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Natural Killer cells and their therapeutic role in pancreatic cancer: a systematic review

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Abstract

Pancreatic cancer is among the three deadliest cancers worldwide with the lowest 5-year survival of all cancers. Despite all efforts, therapeutic improvements have barely been made over the last decade. Even recent highly promising targeted and immunotherapeutic approaches did not live up to their expectations. Therefore, other horizons have to be explored. Natural Killer (NK) cells are gaining more and more interest as a highly attractive target for cancer immunotherapies, both as pharmaceutical target and for cell therapies. In this systematic review we summarise the pathophysiological adaptions of NK cells in pancreatic cancer and highlight possible (future) therapeutic NK cell-related targets. Furthermore, an extensive overview of recent therapeutic approaches with an effect on NK cells is given, including cytokine-based, viro- and bacteriotherapy and cell therapy. We also discuss ongoing clinical trials that might influence NK cells. In conclusion, although several issues regarding NK cells in pancreatic cancer remain unsolved and need further investigation, extensive evidence is already provided that support NK cell oriented approaches in pancreatic cancer.

Key Words (6)

Natural Killer (NK) cells; Pancreatic cancer (PDAC); Systematic review; Immunotherapy; Treatment overview; NK Cell biology;

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Table of abbreviations

5-FU: 5-fluoro-uracil
AAV: adeno-associated virus
ADAM: ADAM metallopeptidase domain
ADCC: antibody-dependent cell-mediated cytotoxicity
CCL: chemokine (C-C motif) ligand
CD: cluster of differentiation
CTLA-4: cytotoxic T-lymphocyte–associated antigen-4
CXCL: C-X-C motif chemokine ligand
DNAM-1: DNAX accessory molecule-1
FcγR: Fcγ receptor
GM-CSF: granulocyte-macrophage colony-stimulating factor
Gzm B: granzyme B
Hsp: heat shock protein
IDO-2,3: indoleamine-2,3-dioxygenase
IFN: interferon
IL: interleukin
ILC: innate lymphoid cell
imILT: immunostimulating interstitial laser thermotherapy
IRE: Irreversible Electroporation
KIR: killer cell immunoglobulin-like receptor
MHC: major histocompatibility complex
MICA/B: major histocompatibility complex class I-related chain A and B
NCR: natural cytotoxicity receptors
NK cell: natural killer cell
NKT cell: Natural killer T cell
OS: overall survival
PCC: pancreatic cancer cell
PD-1: programmed death-1
PDAC: pancreatic ductal adenocarcinoma
PD-L1: programmed death-ligand-1
Perf: perforin
PSC: pancreatic stellate cell
TGFβ: transforming growth factor β
TINK: tumour infiltrating natural killer cell
TLR: Toll-like receptor
TME: tumour microenvironment
TNF: tumour necrosis factor
Treg: regulatory T cell
ULBP1-6: UL16 binding protein 1-6
VLP: virus-like particle

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is among the three most deadliest cancers in Western countries, recently overtaking breast cancer (1). The 5-year survival of 7% has barely changed in 50 years and is stated as the worst of any cancer type (1, 2). PDAC has proven to be an extremely difficult-to-treat cancer because of its rapidly progressive nature and high grade of resistance to all conventional, targeted and immunotherapies (3, 4). This is painfully evidenced by an unforgiving reality test when preclinical insights are tested in the clinic (5, 6). Indeed, 47 clinical trials failed to show improvement over gemcitabine treatment, which is in sharp contrast with recent, encouraging discoveries in cancer immunotherapy in other tumour types (6). Even the highly promising immune checkpoint inhibitors programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have had no significant effect in PDAC (7, 8).

The tumour microenvironment (TME) is believed to be a major confounding factor involved in the failure of all these new approaches (9-13). A hallmark of this TME in PDAC is the strong desmoplastic reaction which results in a dense fibrotic/desmoplastic stroma that surrounds the pancreatic cancer cells (3, 12, 13). By acting as a mechanical and functional shield around the tumour, it is responsible for diminished delivery of anticancer agents because it causes a high intratumoural pressure and low microvascular density. The main orchestrator of this fierce stromal barrier is the pancreas stellate cell (PSC). Once activated, these cells enhance the development, progression and invasion of PDAC through their extensive crosstalk with the tumour, resulting in reciprocal stimulation and therapy resistance (3, 12-14).

Immune cells also comprise part of the TME. In this review we focus on natural killer (NK) cells. Although one of their primary functions is to kill cancer cells, less attention has been paid to these cytotoxic immune cells compared to T cells. NK cells are a subset of innate lymphoid cells (ILCs) and comprise about 5-15% of the circulating cell population (15, 16). They were originally identified as an immune cell population with profound tumour cell killing abilities *in vitro* (17, 18). However, numerous studies have since demonstrated their anticancer effect in different animal models as well as their benefit in human studies (16, 19, 20). By using a well-defined set of activating and inhibitory receptors, NK cells are able to recognize and kill tumour cells while sparing healthy cells, more specifically because they sense a certain lack of major histocompatibility complex (MHC)-I molecules via their killer-cell immunoglobulin-like receptors (KIRs) (15). Moreover, once activated, NK cells can secrete a vast number of cytokines and chemokines such as interferon (IFN)γ, tumour necrosis factor (TNF)α, granulocyte-macrophage colony-stimulating factor (GM-CSF), chemokine (C-C motif) ligand (CCL) 1-5 and chemokine (C-X-C motif) ligand (CXCL) 8, which trigger activation and recruitment of other innate and adaptive immune cells that broaden and strengthen the anti-tumour immune response (21). These unique features make NK cells ideal targets for cancer immunotherapy as evidenced by an increasing number of both preclinical and clinical studies that show promising results in different tumour types (15, 16).

However, the role of NK cells in PDAC has not been well defined, given only a few articles have focused specifically on NK cells. Nonetheless, data on their function and importance in PDAC are available in some studies. This systematic review summarises the current evidence on NK cells in PDAC and highlights several possible approaches that could be pursued in future PDAC research. To our knowledge, this is the first systematic review which focusses on the NK cells in pancreatic cancer.

2. Methodology

We employed the Preferred Reporting Items for Systematic Reviews and Meta – Analyses (PRISMA) methodology to conduct this systematic review (22). We performed a search in the highly relevant MEDLINE database (1973 – present) using a list of four terms:

- “NK cells”, “pancreatic cancer” and the Boolean operator “AND”
- “NK cell”, “pancreatic cancer” and the Boolean operator “AND”

- "natural killer cells", "pancreatic cancer" and the Boolean operator "AND"
- "natural killer cell", "pancreatic cancer" and the Boolean operator "AND"

An overview of the systematic review process we employed is shown in the flow-chart (Fig. 1). We included research articles which had pure data on NK cells in PDAC that provided full-text and were written in English. With 'pure' we mean that we excluded reviews and conference proceedings as well as articles that showed effects of peripheral blood mononuclear cells (PBMC) or lymphokine-activated killer (LAK) cells without mentioning effects of NK cells separately. Furthermore, we performed a screening for clinical trials at clinicaltrials.gov. The term we used was "pancreatic cancer" and this included trials with the status "recruiting", "active not recruiting" and "enrolling by invitation". In total, 725 different clinical trials were screened for involvement of immunotherapeutic compounds or strategies and were subsequently linked to compounds and observations described in this review. This resulted in an inclusion of 34 clinical trials in this manuscript. The search was ended on 16 October 2017.

