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

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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 protects 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)

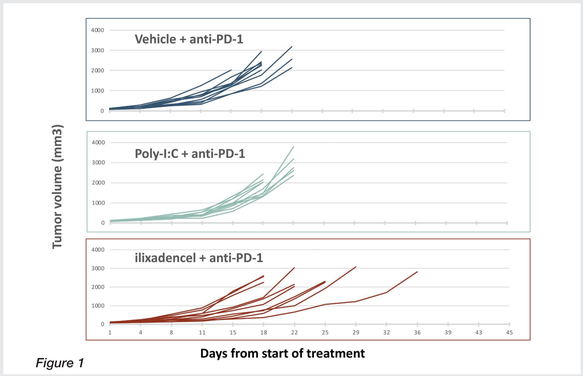

Figure 1

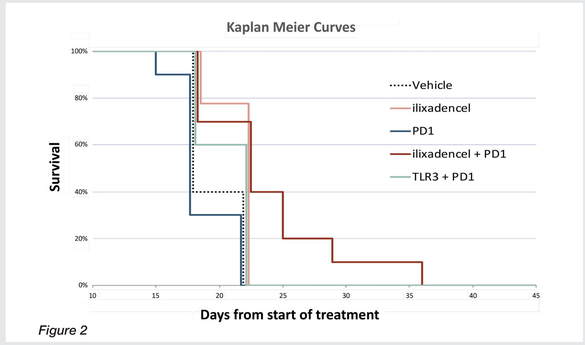

Figure 2

and prolonged survival (Figure 2). Notably, intratumoral immune priming with the TLR3-ligand Poly I:C (injected Day 1,4 and 7) did not overcome resistance to PD-1 blockade. These findings are in line with a recent report indicating that intratumoral administration of Poly I:C has to be combined with Flt3L-induced recruitment of circulating DCs in order to act in synergy with CPI treatment (5).

Intratumoral immune priming with ilixadencel has a synergistic anti-tumor effect when combined with the immune enhancer anti-CD137(4-1BB) (Fig. 3, 4)

Since anti-CD137 has been shown to positively affect both NK cells and primed CD8+ T cell functions (6), and the mode of action of ilixadencel is proposed to be dependent on NK cell/IFN-gamma mediated promotion of cross presentation and type-1 polarization of endogenous, ”bystander” DC (2,3), the combined treatment with ilixadencel and anti-CD137 was also investigated. Ilixadencel was injected at Day 1 (tumor diameter

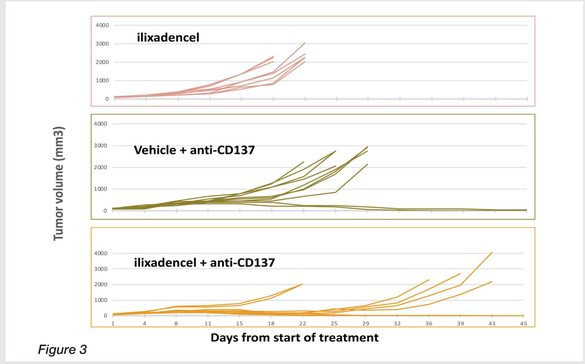

Figure 3

approximately 100 mm3) and Day 7 +/- anti-CD137 at Day 4 and Day 7. This combination was found to synergistically delay tumor progression and increase the number of complete tumor rejection when compared to monotherapy with anti-CD137 (Figure 3) and also prolonged survival (Figure 4). In order to evaluate if tumor rejection was due to adaptive immune mechanisms the mice who had rejected their first tumor were re-challenged with a new tumor cell inoculation. All treatment-naive control mice (n=6) developed progressively growing tumors while all 3 mice in the ilixadencel/anti-CD137 group that rejected the primary tumor resisted tumor re-challenge. These data thus indicate the induction of a systemic adaptive immune memory.

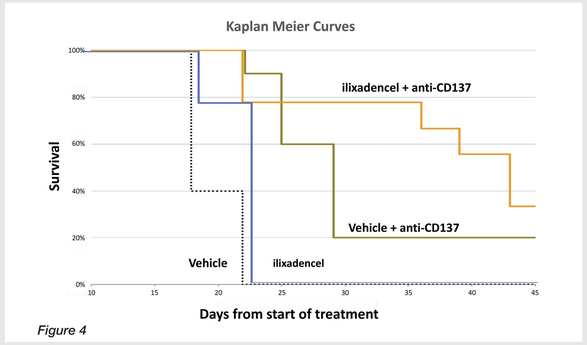

Figure 4

Ilixadencel 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 induce their 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 co-cultured allogeneic PBMCs were shown to substantially upregulate CD137. These data may potentially explain the synergy seen between ilixadencel and anti-CD137 in the CT26 tumor model.

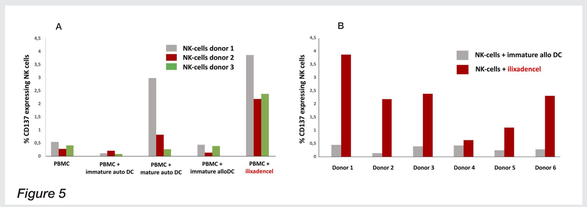

Figure 5

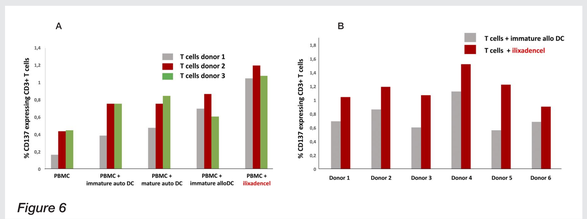

Figure 6

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 an 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 3H3) 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-cultred 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-CD3-FITC and anti-CD56-APC.

Isolation and generation of mouse and human ilixadencel

Bone marrow-derived mouse immature DCs were generated from wild-type C57BL6 (H-2Dd) mice by standard methods (4). The non-adherent immature 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. The cell pellet was resuspended in the appropriate volume of vehicle to obtain approximately 1 million cells per 25 µL (dosing volume per mouse). The vehicle consisted of 10% DMSO in mouse plasma in order to mimic the clinical situation where thawed ilixadencel cells are immediately injected in freezing medium consisting of 10% DMSO in AB-plasma. Human ilixadencel DCs were generated from isolated peripheral blood monocytes by differentiation with IL-4 and GM-CSF and subsequent activation with the COMBIG cocktail (2).

Mouse tumor model

BALB/c (H-2Db) mice were injected s.c. in the right flank with 3x105 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 mm3.
Vehicle 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.

This study was conducted by:

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