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- ONCOTHESIS -

TITLE

Paving the way for immunotherapy in pancreatic cancer by exploring a novel combination immunotherapy consisting of a CD40 agonist and interleukin-15

KEYWORDS

Pancreatic cancer; Pancreatic stellate cell; Immunotherapy; Interleukin-15; IL-15; Natural Killer Cells; NK Cells; CD40 agonist; Combination Therapy

SUMMARY

Pancreatic Ductal Adenocarcinoma (PDAC) has the worst 5-year survival of all cancer types. Treatment options for these patients are limited and consists mainly of chemotherapy. However, the unique tumour microenvironment with its dense, fibrotic shield causes resistance to current and novel therapies. Tackling this stromal shield is therefore deemed crucial for making progress in PDAC treatment. We investigated in this thesis the potential of Natural Killer (NK) cells to address this high medical need. First, our systematic review revealed strong evidence of their importance in PDAC but also how the tumour renders them in a suppressed and less functional state. Based on this information, we sought to stimulate NK cells in such way that they attack both tumour and surrounding stroma. We show that, upon stimulation with IL-15, NK cells are capable of killing both pancreatic cancer and stellate cells, the drivers of the stromal reaction, in a contact-dependant manner. Increased expression of NKG2D and TIM-3 receptors was partially responsible for this enhanced killing. Furthermore, in our search to potentiate IL-15 stimulation, we combined this with an immune priming CD40 agonist and demonstrated profound anti-tumour effects and prolonged survival in PDAC mouse models. Increased intra-tumoural cytotoxic T cells, NK cells and reduced T regulatory cells combined with increased cross-presenting dendritic cells in the tumour draining lymph nodes are the main effectors of the observed anti-tumour effects. Summarised, our data provide a strong rationale for NK cell-driven cancer immunotherapy where immune stimulation is combined with immune priming. Initiation of an early-phase clinical trials with this novel combination immunotherapy for PDAC patients is warranted.

MANUSCRIPT

Introduction

PDAC is the third cause of cancer-related death worldwide with an increasing incidence. Its 5-year survival of 7% has barely changed in 50 years and is stated as the worst of any cancer type ~~(1)~~ [1]. In Belgium, it is the sixth most common cancer in both males and females ~~(2)~~ [2]. The incidence is increasing rapidly, partly due to our Western lifestyle, and is projected to rise by almost 60% by 2025 ~~(3)~~ [3]. This high mortality rate is attributed to high aggressiveness, a lack of reliable screening methods and the absence of early symptoms. Hence, the majority of patients (80-90%) present with either locally advanced and thus unresectable or metastatic disease ~~(4)~~ [4]. In these cases, the first line treatment consists of FOLFIRINOX when patients have a good performance status or gemcitabine + nab-paclitaxel as an alternative option. However, the efficacy of these treatments is only modest due to high resistance ~~5~~ [5].

The unique tumour microenvironment (TME) of PDAC is held responsible for this great amount of therapy resistance towards both current and novel treatment schemes. More specifically, a profound desmoplastic reaction, orchestrated by activated pancreatic stellate cells (PSC), causes shielding of the tumour and a high intra-tumoural pressure. This leads to a virtually impenetrable tumour resulting in this dismal prognosis for PDAC patients ~~(6, 7)~~ [6, 7].

Over the past decades, numerous clinical trials have been performed attempting to increase the survival rates of PDAC patients. Unfortunately, they all failed to show improvement over the current standard-of-care. Even the highly promising anti-PD-1/PD-L1 and anti-CTLA-4 antibodies as immune checkpoint inhibitors failed to show improvement over gemcitabine ~~(8)~~ [8]. Therefore, new treatment options targeting not only the tumour but also this unique TME are urgently needed. We focussed in this PhD project on the potential of Natural Killer (NK) cells to attack both tumour and its TME. NK cells can recognize and kill tumour cells using a specific set of activating and inhibitory receptors. As professional cancer killing cells, they are prime candidates for battling cancer ~~(9)~~ [9]. We show that by using new strategies which combine immune priming with immune stimulation, the power of NK and other immune cells can be harnessed to pave the way for the highly unmet medical need of PDAC patients.

The role of NK cells in PDAC

Although NK cells have a solid track record in cancer immunotherapy as tumour cell killers of the innate immune system, their specific role in PDAC is still not fully elucidated and evidence is often scattered across different articles ~~(10)~~ [10]. Therefore, we performed a systematic review of the literature to gather all bits and pieces of evidence on the

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role and therapeutic potential of NK cells in PDAC ~~(11)~~.[11] First, it became clear the amount of NK cells present in a PDAC patients is positively correlated with their survival, indicating NK cells do have anti-tumour functions in PDAC. However, numbers are not everything since the TME clearly impairs the function of NK cells significantly. More specifically, several activating receptors like NKp46 and NKp30, DNAM-1 within the promising DNAM-1/TIGIT/PVRIG/TACTILE axis, and the NKG2D receptor are downregulated in PDAC patients. Finally, also the NK cell effector molecules granzyme B and perforin are downregulated as is their production capacity of immune activating cytokines IFNγ and TNFα. Hence, NK cell functions in PDAC patients are significantly reduced, hereby offering potential therapeutic targets.

Based on these observations, many different approaches have been or are being investigated whether it is with a direct focus on NK cells or by using a drug that may increase their number and functional activity. The current, classic treatment options like surgery and chemotherapy with gemcitabine have already demonstrated important interactions with NK cell activity. However, our search surprisingly did not reveal any study on the effect of FOLFIRINOX or nab-paclitaxel on the immune system, despite the frequent use of both drugs in the clinic. Yet, it is important that these interactions are well understood when combining chemotherapy and immunotherapy in future PDAC therapies for optimal inclusion and synergy between immunotherapeutic and chemotherapeutic approaches.