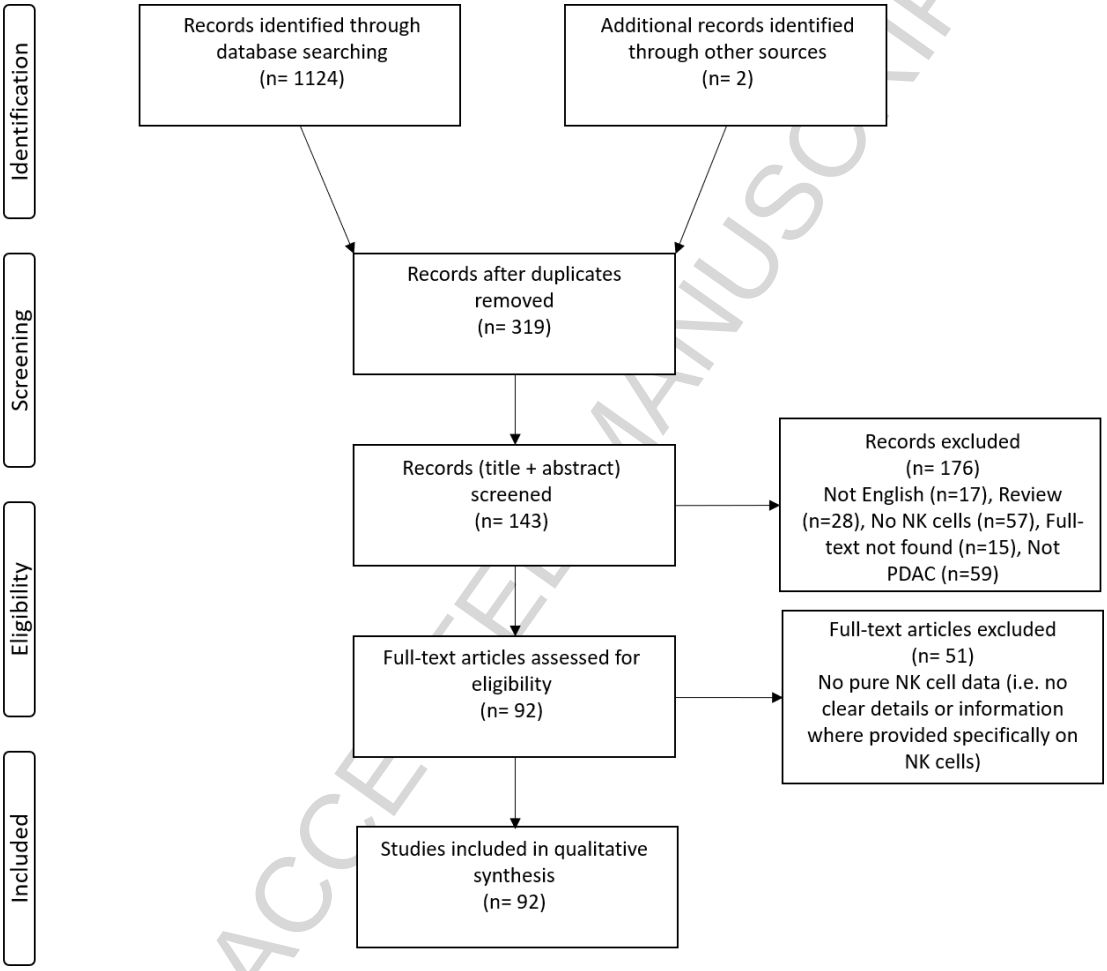

Figure 1. Flow chart delineating the search and screening process of this systematic review.

3. The Biology and physiological changes of NK cells in PDAC

Tumours have an extensive crosstalk with their surrounding microenvironment, including the presence of NK cells and other immune cells (23, 24). Therefore, we first summarise the main facts of NK cells in PDAC and their adaptations caused by the tumour and its TME (Figure 2).

3.1. It's all about the numbers, or not?

The importance of NK cells in pancreatic cancer is shown by the positive correlation between the absolute number of NK cells circulating in treatment-naïve patients and overall survival (OS) (25). Whether treatment-naive patients with PDAC have altered numbers of NK cells in comparison with healthy

volunteers remains a matter of debate, since some studies show that there is no significant difference in percentage of circulating NK cells (26, 27), while other studies demonstrate a significant increase (28-30). These observed differences might be explained by interindividual variations and this issue might be resolved by inclusion of bigger patient cohorts. The value of NK cell activity as a predictive marker for cancer progression is also being investigated in clinical trial NCT02887599.

However, equally important as the quantity of the NK cells is their quality. In general, the cytotoxic capacity of circulating NK cells is reduced in PDAC patients when compared with NK cells from sex- and age-matched healthy controls (25, 26). An important factor in this reduction of cytotoxic activity is less production of granzyme B and perforin, both on a mRNA level as well as on protein level (31-33). Mouse experiments have also shown that increased lactate dehydrogenase activity and lactate production, caused by the altered metabolic phenotype of the tumour, contributed to this reduction in NK cell cytotoxicity (34). In addition, NK cell antibody dependent cell-mediated cytotoxicity (ADCC) is reduced because pancreatic cancer cells secrete IGHG1, a protein which competitively binds with Fcγ receptors on NK cells (35). Finally, their capacity to secrete important immune activating cytokines such as TNFα and IFNγ has also been shown to be reduced (33).

3.2. Downregulation of activating receptors

PDAC has the capacity to alter the balance of signalling in NK cells from an activation to an inactivation state by downregulating the expression of several activating receptors. Here, a first malevolent adaption is the decreased expression of the natural cytotoxicity receptors (NCRs) that are linked to the eradication of malignant cells. Several studies have shown a decreased expression and frequency of NK cells positive for NKp46 and NKp30. This observation that expression of NKp46 and NKp30 is correlated with the pathological state and histological grade of PDAC, supports the importance of these NCRs. Interestingly, this decrease was not observed for NKp44 and NKp80 (32, 33). Tumours also change their TME by production of lactate, a by-product of tumour metabolism. This altered metabolic phenotype also causes a downregulation of NKp46 (34). Finally, the NKp30 ligand B7-H6 is highly expressed on pancreatic tumour cells, driven by the proto-oncogene Myc, indicating that the NKp30/B7-H6 axis could be a potential cancer immunotherapy target (36).

The next important group of NK cell receptors include the nectin and nectin-like binding molecules DNAX accessory molecule (DNAM)-1, T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (TIGIT) and CD96 (TACTILE). Both DNAM-1 (CD226) and TIGIT have been extensively investigated and play a counterbalancing role on NK cells. DNAM-1 has an activating role and potentiates the cytotoxic effect of NK cells against a wide variety of tumour cells while TIGIT limits the IFNγ production and NK cell cytoxicity, hereby preventing damage to self-tissue via inhibitory interactions with MHC-I (37, 38). Less is known about CD96 but a recent study has identified this receptor as a negative regulator of NK cell activity and blocking of this receptor also led to antimetastatic activity. On the other hand, it also promotes NK cell adhesion (37, 38).. In PDAC patients, the activating receptor DNAM-1 and ambiguous receptor CD96, but not the inhibitory receptor TIGIT, have been reported to be downregulated on NK cells and correlated with disease progression (39). In contrast, a higher expression of the ligand CD155 was observed, pointing towards the potential of therapies which upregulate these activating receptors. Binding of CD155 with DNAM-1 and CD96 promotes adhesion of the NK cell to its target cell and also the synthesis of cytotoxic granules that induce the target cell lysis (39). Although this effect is widely proven for DNAM-1, further studies to clarify the detailed function and mechanism of action for CD96 are required.

