PD-L1 on dendritic cells attenuates T cell activation and regulates response to immune checkpoint blockade

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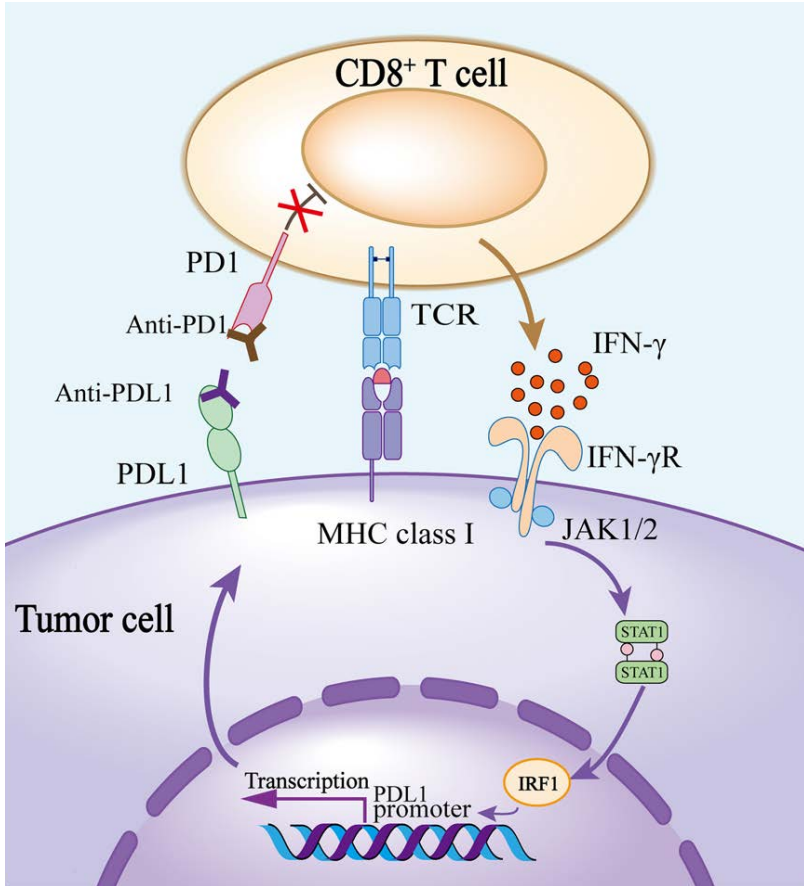
PD1/PDL1 axis in tumour immunology



Lei et al. Resistance Mechanisms of Anti-PD1/PDL1 Therapy in Solid Tumors
Frontiers in Cell and Developmental Biology

Recognition of Tumour antigen activates the CD8+ T cell and results in the release of INF-gamma→ Upregulation of PDL1 on Tumour cell

PD1 on the activated tumour cell is binds PDL1 and leads to an inhibitory Signal → less cytotoxicity

Anti-PD1/ Anti-PDL1 therapy to prevent shutdown of T cell activity

Introduction

- Antigen presentation is a dynamic event of an effective antitumour immune response
- APCs lead to priming, activation and possible reactivation of T cells
- Conventional DCs (cDCs) are the most efficient APCs (cDC1, cDC2)
- During tumour progression cDCs can switch from immunostimulatory to immunosuppressive functions
- PDL1 is highly expressed in the tumour microenvironment
  → relative contribution still unclear

Material and Methods

- Mice: Wild-type C57BL/6 mice, Batf3$^{-/-}$, CD11c-cre mice , Pdl1fl/fl mice
- Cell lines: MC38 (murine colon carcinoma cell line), E.G7 (mouse lymphoma cell line), B16 (murine melanoma cell line)
- Transfection experiments for generation of MC38-EGFP, MC38-OVA, and MC38-SIY cell lines.
- Flow Cytometry
- Tumour model: MC38 or E.G7 cells were subcutaneously injected into the right flank of the mice.
  i.p. treatment with IgG and anti-PDL1

PDL1 on DCs is essential for checkpoint blockade therapy

a

b

→ WT B6 mice were inoculated with $5x10^5$ MC38 cells
Analysis at day 14 post inoculation

c

PDL1 Expression is highest in DCs and Macrophages

Knockout of PDL1 on DCs results in a significant decrease of tumour size.

