Intratumoral administration of pro-inflammatory allogeneic, off-the-shelf, dendritic cells in combination with anti-PD-1 or anti-CD137 has a synergistic anti-tumor effect

**Background**

The future of immuno-oncology lies in combination therapies of adaptive approaches that “release the brake”, such as immune check-point inhibitors (CPIs), with immune activating therapies that “push the gas” on the immune system, such as immune primers, vaccines and/or immune enhancers (e.g. IL-2, 4-1BB). Traditional vaccine approaches based on tumor-associated antigens have shown limited efficacy due to immune tolerance and limited antigen coverage, and recent efforts based on tumor-specific neoantigens through tumor biopsies and ex vivo identification and production requires a highly patient dependent process with significant limitations. The obvious way to circumvent these challenges would be to use the patient’s own tumor in situ, as a direct neoantigen source by intratumoral administration of a potent immune primer.

Immunicum AB has developed a cell-based immune primer named ilixadencel, consisting of pro-inflammatory allogeneic DCs producing high levels of immune cell-recruiting and activating factors for endogenous DCs, NK cells and T cells (1-4). Ilixadencel is currently in a Phase 2 study in renal cell carcinoma, and will now be investigated in a Phase 1b/2 study with checkpoints inhibitors in non-small cell lung cancer, head and neck squamous cell carcinoma, and gastric adenocarcinoma. Here we investigated the impact of mouse ilixadencel on tumor response in a mouse tumor model when ilixadencel is combined with the CPI (anti-PD-1) or the immune enhancer 4-1BB (anti-CD137), in comparison to a TLR- ligand as additional intratumoral immune priming approach. The ability of ilixadencel to up-regulate CD137-expression on co-cultured allogeneic NK cells and T cells in vitro was further investigated.

**Results**

Intratumoral immune priming with ilixadencel overcomes resistance to PD-1 blockade (Fig. 1, 2)

Data from clinical studies with ilixadencel (1,3) suggest that ilixadencel may synergize with drugs that promotes primed T cells from subsequent tumor-derived immunosuppression (i.e. sunitinib and gemcitabine). By using the anti-PD-1 resistant CT26 colon carcinoma model where the tumor is allowed to grow for 12 days before start of anti-PD-1 treatment (Day 1 in the study), the addition of two intratumoral injections of ilixadencel at Day 1 and 4 was found to overcome resistance to monotherapy with anti-PD-1 as shown by a significant delay in tumor progression (Figure 1).

Ilxadencel induces up-regulation of CD137 in co-cultured human allogeneic NK cells and T cells (Fig. 5, 6)

The stimulation of NK cells with IL-2 or ligation of their Fc-receptors has been shown to increase expression of the costimulatory receptor CD137. We therefore hypothesized that ilixadencel, which has been shown to activate co-cultured allogeneic T cells and NK cells in vitro (2), is also able to increase expression of CD137 in NK cells. As shown in Figure 5 and 6, T cells and particularly NK cells within PBMC populations co-cultured with ilixadencel DCs were shown to substantially upregulate CD137. These data may potentially explain the synergy seen between ilixadencel and anti-CD137 in the CT26 tumor model.

**Conclusions**

Our results support the use of intratumoral treatment with ilixadencel, an off-the-shelf cell-based immune primer, in combination with anti-PD-1 or with the immune enhancer anti-CD137. This emphasizes the potential for future Immuno-Oncology (IO) treatment strategies to incorporate the complementary mechanisms of immune primer, immune enhancer, and checkpoint inhibitor, while maintaining a sustainable tolerability profile for such regimens.

**Material and methods**

Reagents

Anti-mouse PD-1 (BioXcell clone RMP1-14) and anti-mouse CD137 (BioXcell clone 3H5) were used for in vivo studies in mice. Poly I:C HMW was purchased from InvivoGen. Expression of CD137 on human T cells and NK cells within PBMC populations co-cultured with immature or mature, autologous or allogeneic DCs for 24 hours was determined by flow cytometry using anti-human CD137-BV421 in combination with anti-CD20-RTC and anti-CD69-APC.

Isolation and generation of mouse and human ilixadencel

Bone marrow-derived mouse ilixadencel DCs were generated from wild-type C57BL/6 (H-2b) mice by standard methods (4). The non-adherent immune DCs were treated for 18 hours with a cocktail consisting of Poly I:C, TLR3- ligand, R848 (TLR7/8-ligand) and IFN-gamma (together, Immunicum’s COMBIG cocktail) before washing and subsequent cryopreservation. Just before dosing, the frozen mouse ilixadencel suspension was briefly thawed and washed.

Mouse tumor model

BALB/c (H-2d) mice were injected s.c. in the right flank with 3×10^5 CT26 syngeneic colon carcinoma cells. Twelve days after tumor cell injection, on Day 1 of the study, mice were sorted into study groups with group mean tumor volume of approximately 100 mm^3.

Vehicles and ilixadencel (0.9 million DCs/dose) were injected intratumorally (i.t.) on Day 1 and 7. Poly I:C (50 μg) was injected i.t. at Day 1, 4 and 7. Both anti-PD-1 and anti-CD137 were administered i.p. at 5 mg/kg. Anti-PD-1 was administered twice a week for two weeks starting on Day 1. Anti-CD137 was administered on Day 4 and 7. Animals were monitored individually for tumor growth until Day 45.

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**References**