One of the best characterised NK cell parameters in pancreatic cancer is the activating receptor NKG2D. This receptor is normally expressed on NK cells cells but also on CD8 T cells and subsets of γδ T cells where it provides a co-stimulatory signal (40). Its ligands consist of major histocompatibility complex class I-related chain A/B (MICA/B) and UL16 binding protein (ULBP) 1-6 (41). It has been demonstrated that MICA/B is expressed on tumour cells and pancreatic stellate cells (PSC) in more than 70% of patients while

almost absent in healthy volunteers and patients with chronic pancreatitis (42-46). Additionally, MICA/B also correlates with the disease stage since higher expression is observed in poorly differentiated PDAC (44). A form of tumour escape results from MICA/B being shed into the TME and serum causing an impairment of NK cell function. Again, higher serum levels of MICA/B correlate with advanced disease and distant metastasis as shown by multiple studies but one, indicating that accumulation of these ligands may serve as an evasion mechanism for immune surveillance where NK cells become anergic (42-44, 47). The mechanism of this impairment is still under investigation but several studies point toward involvement of ADAM metallopeptidase domain (ADAM) 10 and ADAM17 causing the shedding, followed by internalisation and degradation upon binding of MICA/B to its receptor NKG2D (47-50). Also the hypoxic TME has been reported to contribute to shedding of MICA/B (48). Recently we and others have showed that NKG2D is downregulated in patients with pancreatic cancer leading to loss of cytotoxic activity (32, 33, 42, 45). To summarize, new strategies that upregulate NKp30, NKp46, NKG2D and/or DNAM-1 on NK cells in PDAC patients are worthy of further investigation.

3.3. Factors in PDAC that influence NK cell activation

In addition to hypoxia in the TME, several other factors including the differentiation status of the tumour, exosomes, PSC and the presence of other immune cell types may influence both NK cell numbers and function in PDAC. For instance, NK cells tend to target cancer stem cells, also in PDAC. These cells are more sensitive to NK cell killing because of their enhanced expression of NKG2D ligands (MICA/B) (51). This phenomenon has been confirmed by the fact that more differentiated tumour cells are more resistant to NK cell killing, caused by upregulation of MHC-I and CD54 and downregulation of CD44 and NKG2D. However, higher differentiated cancer cells were more susceptible to chemotherapy (52).

Similar to the differentiation status, exosomes that are nanometer-sized vesicles of endocytic origin that are released into the extracellular space can also play a role in NK cell evasion of pancreatic cancer. Salivary exosomes from tumour bearing mice were found to reduce the activation levels and suppress cytotoxic capacity of NK cells caused by downregulation of NKG2D (53). In contrast, exosomes originating from heat shock protein (HsP)70/Bag-4 positive pancreatic carcinoma cell lines induced migration and cytolytic activity of NK cells, caused by Hsp70 contact (54). This was also found to be the case when exosomes of a rat PDAC were used (55).

Not only cancer cells but also cells of the TME may influence NK cell activity in PDAC. An important cell in the TME is the PSC, a myofibroblast-like cell which becomes activated upon tumour occurrence and is responsible for formation of the pronounced desmoplastic reaction in PDAC. It has been shown that NK cells tend to migrate more towards activated PSC and not to quiescent PSC. As a consequence, NK cells are sequestrated to the panstromal compartment and not to the juxtatumoural area preventing NK cell anti-tumour function given their lack of close proximity to the tumour cell (56). Better understanding of the interaction between activated PSC and NK cells will be important for future therapeutic approaches. Furthermore the presence of NKT cells has been reported to have a beneficial effect on NK cells. Depletion of these cells resulted in less NK cell activity, given the loss of transactivation by NKT cells. In contrast, reduction of regulatory T cells (Treg), restored NK cell cytotoxicity (57).

It is now clear that NK cells play an important role in PDAC but, as in many tumour types, their function and beneficial effects are rendered suboptimal due to the negative influence of the tumour and its immunosuppressive microenvironment. Therefore, therapies targeting these immunosuppressive effects might be of great potential for future treatments of PDAC. For example, interventions blocking or preventing the shedding of MICA/B or the ULBPs may help in enhancing the NKG2D axis. Furthermore, immune-stimulatory agents, such as interleukin (IL)-15 as we have shown, that induce upregulation of NKG2D, may help reinvigorate this effect. More in-depth research is warranted to untangle the complicated mechanisms of currently poorly understood factors like the CD96 receptor, exosome involvement or the strategic role of PSC and hypoxia in the tumour microenvironment. Additionally, a lot of information is still

to be gained by detailed analysis of the phenotype and activation status of NK cells at the primary tumour site. Future research investigating this gap in our current knowledge will undoubtedly provide valuable insights in how NK cells interact with the tumour microenvironment and how they can be targeted with new therapeutic approaches. This may even reveal new targets and approaches to tackle PDAC on several fronts at the same time.

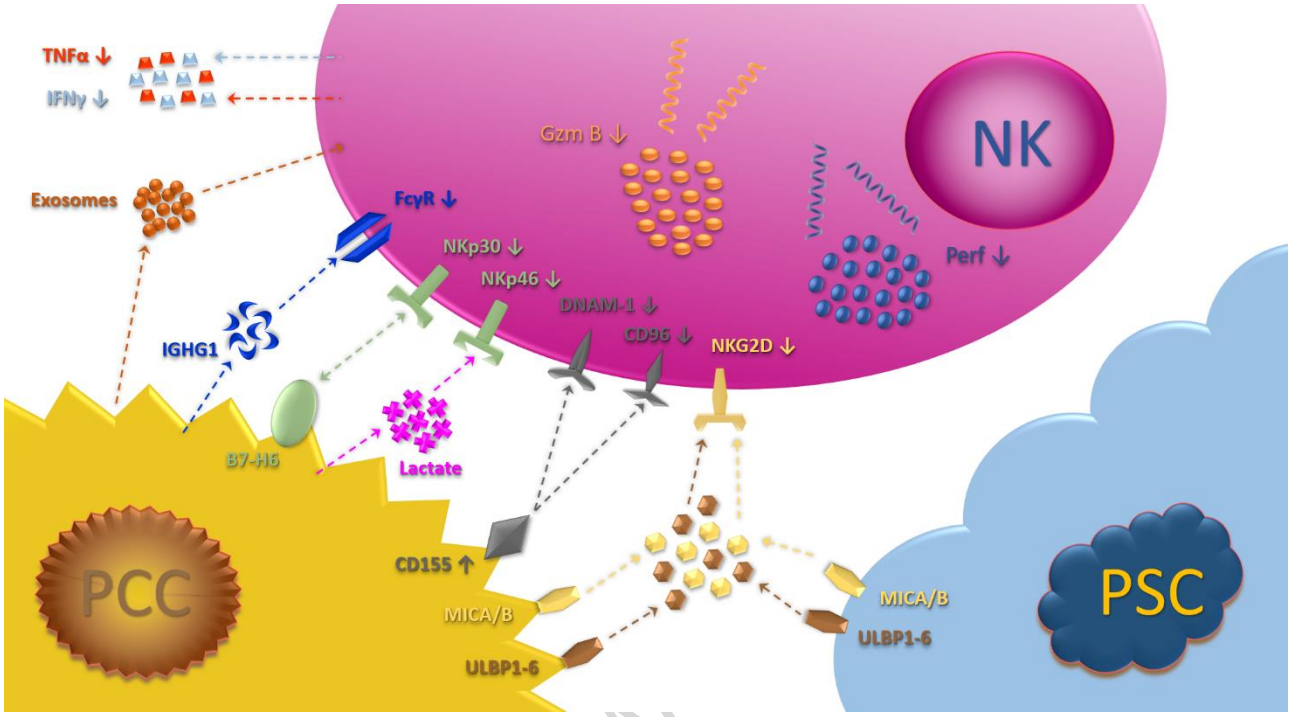

Figure 2. Overview of the pathophysiological changes and interactions of NK cells in PDAC. Abbreviations: GzmB, granzyme B; NK, natural killer cell; PCC, pancreatic cancer cell; Perf, perforine; PSC, pancreatic stellate cell.