PDL1 on DCs is important for T cell primimg during anti-tumour responses

d

e

f

MC38 tumour tissues were collected on day 14 post inoculation

D) PDL1 levels on tumour cells were measured by flow cytometry

E) CD11c-cre;Pdl1fl/fl were inoculated with E.G7 cells, OVA-specific T cells in PBMCs at day 12

F) Inoculation with MC38-SIY cells → DCs isolated from draining lymph nodes and INF production was assessed after co-culture with T cells

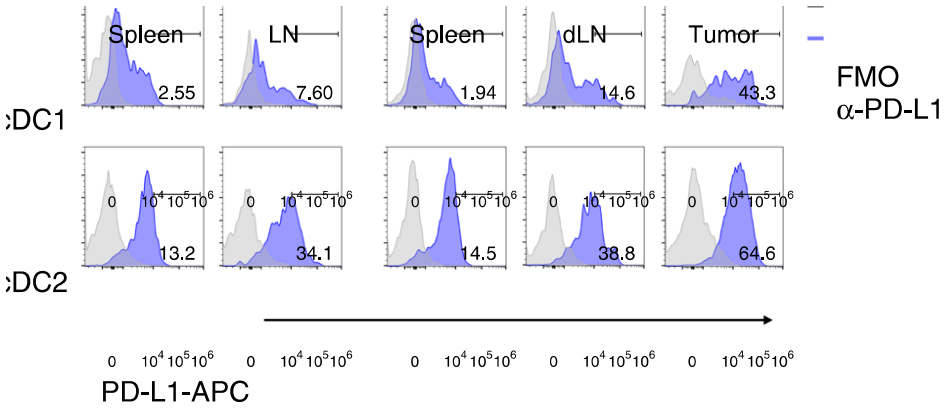
PDL1 on DCs is essential for the response to PDL1-blockade therapy

Established MC38 tumours were treated with IgG od anti-PDL1 on days 8 and 12 and evaluated for tumour growth

Batf3$^{-/-}$ mice fail to generate conventional DCs, important for antigen cross-representation

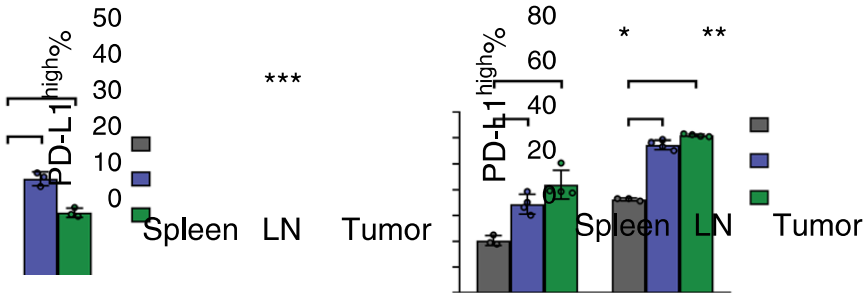
Effect of anti-PDL1 therapy on tumour size is significantly decrease in Batf3$^{-/-}$ mice

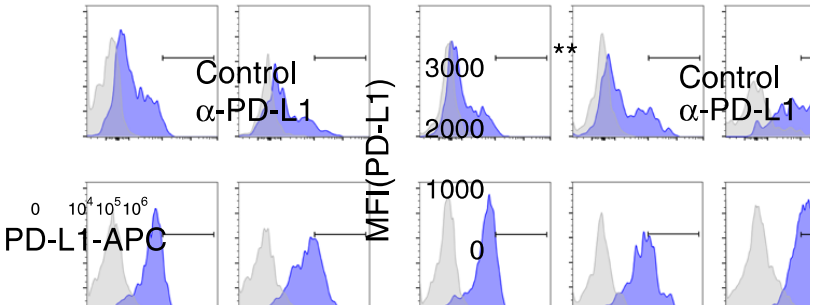
PDL1 is upregulated by IFN-gamma and T cells in tumour



