# DCOne-derived mature DCs opsonized with anti-PD-L1 antibodies as potential intratumoral immune primers

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## Introduction

Immune checkpoint blockade (ICB) therapies are predominantly effective in inflamed and lymphocyte-infiltrated tumors termed as “hot tumors”. In a recent study an essential role for functional Fc-gamma-receptors (FgR) on intratumoral NK cells and macrophages in the induction of a “hot” tumor was shown. Notably, this inflammatory response led to a self-sustaining systemic tumor-specific T cell activation driven by ICB.

Mature dendritic cells derived from the leukemic cell line DCOne (DCOne mDC) strongly activate co-cultured allogeneic T cells, while expressing PD-L1. We therefore aimed to evaluate how opsonization of these cells with anti-PD-L1 antibodies could affect the activation of FgR-expressing NK cells and monocytes within co-cultured allogeneic PBMCs.

## Methods

PBMCs from healthy CMV seropositive donors were co-cultured with DCOne mDCs in the absence or presence of different anti-PD-L1 antibodies for 4-7 days and without stimulating cytokines. Proliferation (CFSE), cytotoxicity (granzyme B; GrB) and immune cell phenotype was monitored with flow cytometry and cytokine/chemokine production with Luminex. The defucosylated anti-PD-L1 antibody used in this study was kindly provided by Glycotope GmbH.

## Results

Addition of anti-PD-L1 IgG1 (high FcR affinity; HA) to DCOne mDC/PBMC co-cultures induce expansion of NK cells, including NKG2C+CD57+ memory NK cells

![Graph showing NK cell frequency and NKG2C+CD57+ memory NK cells](image)

**Figure 1.** PBMC were cultured cytokine-free media in the absence or presence of DCOne mDC in combination with defucosylated anti-PD-L1 antibody. (A) Dot plots show the frequency of T cells and NK cells at day 7. (B and C) Fold expansion of total NK cells (B) and NKG2C+CD57+ memory NK cells (C) at day 7. *p<0.001; significant differences between indicated groups, using one-way ANOVA.

Addition of anti-PD-L1 antibodies to DCOne/PBMC co-cultures induce increased production of immune cell recruiting chemokines and proinflammatory cytokines

**Figure 2.** PBMC were cultured cytokine-free media in the absence or presence of DCOne mDC in combination with defucosylated anti-PD-L1 antibody. Supernatants were harvested on day 4 and analyzed for secreted cytokines and chemokines using Luminex technology.

**Figure 3.** Tumor cell line K562 was labeled with fluorescent cell linker dye TF4 and co-incubated with NK cells from different co-cultures for 1 hour at an effector:target ratio of 10:1 in the presence of fluorescenc granzyme B substrate. Fluorescent Granzyme B activity in the target tumor cells after cleavage of the granzyme B substrate was measured by using the GranToxiLux™ kit (OncoImmunin, Inc., MD).

## Conclusions

- **DCOne-derived mDCs opsonized with anti-PD-L1 antibodies with high Fc-receptor affinity increase the expansion of co-cultured allogeneic NK cells, including memory NK cells.**
- **Co-culture supernatants show increased release of proinflammatory factors, including immune cell recruiting chemokines and immune cell activating cytokines.** Notably, IL-1b is strongly increased, indicating that FgR-expressing myeloid cells (monocytes and DCs) are also activated.
- **NK cells activated with anti-PD-L1 opsonized DCOne mDCs show increased cytotoxicity.**

Taken together these data indicate that DCOne-derived mDCs opsonized with anti-PD-L1 antibody (HA), if administered intratumorally, will induce FcYR dependent activation of intratumoral macrophages and NK cells that may initiate critical steps including local tumor cell killing and recruitment of immune cells, such as cross-presenting DCs, that could sensitize for concomitant ICB therapy.