4. The effect of the current standard of care in PDAC on NK cells

At present, the standard of care for pancreatic cancer remains surgical removal of the tumour with consecutive chemotherapy (10-20% of the patients) or chemotherapy alone when the patient has locally advanced or metastatic disease (58). Current chemotherapy approaches comprise 2 options: the first one is FOLFIRINOX, a combination regimen of 4 different chemotherapeutic agents (5-FU, oxaliplatin, irinotecan and leucoverin), used in adjuvant setting for patients with metastatic disease or in neo-adjuvant setting for patients with locally advanced disease in attempt to make patients candidate for surgery. The second option is gemcitabine with or without nab-paclitaxel in most cases where the patient is not fit enough to receive FOLFIRINOX (59). However, little is known about the impact of these standard of care approaches on the immune cells. Our search revealed several articles that looked into this important matter (Tables 1 and 2).

4.1. Surgery

Surgical resection of the tumour has been shown to have an effect on the number of peripheral NK cells given an increase was observed 30 days post resection in some patients. This same study also showed that a higher number of NK cells at that 30-day point was correlated with better survival since patients who survived 2 years since surgery had higher median NK cells levels at that 30-day point than deceased patients (60). At present, several clinical studies are ongoing where the effect of surgery (with or without additional physical intervention) on numbers of NK cells is being investigated. These results will provide a solid rationale for future NK cell targeted immunotherapy in the (neo-)adjuvant setting with surgery. This may decrease the frequency of relapse and microscopic residual disease. Decreased cytotoxic activity was

also observed shortly after surgery but normalised by day 30 (60), although an older study did not observe this same effect (61).

Table 1. Overview of ongoing clinical studies investigating immune cell (including NK cell) responses in patients undergoing surgery or other physical intervention.

Study purpose	Intervention	NCT number	Status
Determination of immunologic signatures following surgery for pancreatic cancer	Surgery	NCT03001518	Recruiting
To profile the immune response to Irreversible Electroporation (IRE) of unresectable pancreatic cancers	IRE with NanoKnife LEDC System	NCT02343835	Recruiting
To investigate the functionality and safety as well as understanding of the subsequent immunological effects of Immunostimulating Interstitial Laser Thermotherapy (imILT)	imILT	NCT02973217	Recruiting

4.2. Chemotherapy

In an orthotopic pancreatic cancer mouse model, it has been shown that upon tumour resection and after adjuvant treatment with gemcitabine, NK cells were increased significantly at the resection margins and played an important role in the therapeutic benefit of adjuvant treatment with gemcitabine, since ablation of NK cells (but not CD8 T cells) abrogated this effect of preventing local recurrence and prolonged survival in mice (62). In another study gemcitabine caused increased expression of MICA/B and production of uric acid that sensitized PDAC cells to NK cell killing (43). Furthermore, gemcitabine also inhibited the shedding of ULBP-2 through downregulation of ADAM10, and increased its membrane expression. This resulted in enhancement of NK cell killing of these cell lines (63). The number of NK cells in the peripheral blood was barely affected by gemcitabine since the initially observed reduction was only transient and even restored to healthy control levels, which was not the case for CD4 and CD8 T cells (26, 64-66). In conclusion, gemcitabine seemed to have a positive effect on NK cell activation in PDAC.

In other studies, gemcitabine was shown to have a positive effect in combination with pemetrexed where the latter initially had a negative impact on innate immunity, however the addition of gemcitabine dampened this effect (25). Addition of cetuximab and bevacuzimab to gemcitabine, 5-FU and cisplatin, was reported to induce a significant increase in the number of NK cells in the spleen of mice and led to reduced tumour burden (67). Presently, several clinical trials are investigating the effect of bevacizumab with gemcitabine, FOLFIRINOX or other chemotherapeutic compounds in PDAC patients (Table 3). Interestingly, the combination of lenalidomide and gemcitabine did not alter the absolute number or frequency of NK cells in the blood of patients with advanced PDAC (30).

Table 2. Overview of studies that investigated the effect of chemotherapy on NK cells in PDAC.

Ref	Species	Compound	Effect on NK cells
Homma et al. ***(65)***	Human PDAC patients (n=32)	Gemcitabine	No effect on circulating NK cells
Daikeler et al. ***(66)***	Human PDAC patients (n=3)	Gemcitabine	No effect on circulating NK cells
Soeda et al. ***(64)***	Human PDAC patients (n=28)	Gemcitabine	Transient ↘ # of NK cells
Bang et al. ***(26)***	Human PDAC patients (n=13)	Gemcitabine + Cisplatin	↗ NK cells compared to healthy control levels
Davis et al. ***(25)***	Human PDAC patients (n=16)	Gemcitabine + Pemetrexed	↗ NK cells 7 days after pemetrexed. # of NK cells correlates with OS. ↗ IFNγ producing NK cells after pemetrexed but gemcitabine reduced this effect.
Ullenhag et al. ***(30)***	Human PDAC patients (n=10)	Gemcitabine + Lenalidomide	No effect on circulating NK cells
Tai et al. ***(67)***	BALB/cAnN.Cg-Foxn1^{nu}/CrlNarl nude mice bearing Panc-1 tumours	Gemcitabine + Cetuximab + Bevacuzimab + Cisplatin + 5-FU	↗ NK cells in spleen
Gürlevik et al. ***(62)***	B6.129SKras^{tm4Tyj} x B6.129P2-Trp53^{tm1Brn}/J transgenic mice	Gemcitabine after resection	↗ survival Prevention of local recurrence but not distant metastasis. ↗ # of NK cells at the resection margin
Lin et al. ***(63)***	2 human pancreatic cancer cell lines	Gemcitabine	↗ cytotoxicity ↘ shedding sULBP-2 via ↘ ADAM10 expression
Xu et al. ***(43)***	7 pancreatic cancer cell lines	Gemcitabine + Allopurinol	↗ cytotoxicity due to MICA/B production due to ↗

			uric acid. Allopurinol abrogates this effect.

Table 3. Ongoing clinical trials with chemotherapy investigating NK cells in PDAC.

Compound	Other interventions	PDAC type	Phase	NCT number	Status
Bevacizumab + Andecaliximab + Gemcitabine + nab-Paclitaxel + Permetrexed + Leucoverin + Oxaliplatin + 5-FU + Irinotecan		Not specified	I	NCT01803282	Recruiting
Bevacizumab + 5-FU + nab-Paclitaxel + Leucoverin + Oxaliplatin		Metastatic	I/II	NCT02620800	Active, not recruiting
Bevacizumab + 5-FU + Gemcitabine + Oxaliplatin	Surgery + radiation therapy	Locally Advanced	II	NCT00602602	Active, not recruiting
Bevacizumab + Gemcitabine	Surgery + radiation therapy	Locally Advanced	II	NCT00460174	Active, not recruiting

As chemotherapy is the only treatment option in the majority of PDAC cases, its impact on the immune system seems crucial to understand. However, only a modest number of articles have reported on the effect of gemcitabine, combined or not with another treatment strategy, on NK cell function. Furthermore, 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.

5. Immunomodulating therapies in PDAC with an effect on NK cells

Several agents have been tested in the past for their effect in PDAC resulting in enhancing NK cell function.. This includes the use of cytokines and chemokines.