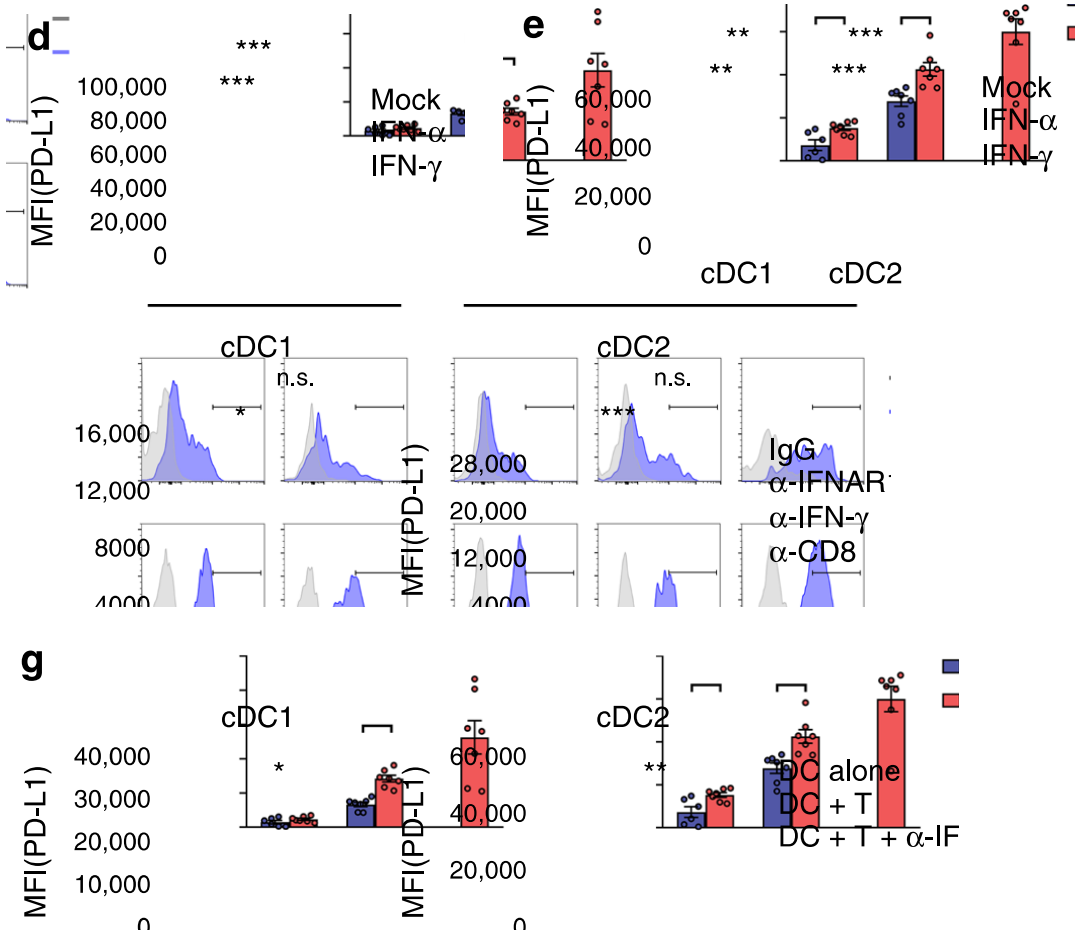
PDL1 expression on cDC1 and cDC2 Isolated from spleen, draining LN and tumour

Bone marrow derived DCs generated with FLT3-L to check effects of type I and type II interferons on PDL1 dynamics