Several new compounds are currently being tested in (pre)clinical settings. Here, the potential of cytokine-based treatments looks very appealing, especially with the rise of new superagonist compounds like N-803. Also, the use of oncolytic viruses and NK cell-based cell therapies may hold substantial power in the struggle against PDAC. Given the evidence for NK cell involvement in PDAC, expanding NK cell-centred approaches and inclusion of NK cell analysis in PDAC studies is of great importance to further explore their power and gain more profound insight into the role they play in PDAC.

IL-15 stimulated NK cells attack both tumour and pancreatic stellate cells

Since PDAC resistance is mainly caused by the stromal shield, we aimed to stimulate NK cells in such a way that they not only kill pancreatic cancers cells (PCC) but also the PSC, a feature that had not been investigated before. To accomplish this, we chose interleukin (IL)-15, as attractive cytokine since it increases both proliferation and persistence of NK and T cells without stimulating – in contrast to IL-2 – T regulatory cells ~~(12)~~.[12] Moreover, its clinical potential is highlighted since the Cancer Immunotherapy Trials Network (CITN) ranked it third on their priority list of promising immunotherapeutic agents after PD-1/PD-L1 blocking and the CD40 agonists [13]. We showed that IL-15 stimulated NK cells are capable of killing PCC lines (range 9-35%) and interestingly also PSC lines (range 20-50%) in a contact-dependent manner and to a significantly higher extent than unstimulated NK cells ~~(14)~~.[14] Further investigation revealed that this elevated killing was partly dependent on IL-15 induced upregulation of the NKG2D and TIM-3 receptors, which is surprising for the latter since TIM-3 is mostly known as an inhibitory lymphocyte receptor. *Ex vivo* autologous experiments confirmed the killing of primary, patient-derived PSC by IL-15 activated NK cells, thereby underscoring the translational potential of our approach. In addition, our screening for potential targets to further boost immune cell activation as part of a combination strategy revealed the expression of both activating (MICA/B, ULBPs and Galectin-9) and inhibitory (PD-L1, PD-L2) ligands on primary PSC. These data highlight the therapeutic potential of IL-15 to tackle both PDAC tumours and their stromal shield and revealed promising targets to tackle the remaining PSC.

The power of combining immune priming with immune stimulation

Despite our encouraging data on the potential of IL-15, single-compound treatments rarely are completely successful, especially in treating PDAC. Therefore, we sought to enhance the potential of IL-15 by combining it with an immune priming agent, i.e. a CD40 agonist. This choice was based on the fact that CD40 agonists have already shown to upregulate IL-15Rα on antigen presenting cells ~~(15)~~.[15] Additionally, CD40 agonists have proven anti-cancer and anti-stromal properties in PDAC ~~(16)~~.[16] When we combined IL-15 and a CD40 agonist, we showed in two different PDAC mouse models that they exert synergistic effects in terms of strong anti-tumour responses and significantly prolonged survival with most mice even becoming completely tumour-free ~~(17)~~.[17] More in-depth immunological experiments revealed strongly increased numbers of NK cells and cytotoxic T cells in the tumour commensurate with a strong reduction of regulatory T cells. Interestingly, we also observed increased numbers of $CD103^+$ dendritic cells, which have high cross-presentation ability, in the tumour draining lymph nodes. Additional immune cell depletion experiments demonstrated a critical role for cytotoxic T cells and clear involvement of NK cells in the anti-tumour effect. Importantly, this combination regimen also induced strong and long-lasting immune memory, with an increase in memory cytotoxic T cells only when both IL-15 and CD40 agonist were combined. Our preclinical data shows great potential and provides a solid base for an early-phase clinical trial in patients PDAC.

Conclusions

PDAC is till today a disease with a dismal prognosis and due to its unique TME, it also requires a unique approach: not only the tumour itself but also the stromal shield needs to be in the crosshair of our treatments. In this thesis, we provided a solid and extensive overview on the role NK cells play in PDAC and it is clear that they have gained their

rightful spot in the field of cancer immunotherapy. Our studies show evidence for inclusion of NK cell targeting therapies in the treatment of PDAC. Here, IL-15 shows great potential to accomplish improved treatment outcome since it empowers NK cells to target both tumour and stromal stellate cells. Moreover, when combined with an immune priming CD40 agonist, our data shows strong synergistic effects which warrant further investigation of this novel combination in an early-phase clinical trial.

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KEY MESSAGES FOR CLINICAL PRACTICE

- NK cells have a significant role to play in PDAC tumour control. To expand our understandings and insights in their functions in PDAC, it is important to investigate the effects of current and novel therapeutic approaches on NK cells. This will provide crucial information on how standard-of-care treatments can be synergised with novel immunotherapy approaches.
- IL-15 is potent stimulator of the immune system with great potential for cancer immunotherapy. Recent development of IL-15 superagonists with better pharmacokinetic properties are greatly enhancing the potential for clinical applications.
- The combination of immune priming with immune stimulation holds great potential for cancer immunotherapy approaches. Our *in vivo* data warrant a first-in-human, early-phase clinical trial for evaluation of the potential of combined IL-15 and CD40 agonist therapy in patients with PDAC.

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DISCLOSURE

The authors have no disclosures and declare to have no conflict of interest.

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