5.1. Chemokines and Cytokines

Several cytokines including IL-2, IL-10, IL-12, IL-15, IL-18, IL-21, IL-22 and IL-23 and the chemokine CCL-21 were tested in *in vitro* and/or *in vivo* studies in PDAC (Table 4). The most abundant cytokine used in these studies was IL-2. As a stand-alone therapy, it was used via subcutaneous injections pre- and post-surgery resulting in significantly higher levels of circulating NK cells until 1 month after surgery, although no increase in NK cells infiltrating the tumour was found (68). In contrast, recruitment of NK cells towards the tumour was observed when IL-2 was delivered via a parvovirus (69). Furthermore, IL-2 combined with several other compounds such as allicin (organosulfur compound obtained from garlic) (70), L19 (an antibody fragment that targets the extracellular domain of fibronectin) (71), or the chemokine CCL7 (69) in different *in vivo* studies was investigated. Increased numbers and functional activity of NK cells and greater NK cell infiltration into the tumour were observed in all these combination studies. Despite these encouraging results in preclinical models, no clinical trials using IL-2 in PDAC are ongoing (Table 5).

Table 4. Overview of finished studies with chemokines and cytokines influencing NK cells in PDAC.

Ref	Species	Compound	Effect on NK cells
Turnquist et al. ***(72)***	C57BL/6 and $Rag2^{-/-}Pfp^{-/-}$ mice bearing Panc02 tumours	CCL-21	↘ tumour growth and metastasis ↗ TINK
Degrate et al. ***(68)***	Human PDAC patients (n=17)	IL-2	No postoperative NK cell decrease No increase of TINKs
Wagner et al. ***(71)***	3 PDAC orthotopic xenograft models in NMRI nude mice	IL-2 – L19	↘ tumour growth and metastasis ↗ TINKs
Wang et al. ***(70)***	C57Bl/6 nude mice bearing BxPC-3 tumours	IL-2 + allicin	↗ NK cells, ↗ IFNγ production
Bhat et al. ***(73)***	1 human PDAC cell line	IL-2 and IL-15	↗ NK cell cytotoxicity No synergy between IL-2 and IL-15

Dempe et al. ***(69)***	Balb/c nude mice bearing MiaPaCa-2 tumours	IL-2 delivered by parvovirus	↘ tumour growth ↗ TINKs
Chard et al. ***(74)***	2 murine PDAC cell lines C57Bl/6 mice	IL-10 armed Vaccinia virus	No NK cell involvement
Zaharoff et al. ***(75)***	CEA.Tg and C57Bl/6 mice bearing Panc02 tumours	IL-12 + Chitosan	NK mediated tumour regression
Yoshida et al. ***(76)***	BALB/$c^{nu/nu}$ and BALB/$c^{scid/scid}$ mice bearing AsPC-1 tumours	IL-12 and/or IL-15 producing PDAC cells	↘ tumour growth and ↗ survival Involvement of NK cells
Péron et al. ***(77)***	athymic mice bearing Capan-1 tumours	IL-12 producing fibroblasts	↘ tumour growth and ↗ survival ↗ TINKs
Van Audenaerde et al. ***(45)***	3 human PDAC cell lines, 3 human PSC cell lines, 5 primairy PSC cell lines	IL-15	↗ NK cell cytotoxicity ↗ NKG2D and TIM-3 expression
Jing et al. ***(78)***	C57Bl/6 mice bearing Panc02 tumours	IL-15 producing MSC	↘ tumour growth and ↗ survival ↗ TINKs
Guo et al. ***(79)***	C57Bl/$6^{wt/wt}$, BALB/$c^{wt/wt}$ and C57Bl/$6^{IL-18-/-}$ mice	IL-18	↗ in plasma = ↗ OS ↗ intratumoural = ↘ OS ↗ NK cell cytotoxicity ↗ cancer cell proliferation, invasion and metastasis ↗ anti-tumour effect with NF-κB blockade
McMichael et al. ***(80)***	4 human PDAC cell lines 01B74 nude athymic nude mice	IL-21 + Cetuximab	IL-21 ↗ NK cell cytotoxicity ↗ ADCC with combination ↗ IFNγ and chemokine production
Ugai et al. ***(81)***	BALB/$c^{nu/nu}$ and BALB/$c^{scid/scid}$ mice bearing AsPC-1 tumours	IL-21 and IL-23	NK mediated ↘ tumour growth with IL-21 No NK involvement in ↘ tumour growth with IL-23 No synergistic effects between IL-21 and IL-23
Curd et al. ***(82)***	1 human PDAC cell line	IL-22	↘ NK cell cytotoxicity via IL-10 and TGFβ1

Besides IL-2, other cytokines have shown beneficial effects mediated by NK cells towards PDAC *in vitro* and/or *in vivo*. For example, several studies have shown that IL-12 is a strong activator of NK cells and when combined with chitosan, a linear polysaccharide, slowed the growth of PDAC causing prolonged intratumoural retention of IL-12 (75-77). Another strong activator of NK cells is IL-15 and numerous studies have shown this cytokine causes tumour cell killing *in vitro* as well as massive intratumoural NK cell infiltration and tumour growth retardation *in vivo (45, 73, 76, 78)*. In addition, IL-15 stimulated NK cells are also capable of killing PSC, even in an autologous setting. This increase in the therapeutic potential of IL-15 for PDAC treatment is significant given the important immunosuppressive role of PSC (45). IL-21 is another cytokine that has anti-tumour properties mediated by NK cells that are further increased in combination with cetuximab via enhanced ADCC (80, 81). CCL21 causes NK cell mediated tumour reduction both locally as well as in distant locations in mice (72). However, for IL-18 the story is somewhat less straightforward since it has been reported in one study to augment NK cell activity in the peripheral blood and lymph nodes, but also induces proliferation and invasion of tumour cells via the NF-κB pathway (79). Interestingly, blocking the NF-κB pathway resulted in abrogation of these detrimental effects without affecting the beneficial immune-stimulatory effects of IL-18 (79). Notably, some cytokines have been reported to induce an anti-tumour effect although not mediated through NK cells. For example, one study reported that the anti-tumour effect of IL-23 was not NK cell dependent (81) with a similar scenario for IL-10 delivered via a vaccinia virus in murine PDAC (74). Finally, not all cytokines exert anti-tumour effects since IL-22 has been shown to protect PDAC from NK cell mediated killing, promote angiogenesis and anti-apoptotic factors and induce the production of the immunosuppressive substances including IL-10 and transforming growth factor (TGF)β1 (82).

Although IL-2 has been historically the most extensively investigated, it appears to have lost some traction, possibly because of its less favourable clinical safety profile with several side effects such as vascular leak syndrome. Moreover, it can stimulate Tregs, an immune cell population which favours PDAC instead of fighting it. In that light, IL-15 has a more manageable profile because it does not induce vascular leak syndrome, or stimulate Tregs and above all induces CD8 memory T cells. Interestingly, a promising superagonist formulation of IL-15, ALT-803, has demonstrated enhanced NK cell activity ex vivo against human ovarian cancer and multiple myeloma (83, 84). This compound also protects and rescues NK cells

from TGFβ1 mediated immunosuppression and increases graft-versus-tumour effect in murine B cell lymphoma (85, 86). Moreover, it is now being tested in several clinical trials in pancreatic cancer (Table 5).

Furthermore, IL-12 is also being evaluated in several clinical trials. Cytokines clearly have great potential in future treatments of PDAC and optimised formulations might bring great advantage to their clinical safety profile. Combination of these molecules with chemotherapy or surgery may raise more powerful anti-tumour responses.