PDL1 is upregulated by IFN-gamma and T cells in tumour

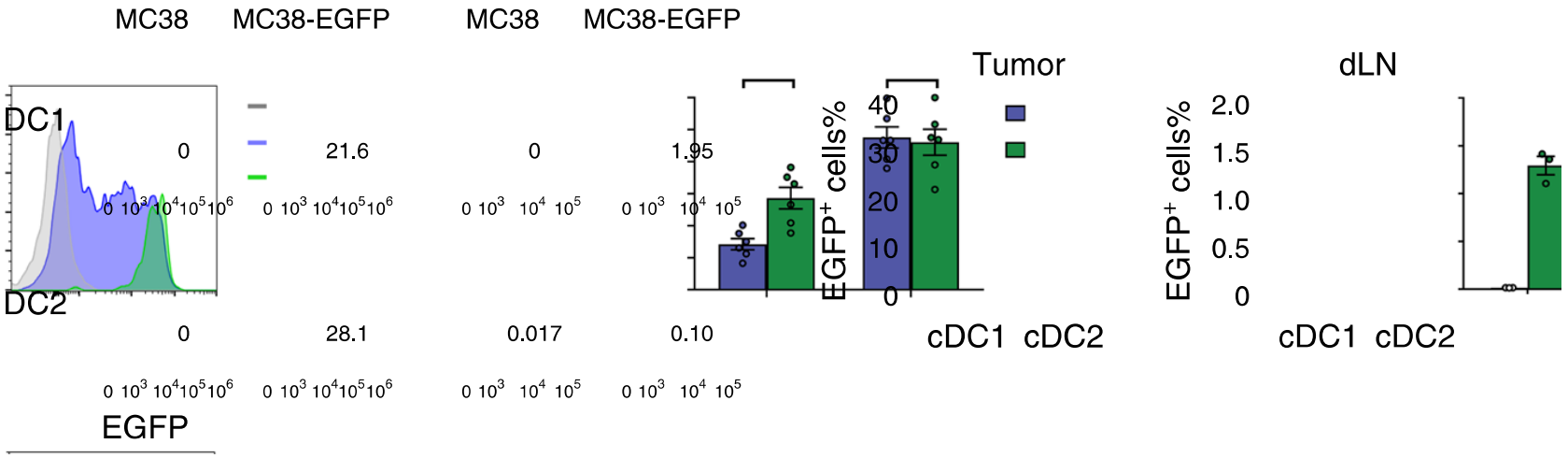


Purified BMDC (d) and DCs (e) were treated with Interferons
→ Increase of PDL1

Inhibition of IFNs and T cells resulted in decreased levels of PDL1

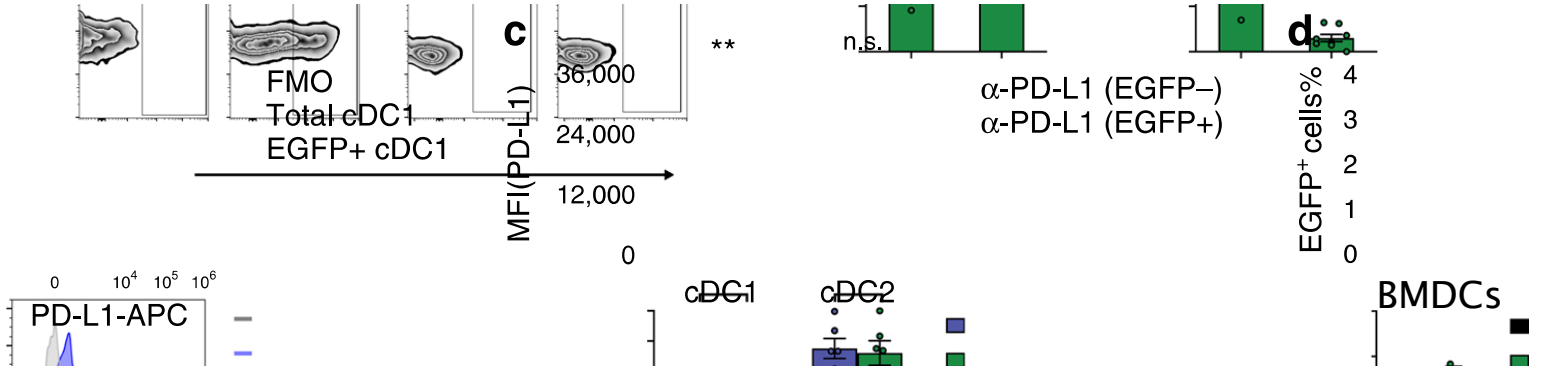
Co-culture of WT cDCs with activated T cells
In combination with anti-IFN threapy

