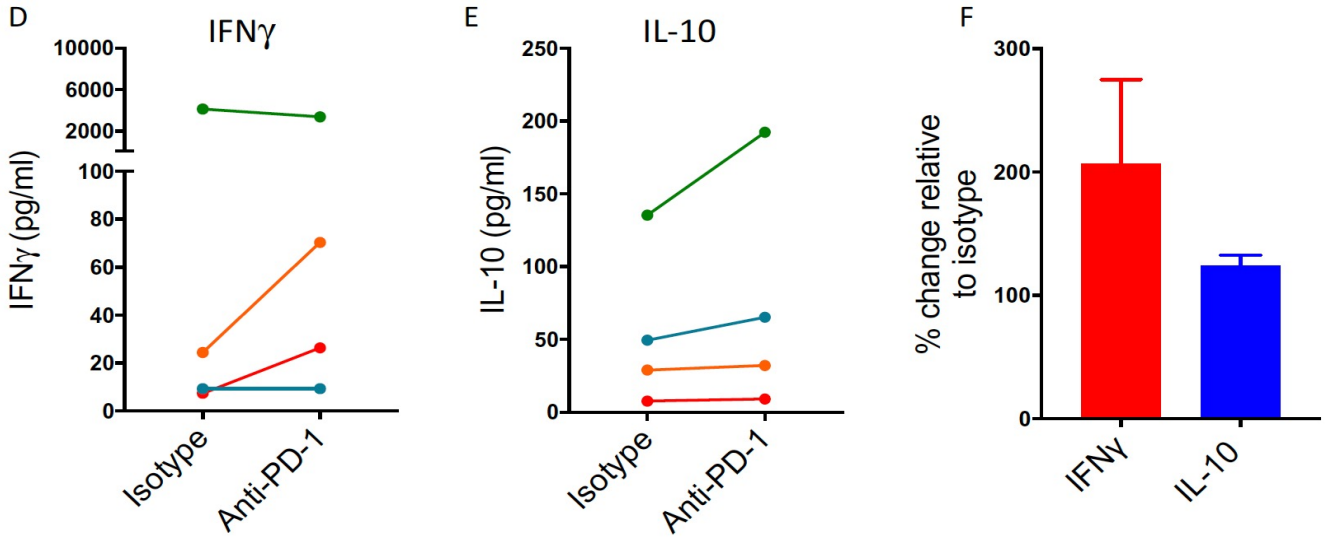
α-PD-1 enhanced IFNγ production over IL-10 release from lung or colon tumors

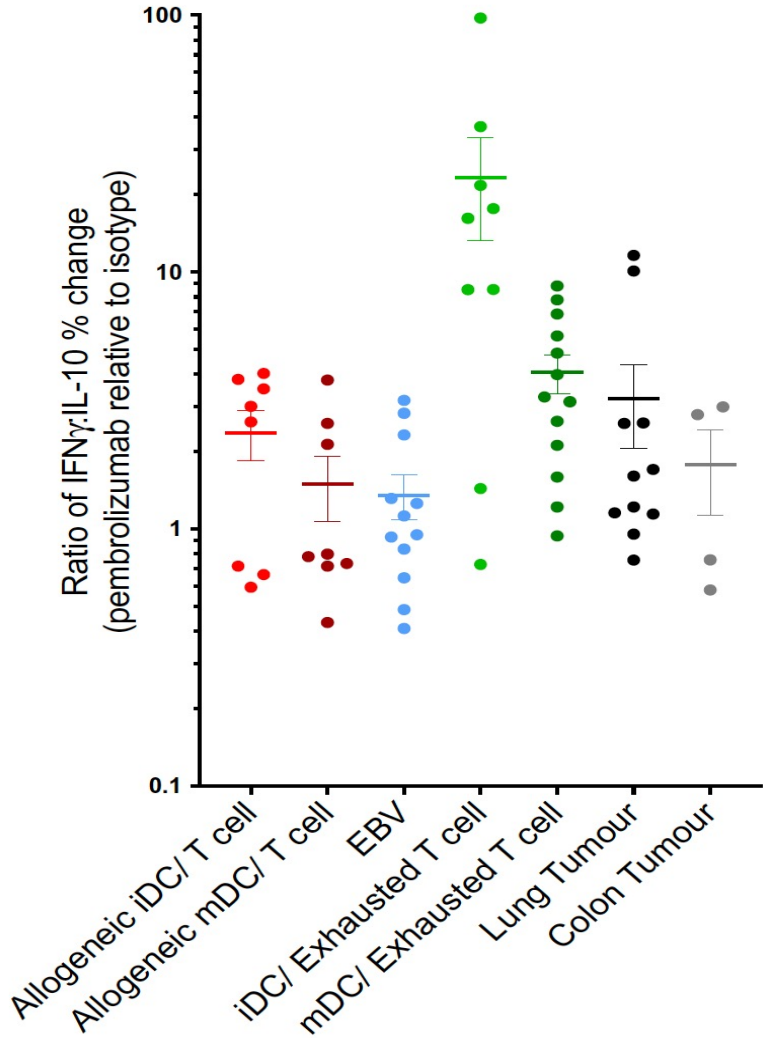
Lung carcinoma



Cancer colon



pembrolizumab skewed the cytokine response in favour of IFNγ over IL-10



Conclusion

- α-PD-1 response depends on the magnitude and activation status of the target T cell.

- They have identified in vitro assays with response profiles that mimic features of dissociated cell populations from primary tumours that could be exploited for the screening of immune checkpoint inhibitors in current and future development.

- The expression of PD-L1 seems to be associated with enhanced responses to anti-PD-1/PD-L1 therapy


спасибо
谢谢
GRACIAS
THANK YOU
ありがとうございました
MERCI
DANKE
धन्यवाद
شُكراً
OBRIGADO

Questions?