Table 5. Ongoing clinical trials with cytokines influencing NK cells in PDAC

Compound	Other interventions	Tumour type	Phase	NCT number	Status
IL-10 (AM0010)	Paclitaxel or Docetaxel and Carboplatin or Cisplatin; FOLFOX (Oxaliplatin/Leucovorin/5-Fluorouracil); gemcitabine/nab-paclitaxel; Capecitabine; Pazopanib; Pembrolizumab; Paclitaxel; nivolumab; Gemcitabine/carboplatin	Advanced solid tumours	I	NCT02009449	Active, not recruiting
IL-10 (AM0010)	FOLFOX (Oxaliplatin/Leucovorin/5-Fluorouracil)	Metastatic Pancreatic Cancer	III	NCT02923921	Recruiting
IL-12 (INO-9012)	INO-1400 (hTERT)	Solid tumours at high risk of relapse	I	NCT02960594	Recruiting
Oncolytic Adenovirus producing IL-12	5-FC	Metastatic PDAC	I	NCT03281382	Recruiting
IL-15 Superagonist (ALT-803)	Gemcitabine + Nab-paclitaxel	Advanced pancreatic cancer	I	NCT02559674	Active, not recruiting
IL-15 Superagonist (ALT-803)	CEA expressing adenovirus (ETBX-011)	CEA expressing cancer	I/II	NCT03127098	Active, not recruiting
IL-15 Superagonist (ALT-803)	cyclophosphamide, oxaliplatin, capecitabine, fluorouracil, leucovorin, nab-paclitaxel, bevacizumab, avelumab, NK-92, GI-4000, and ETBX-011.	pancreatic cancer who have progressed on or after previous Standard of Care first line therapy and chemotherapy	I/II	NCT03136406	Recruiting

5.2. IFNα: lessons from the CapRI trials and beyond

The therapeutic approach in the CapRI trial was chemoradioimmunotherapy consisting of 5-FU, cisplatin, IFNα and irradiation (Table 6). First, IFNα as a single agent showed several beneficial effects on NK cells such as an increased activation status and tumour infiltration, as well as higher killing capacities mediated by increased granzyme B release in several studies (46, 87-92). Moreover, *in vivo* mouse experiments showed that addition of 5-FU made PDAC cells more susceptible for NK cell mediated killing by increasing the expression levels of NKG2D ligands Mult-1 and Rae-1 (91). Human trials confirmed these results by also showing an increased activation status of circulating NK cells, however, no increase in NK cell numbers nor their cytotoxic activity was observed (90). In contrast to the positive effects observed in the CapRI trial, a retrospective analysis showed a negative correlation between the frequency of peripheral NK cells and OS. However, the authors could not exclude that this was merely an epiphenomenon (93). Finally, two further studies showed that when combined with either a CEA-expressing poxvirus or the antibiotic doxorubicin, the anti-tumour effect of IFNα was significantly enhanced (88, 94). The results of the CapRI trial and surrounding studies were not clear cut in favour of NK cells, since NK cell involvement was not always shown. Although a clinical trial has been performed and *in vivo* preclinical experiments have shown encouraging results, no present clinical trials are ongoing with IFNα. However, IFNα may still be a useful component in treatment schedules for PDAC, particularly in combination with other modalities.

Table 6. Overview of non-clinical studies with IFNα influencing NK cells in PDAC.

Ref	Species	Compound	Effect on NK cells
Karakhanova et al. ***(90)***	Mice bearing Panc02 tumours Human PDAC patients (n=17)	5-FU + Cisplatin + IFNα + radiation	↗ NK cell activation status ↗ NKG2D expression

			No change in NK cell numbers or cytotoxicity
Hara et al. ***(89)***	Syrian hamsters bearing PGHAM-1 tumours	Adenoviral vectors expressing IFNα	↘ local and distant tumour growth ↗ TINK
Hance et al. ***(88)***	C57BL/6 bearing Panc02 tumours	IFNα + CEA-directed Vaccinia virus	↘ tumour growth and ↗ survival ↗ amount of NK cells ↗ NK cell cytotoxicity ↗ Granzyme B and IFNγ ↗ TINKs
Wang et al. ***(94)***	C57BL/6 bearing Panc02 tumours	IFNα + Doxorubicin	↘ tumour growth ↗ NKG2D ligands on tumour cells
Khallouf et al. ***(91)***	C57BL/6 and CD11c.DOG mice bearing Panc02 tumours	5-FU + IFNα	↘ tumour growth ↗ NK cell cytotoxicity ↗ NKG2D ligands on tumour cells
Schmidt et al. ***(92)***	8 human PDAC cell lines	5-FU + Cisplatin + IFNα + radiation	↗ Granzyme B release ↗ NK cell cytotoxicity
Ohashi et al. ***(46)***	4 human PDAC cell lines BALB/c nude mice bearing AsPC-1 tumours	Adenoviral vectors expressing IFNα	↘ local and distant tumour growth ↗ NK cell activation ↗ NKG2D ligands on tumour cells

5.3. Viro- and bacteriotherapy

Another way to combat PDAC is by using viral particles or modified bacteria to elicit a specific immune response against the tumour. Regarding the use of viruses, several modified viruses have been used including the human Reovirus in combination with the chemotherapeutics paclitaxel/carboplatin (95), a Baculovirus for activation of dendritic cells (96), an adeno-associated virus (AAV)-2 (97), the Newcastle disease virus (98), an oncolytic parvovirus (73) and also Virus-like particles (VLPs) expressing the glycoprotein Trop2 (99) (Table 7). In general, all these approaches resulted in increasing NK cell numbers, and their activation, cytotoxic capacity and infiltration into the tumour. Moreover, the oncolytic parvovirus induced upregulation of NKG2D and DNAM-1 ligands and downregulation of MHC-I and increased IFNγ expression was observed (73). However, an important comment to make for the studies using VLPs and the Newcastle disease virus is that a subsequent adequate adaptive immune response was necessary to sustain the long-term anti-tumour effect, since the presence of abundant NK cells in the tumours led to the rise of an NK cell-resistant tumour cell population with inhibitory properties (98, 99). When considering the use of bacteria, two studies using either a live attenuated Listeria vaccine or the lysate of *Streptococcus pyogenes* show delayed tumour growth caused by both specific and non-specific immune responses, including NK cells (100, 101). However, another study reported that treatment with probiotic bacteria resulted in stronger resistance to NK cell mediated killing of the PDAC given the tumour cells had a more differentiated phenotype (102).

In summary, the described studies demonstrate a potent effect of oncolytic viruses and modified bacteria against PDAC. An additional beneficial side-effect was that these approaches also attracted and activated NK cells, probably because killing of virally infected cells is a basic function of NK cells. A hurdle for the use of oncolytic viruses and bacteria in the clinic might be a certain restraint of the patients towards treatment with a virus. Nevertheless, there is an increasing number of clinical studies examining efficacy of these treatments in PDAC patients (Table 8).

Table 7. Overview of finished studies using viral or bacteriotherapy influencing NK cells in PDAC.