PDL1 on cDC1 is upregulated during antigen presentation by IFNG-gamma

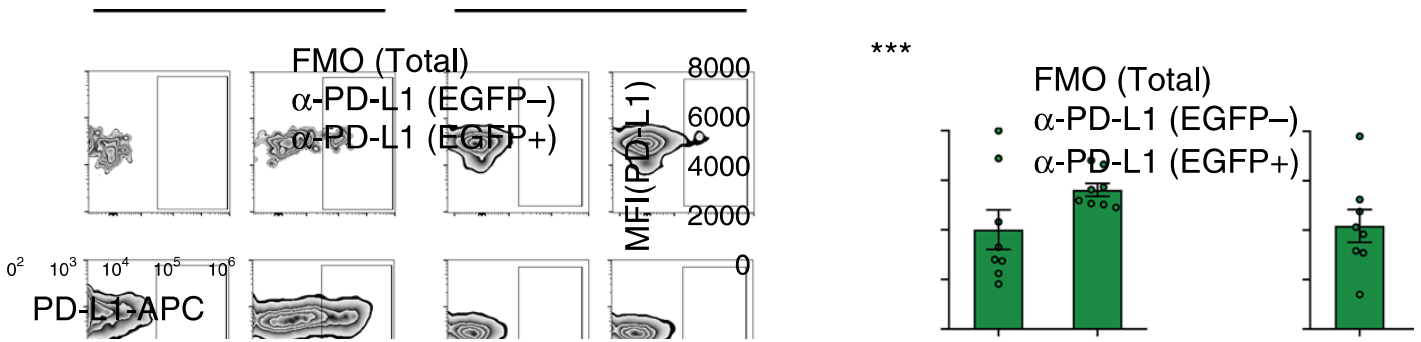


WT B6 mice were inoculated with $5x10^5$ MC38-EGFP+ cells. After tumour establishment tissue and dLN were collected, EGFP+ cDCs were measured by flow cytometry

PDL1 on cDC1 is upregulated during antigen presentation by INFG-gamma

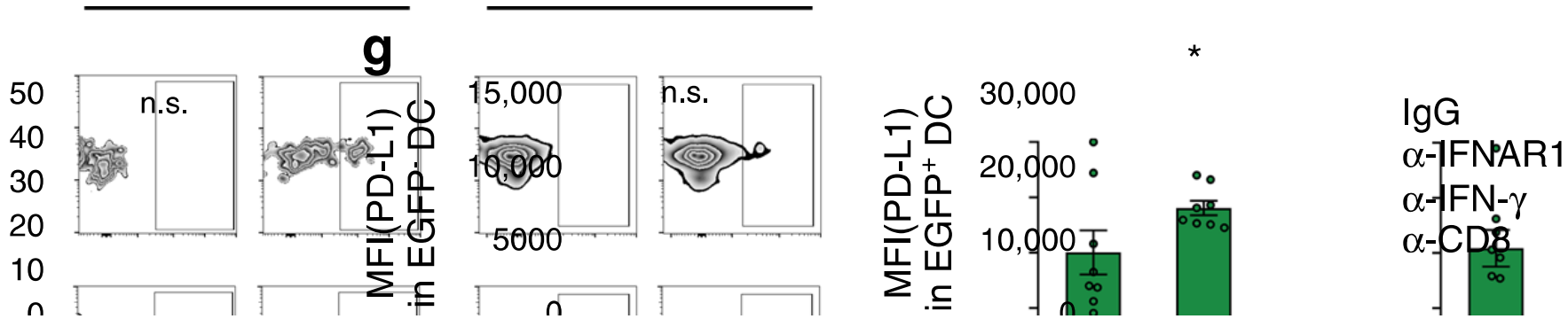


EGFP+ cDCs show highest PDL1 expression → suggesting a link between antigen uptake and PDL1 expression



Less difference for BMDCs (e) when compared to cDCs (b)

