Introduction

The future of immuno-oncology lies in combination therapies of adaptive approaches that “release the brake” (e.g., checkpoint inhibitors) with immune activating therapies that “push the gas” on the immune system (e.g., immune primers). Traditional vaccine approaches priming the immune system to tumor-associated antigens have shown limited efficacy, and recent efforts focus on tumor-specific antigens, or neoantigens. Tumor biopsies and ex vivo identification and production of neoantigens for immune priming approaches is however a patient-dependent process with significant technological and practical limitations.1,2 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.3

In specific, a crucial and emerging immune priming role is to recruit and activate endogenous “bystander” dendritic cells (DCs) to the immune cell-exhausted tumor tissue to capture tumor cell debris and neoantigens4 to optimally initiate the cancer immunity cycle. Immunuc AB has developed a cell-based immune primer, named ilixadencel consisting of pro-inflammatory allogeneic DCs producing high levels of DC-recruiting and DC-activating factors. This concept does not require MHC compatibility between injected cells and the patient and therefore introduces the possibility of using patient-independent, pre-produced cryopreserved DCs from healthy blood donors as an off-the-shelf immune primer. Ilixadencel has been studied in two Phase II clinical trials, which included 12 patients with metastatic renal cell carcinoma (RCC)5 and 18 patients with advanced/metastatic hepatocellular carcinoma. Two additional clinical studies are ongoing: an international multi-center, randomized Phase II study, in RCC (MERECA; in Europe and US) are ongoing: an international multi-center, randomized phase II study, in RCC (MERECA; in Europe and US) and a Phase II study in gastrointestinal stromal tumors (GIST) in Sweden.

Here we present in vitro data using GMP-produced pro-inflammatory DCs (INN: ilixadencel) supporting the proposed mode of action.

Material and methods

GMP-production of ilixadencel

Ilxixadencel investigated in this study was manufactured in the GMP facilities of BioNTech IMFS GmbH, Germany. Fresh leukapheresis product from a healthy donor was used as starting material. Isolated monocytes were cultivated in serum-free media, supplemented with IL-4 and GM-CSF. On day 4, the differentiated immature DCs were stimulated with a cocktail consisting of R848 (TLR7/8 ligand), Poly I:C (TLR3 ligand) and IFN-gamma. On day 5, the pro-inflammatory DCs were harvested, washed, formulated in AB plasma and DMSO, and transferred to vials for cryopreservation.

In vitro assays

Thawed ilixadencel was cultured in AIM-V medium for 24 hours, either alone or mixed with allogeneic PBMCs (alloPBMCs; 1:5) or allogeneic NK cells (1:1) isolated by negative selection. Ilxixadencel supernatants (SN) were collected after 24 hours and chemokine and cytokine production was measured by ELISA or Luminox technique. Phenotypic characterization of NK cells, T cells and bystander DCs was performed by flow cytomtery. For measurement of NK cell cytotoxicity, NK cells were first cocultured with ilixadencel (1:1) for 24 hours followed by washing and subsequent coculture with luciferase-tagged K562 target cells (10:1). Luciferase activity from viable K562 was measured 18 hours later by a luminometer. IL-2 (200 IU/ml) activated NK cells were used as a positive control. Phenotypic maturation of bystander DCs induced by different cell culture SNs was measured after 48 hours. Bystander immature DCs or DC matured with different cell culture SNs were subsequently washed after 48 hours and subsequently exposed to Jurkat D1.1 cells expressing CD40 ligand expressing CD40 ligand in a ratio of 1:1. Supernatants were collected 18 hours later and tested for IL-12 (IL-12 p70) by ELISA.

Results

Ilixadencel is characterized by a sustained production of Th1-associated chemokines and cytokines after thawing

Since tumors are poorly infiltrated by DCs that are able to transport intact tumor antigens to the draining lymph node, a crucial function of an intratumorally injected immune primer is to induce recruitment of immune cells, including DCs/DC-precursors into the tumor.4 As shown in table 1, ilixadencel used in the in vitro studies produced substantial amounts of Th1-associated chemokines and cytokines during the first 24 hours after thawing.

Table 1

<table>
<thead>
<tr>
<th>Proinflammatory factor</th>
<th>pg/ml/10⁶ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-alpha</td>
<td>84</td>
</tr>
<tr>
<td>IL-1 beta</td>
<td>685</td>
</tr>
<tr>
<td>IL-12 p70</td>
<td>1470</td>
</tr>
<tr>
<td>MIP-1 beta</td>
<td>3420</td>
</tr>
<tr>
<td>RANTES</td>
<td>20940</td>
</tr>
</tbody>
</table>

Figure 1

Ilixadencel induces strong production of IFN-gamma in cocultured NK cells

To elucidate whether NK cells participated in the IFN-gamma production seen in the ilixadencel/alloPBMC population, isolated NK cells were cocultured with ilixadencel. As shown in figure 2, ilixadencel induced significant NK cell activation (2A), leading to strong release of IFN-gamma (2B).

Figure 2

Ilixadencel induces a strong cytolytic capacity in cocultured allogenetic NK cells and ilixadencel/alloPMBC cocultures induces Th1 polarized bystander DCs

In order to evaluate if recruited bystander DCs become matured in bystander DCs. Supernatants from cultured ilixadencel or ilixadencel/alloPBMC cocultures induces Th1 polarized bystander DCs

Finally, we also investigated if bystander DCs matured with different SNs where responsive to CD40-mediated “licensing” leading to subsequent production of IL-12 p70, a cytokine that directly regulates effector cell development in human CD4+ Th1 cells7 and CD8+ T cells8. As shown in Figure 4B, bystander DCs matured with ilixadencel or ilixadencel/alloPBMC SNs produced substantial amounts of IL-12 when subsequently stimulated with CD40 ligand.

Figure 4

Collectively, the presented in vitro results indicate that intratumorally injected ilixadencel will create an optimal immune priming environment leading to NK cell mediated tumor cell death with release of tumor-derived antigens, including neoantigens, as well as maturation of bystander endogenous DCs into Th1 helper 1 polarizing mature DCs. This fulfills the ideal profile of an effective immune primer in the converging field of innate and adaptive immuno-oncology combination therapies.

Figure 5

References

2. Fritsch et al, Oncoimmunology 2014; Jun 25;3(8):29311