Ref	Species	Compound	Effect on NK cells
Noonan et al. ***(95)***	Human PDAC patients (n=76)	Oncolytic virus Pelareorep	↗ # of NK cells associated with DCR No differences in immunophenotypic biomarkers
Le et al. ***(100)***	ANZ-100: human PDAC patients (n=2) CRS-207: human PDAC patients (n=7)	Live Attenuated Listeria Virus Vaccine (ANZ-100), expressing Mesothelin (CRS-207)	3 patients reached long-term survival (>15 months) Transient ↘ circulating NK cells ↗ NK cell activation
Schwaiger et al. ***(98)***	C57BL/6N mice bearing Panc02 or DT6606PDA tumours	Newcastle disease virus	↗ NK ligands ↘ MHC Class I ↘ Tumour growth ↗ TINKs ↗ systemic NK cell activation
Cubas et al. ***(99)***	C57BL/6 wt, CD4ko, CD8ko, FcgRIIIko, RagI ko mice bearing	Chimeric Trop VLP's	↘ Tumour growth ↗ NK cell activation and infiltration

	Panc02 tumours		
Linnebacher et al. ***(101)***	C57BL/6 mice bearing Panc02 tumours	*S. pyogenes* serotype M49 lysate	↘ Tumour growth ↗ NK cells, ↗ IFNγ and GM-CSF production
Fujihira et al. ***(96)***	BALB/c nu/nu mice bearing AsPC-1 tumours	Baculovirus infected DC	↗ NK cell activation and cytotoxicity ↗ IFNγ
Eisold et al. ***(97)***	Lewis rats bearing DSL6a tumours	Wildtype AAV-2	↗ TINKs No NK cell mediated killing
Bhat et al. ***(73)***	5 human PDAC cell lines	Oncolytic parvovirus H-1PV	↗ IFNγ, TNFα and MIP1-α/β ↗ NK cell cytotoxicity ↗ NKG2D, NKp46 and CD16
Bui et al. ***(102)***	1 human PDAC cell line	Probiotic bacteria	↘ NK cell cytotoxicity by IFNγ and TNFα

Abbreviations: DCR, disease control rate.

Table 8. Ongoing clinical trials with oncolytic viruses influencing NK cells in PDAC

Compound	Other interventions	Tumour type	Phase	NCT number	Status
Oncolytic Adenovirus (Theragene®)		Locally advanced pancreatic cancer	I	NCT02894944	Recruiting
Oncolytic Adenovirus expressing PH20 hyaluronidase (VCN-01)	Gemcitabine + nab-Paclitaxel	Advanced pancreatic cancer	I	NCT02045589	Active, not recruiting
Oncolytic Adenovirus (LOAd70)	Gemcitabine + nab-Paclitaxel	Pancreatic cancer	I/II	NCT02705196	Recruiting
Recombinant Fowlpox (Falimarev) + Recombinant Vaccinia (Inalimarev)	Sargramostim (GM-CSF)	Pancreatic cancer that cannot be removed by surgery	I	NCT00669734	Active, not recruiting
Attenuated mutant of Herpes Simplex Virus Type 1 (TBI-1401(HF10))	Gemcitabine + nab-Paclitaxel	Unresectable pancreatic cancer.	I	NCT03252808	Recruiting
GM-CSF producing attenuated mutant of Herpes Simplex Virus Type 1 (Talimogene Laherparepvec)		Pancreatic cancer	I	NCT03086642	Recruiting
Modified Vaccinia Virus Ankara Vaccine Expressing p53	Pembrolizumab (anti-PD-1)	Solid tumours that have failed prior therapy	I	NCT02432963	Recruiting
Parvovirus H-1 (H-1PV)		Metastatic inoperable pancreatic cancer	I/II	NCT02653313	Recruiting
Wild-type Reovirus	Paclitaxel + Carboplatin	Recurrent or metastatic pancreatic cancer	II	NCT01280058	Active, not recruiting

5.4. Other compounds

Finally, several other therapies that influence NK cells have been tested against pancreatic cancer in preclinical studies (Table 9), resulting in positive NK cell modulation via NKG2D ligand modulation (103-105) or stimulation of different Toll-like receptors (TLRs) (106-108). Furthermore, the effect of checkpoint inhibitors PD-1/PD-L1 and CTLA-4 in vivo has also been investigated, however without any evidence of an impact on NK cell activity (109, 110). Another checkpoint inhibitor, targeting indoleamine 2,3-dioxygenase (IDO-2,3), did show beneficial anti-tumour effects partially mediated by NK cells (33). In addition, delivery of CD40L and 4-1BBL using an oncolytic virus resulted in profound tumour cell killing and immune activation, including NK cells (111). Other studies have used compounds such as curcuminoids, flavone acetic acid, RP101, cobra venom factor coupled with a ^{99m}Tc labelled monoclonal antibody against CA19-9 and bifunctional siRNA which combined TGFβ silencing with RIG-I activation, all demonstrated activation of NK cells resulting in promising anti-tumour effects (112-116). Gene transfer of the Flt3 ligand has also been shown to significantly increase NK cell numbers in the spleen, that was not observed in non-responders, however the anti-tumour effect was not NK cell-mediated (117). Finally, some compounds have had an inhibitory effect on PDAC although there was no significant involvement of NK cells. This includes the antioxidant SkQ1 (118), *in vivo* gene therapy with the Somatostatin Receptor sst2 (119), treatment of low-dose ifosfamide, irradiation and micro-encapsulated cells producing CYP2B1 (120). Of all these different

compounds, only two are currently being tested in clinical trials: a TLR8 agonist and an IDO-2,3 inhibitor (Table 10). Regarding the use of all these different compounds, it is hard to predict which others may make it to the clinic. However, some of these compounds such as RP101, valproic acid and the adenovirus loaded with CD40L and 4-1BBL have demonstrated encouraging results in various *in vivo* mouse models and probably warrant clinical testing.

Table 9. Overview of non-clinical studies using other compounds influencing NK cells in PDAC.

Ref	Species	Compound	Effect on NK cells
Frankel et al. ***(110)***	Human PDAC patients (n=1)	Ipilimumab (anti-CTLA4 antibody)	High amount of NK cells in TIL
Fahrig et al. ***(114)***	Human PDAC patients (n=13 and n=21) 3 human PDAC cell lines	RP101 (+gemcitabine + cisplatin)	RP101 ↗ NK cell cytotoxicity in vitro ↘ chemoresistance ↗ chemotherapy efficacy
Ryschich et al. ***(120)***	male Lewis rats bearing DSL6A tumours	micro-encapsulated cells producing CYP2B1 + ifosfamide + irradiation	↘ tumour growth No NK cell involvement
Ryschich et al. ***(117)***	male Lewis rats bearing DSL6A tumours	FLt3 ligand gene transfer	↘ tumour growth ↗ amount intrasplenic NK cells no increased TINKs
Juhl et al. ***(116)***	Nude rats bearing PancTu-1 tumours	Monoclonal antibody-cobra venom factor conjugate + ^{99m}Tc-labeled Anti-CEA antibody	↗ TINKs
Carrere et al. ***(119)***	Nude mice bearing Capan-1 tumours Syrian golden hamsters bearing PC1.0 tumours	Somatostatin Receptor sst2 gene therapy	↘ tumour growth No NK cell involvement
Schettini et al. ***(106)***	nu/nu Foxn1nu mice bearing KCM tumours	CpG-conjugated anti-MUC1 antibody	↘ tumour growth ↗ ADCC ↗ Perforin
Salazar et al. ***(109)***	C57BL/6 mice bearing KPC tumours	Anti-PD1	↗ survival Role of NK cells not clear
Damia et al. ***(112)***	C57Bl/6 mice bearing PAN/03 tumours	Flavone acetic acid	Inhibition of tumour growth NK cells regulate anti-tumour effect
Eriksson et al. ***(111)***	C57BL/6 Nu/Nu mice bearing Panc01 tumours	LOAd703 (Adenovirus armed with trimerised CD40L and 4-1BBL	↘ tumour growth ↗ NK cell amount ↗ NK cell attracting chemokines
Schneider et al. ***(107)***	C57BL/6 mice bearing Panc02 tumours	MALP-2	↘ tumour growth and ↗ survival ↗ NK cells in spleen
Ellermeier et al. ***(113)***	C57BL/6 mice bearing Panc02 tumours	ppp-TGF-β	↗ NK cell activation Control of tumour growth No NK cell involvement
Bazhin et al. ***(118)***	C57BL/6 mice bearing Panc02 tumours	antioxidant SkQ1	no anti-tumour effect no effect on NK cells
Narumi et al. ***(103)***	C57BL/6 mice bearing Pan02 and Pan02-S100A8/A9 tumours	S100A8/A9	↘ tumour growth ↗ survival ↗ TINKs ↗ NK cell activation ↗ NKG2D ligand-mediated activity
Schölch et al. ***(108)***	C57BL/6 mice bearing Panc02 tumours 2 human PDAC cell lines	3M-011 (TLR7/8 agonist) + Radiotherapy	↘ tumour growth and metastasis ↗ NK cell activation and cytotoxicity via IL-6 DC mediated priming is required
Shi et al. ***(104)***	3 human PDAC cell lines	Valproic acid	↗ NK cell cytotoxicity ↗ Tumour MICA/B expression
Cata et al. ***(105)***	1 human PDAC cell line	Lidocaine	↗ NKG2D expression ↗ NK cell cytotoxicity
Halder et al. ***(115)***	2 human PDAC cell lines	Cucuminoids + ω-3 fatty acids + anti-oxidants	↗ NK cell cytotoxicity ↘ IFNγ
Peng et al. ***(33)***	1 normal human pancreatic ductal cell line 2 human PDAC cell lines	1-MT1 (IDO-2,3 inhibitor) and TIMP-1 (MMP-9 inhibitor)	Restauration of NK cell cytotoxicity Restauration of NKG2D, NKp30, NKp44 and NKp46, DNAM-1, Perforin and Granzyme B No synergy observed