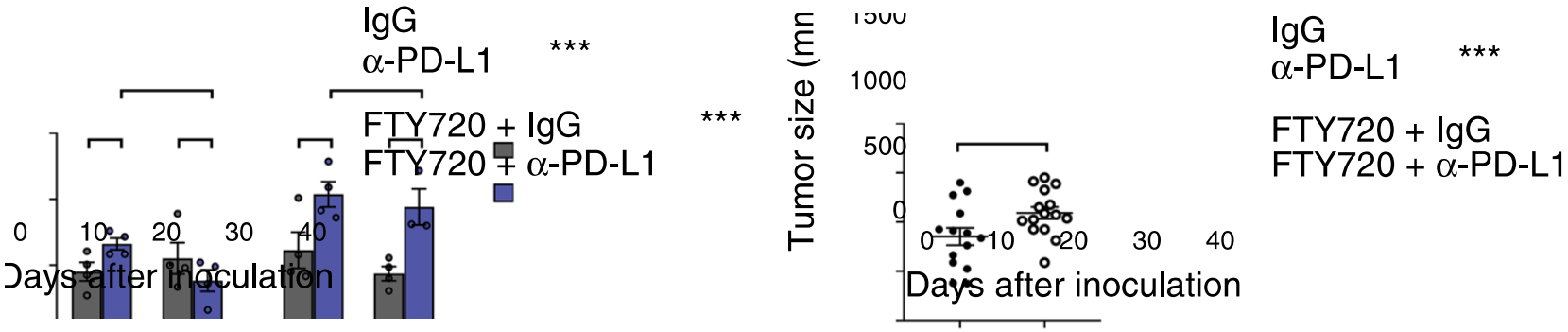
PDL1 on cDC1 is upregulated during antigen presentation by INF-gamma



Antigen uptake is not affected by addition of inhibitors

Upregulation of PDL1 in EGFP+ DCs is dependant of T cells and INF-gamma

PDL1 blockade reactivates T cells in tumour microenvironment for tumour control

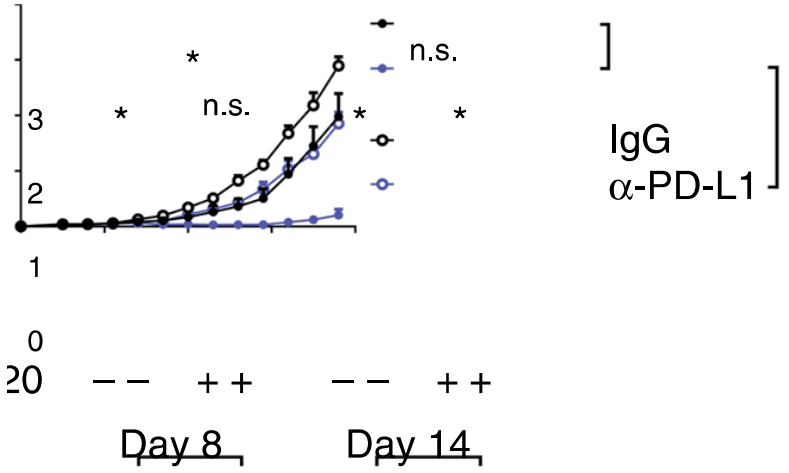


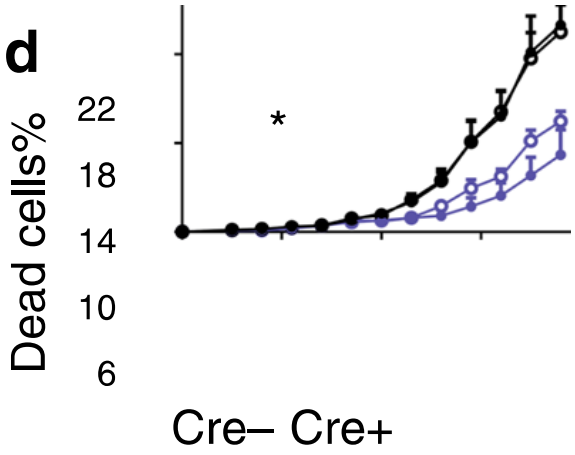
C57BL/6 mice were inoculated with 2.5 x 10^5 MC38-Ova cells and treated with (a) 200 µg IgG or anti PDL1 on days 8 and 12
(b) 250 µg IgG or PDL1 on days 14 and 18

Fingolimod (FTY720) immunomodulator, prevents lymphocyte egress from LNs

Early phase blocking resulted in a loss of anti-PDL1 therapy on tumour size

PDL1 blockade reactivates T cells in tumour microenvironment for tumour control





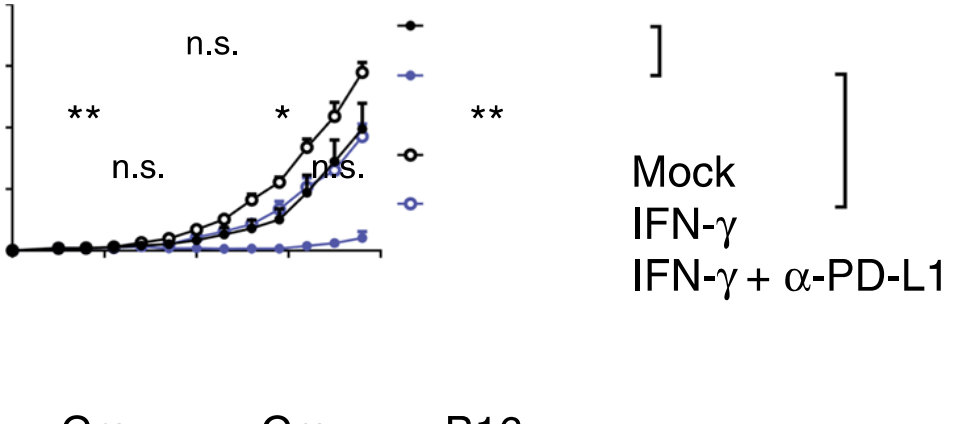
PDL1 blockade therapy increased the number of activated T cells at tumour site.

Inhibition of T cell infiltration at an early Stage resulted in less activated T cells

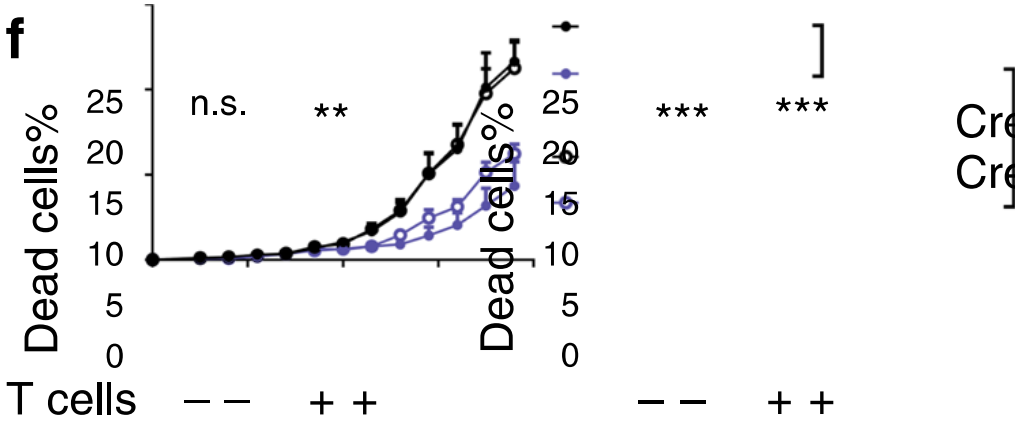
Reactivation of T cells by anti- PDL1 therapy in prolonged disease

Number od dead cDCs is significantly higher after PDL1 knockout

PDL1 blockade reactivates T cells in tumour microenvironment for tumour control



DCs purified from conditional knockout mice or B16 cells were treated with IFN-γ for 96 h. Cell viability was determined by MTT assay



DCs isolated from spleens of CD11c-cre;$Pdl1_{fl/fl}$ or control mice were loaded with OT-1 peptide and incubated with activated OT-1 T cells.

Cell death of cDC1 and cDC2 cells were measured by flow cytometry

Summary

- Therapeutic effects of PD-L1 blockade therapy disappear completely in DC-conditional knockout mice, even though other cells still express high levels of PD-L1.
- PD-L1 upregulation on cDC1s is mediated by IFN-γ produced by activated T cells.
- Identified a critical and dynamical role of PD-L1 on DCs in T cell (re-)activation and immune checkpoint blockade therapy

Thank you for your attention