Table 10. Ongoing clinical trials with other compounds influencing NK cells in PDAC

Compound	Other interventions	Tumour type	Phase	NCT number	Status
TLR8 Agonist (VTX-2337)	Pegfilgrastim + Cyclofosfamide	Metastatic, Persistent, Recurrent, or Progressive	I	NCT02650635	Active, not recruiting

		Solid Tumours			
IDO-2,3 inhibitor (Indoximod)	Gemcitabine + nab-Paclitaxel	Metastatic Pancreatic Cancer	I/II	NCT02077881	Recruiting

6. Cell Therapies in PDAC

In this last part, we discuss cell therapies involving NK cells in pancreatic cancer. Several studies have explored adoptive NK cell therapy in PDAC (Table 11). In one study, NK cells were *ex vivo* expanded with antibodies directed against CD3 and CD52 that resulted in higher *in vitro* cytotoxicity and tumour suppression and resulted in better survival in patients up to 13 months following their adoptive transfer. In these patients metastatic lesions also shrunk as well as tumour markers (121). Another *in vivo* study in mice showed promising results on both primary and metastatic PDAC tumour reduction following adoptive transfer of NK cells stimulated *ex vivo* with IL-2 and TKD, an Hsp70-peptide (122). Finally, 2 clinical studies were performed using allogeneic NK cell transplantation in combination with percutaneous irreversible electroporation (IRE). Both studies showed promising results with significantly increased progression-free survival and OS in stage III PDAC and extended OS in stage IV PDAC (123, 124). Currently, 3 clinical trials are ongoing using NK cells of which 2 are in PDAC and 1 in solid tumours including PDAC. Another study reported use of cytokine induced killer cells (Table 12). Overall, the use of NK cell adoptive transfer has so far delivered promising results. Although this type of therapy is often expensive and labour-intensive, it holds great potential in the treatment of PDAC patients in the future.

Dendritic cell (DC) vaccines have been shown to have an impact in PDAC in preclinical models. In an *in vivo* study, mice were vaccinated with DCs, pulsed with tumour RNA, resulting in significant anti-tumour responses driven by NK cells and T cells. However, the RNA pulsed DCs induced significantly less NK cell activity than unpulsed DCs (125). Another *in vitro* study investigated DCs, either pulsed with tumour cell lysate or apoptotic tumour cells, that were injected intratumourally. They observed that DCs pulsed with apoptotic tumour cells instead of cell lysate showed significantly better responses. Interestingly, in this study, the NK cell response was dependent on tumour cell contact and IL-12 produced by the DCs (126). Presently, 4 clinical studies using DCs are ongoing, either with pulsed DCs alone or in combination with chemotherapy or other killer immune cells. Given the promising preclinical results, we are eagerly awaiting the results of these clinical studies.

Table 11. Overview of finished studies using cell therapy involving NK cells in PDAC.

Ref	Compound	Effect on NK cells	Species
Schnurr et al. ***(126)***	Apoptotic tumour cells or cell lysates pulsed DC	Superior DC priming with apoptotic tumour cells ↗ NK cell cytotoxicity ↗ IFNγ	1 human PDAC cell line
Schmidt et al. ***(125)***	Tumour RNA pulsed DC	↘ tumour volume with intratumoural injection ↘ NK cells with pulsed DC	C57BL/6 mice bearing Panc02 tumours
Masuyama et al. ***(121)***	Ex vivo CD3 / CD52 expanded NK cells	↗ NK cell propagation ↗ Activating receptors and KIR2DL1 ↗ NK cell cytotoxicity (in vitro) ↗ anti-tumour effect and survival (in vivo) ↘ tumour markers and metastasis (patient)	2 human PDAC cell lines NRG mice bearing BxPC-3 tumours Human PDAC patients (n=1)
Stangl et al. ***(122)***	Ex vivo IL-2/TKD activated NK cells	↗ NK cell cytotoxicity ↘ tumour growth and ↗ survival ↘ metastasis ↗ TINK	SCID/beige mice bearing Colo357 tumours
Lin et al. ***(123)***	Allogeneic NK cells + percutaneous irreversible electroporation	↗ PFS ↗ OS	Human PDAC patients (n=67)
Lin et al. ***(124)***	Allogeneic NK cells + percutaneous irreversible electroporation	↗ lymphocyte count and function ↘ tumour markers ↗ QOL	Human PDAC patients (n=40)

Table 12. Ongoing clinical trials with DC/NK cell therapy in PDAC.

Cell therapy	Other interventions	Tumour type	Phase	NCT number	Status

DC Vaccine	FOLFIRINOX) or nab-paclitaxel/gemcitabine	Pancreatic Cancer	I	NCT02548169	Recruiting
NK cells using the CliniMACs CD3 and CD56 systems		Lung or prostatic neoplasms, colorectal or kidney neoplasms, pancreatic neoplasms, leukemia, myelogenous, chronic lymphocytic leukemia, BCR-ABL positive melanoma	I	NCT00720785	Recruiting
NK cells	IRE	Advanced pancreatic cancer	I/II	NCT02718859	Recruiting
High-activity NK cells		Small metastases of pancreatic cancer	I/II	NCT03008304	Recruiting
DC activated Cytokine induced killer treatment	S-1	Unresectable locally advanced pancreatic Cancer	I/II	NCT01781520	Recruiting
DCs pulsed with tumour lysate or DCs pulsed with MUC-1/WT-1 peptides		Pancreatic cancer	I/II	NCT03114631	Enrolling by invitation
iAPA-DC/CTL adoptive cellular immunotherapy	Gemcitabine	Advanced Pancreatic Cancer	I/II	NCT02529579	Recruiting
Autologous Cytokine-induced Killer Cells	Tegafur, Gimeracil, Oteracil and Potassium	Advanced Pancreatic Cancer	II	NCT03002831	Recruiting

7. Conclusion

This systematic review provides substantial evidence for the important role NK cells play in PDAC and their potential therapeutic impact. PDAC substantially impairs NK cell functions by downregulation of effector molecules, reduced cytokine secretion capacity and decreased expression of virtually every activation receptor, including the NCRs, nectin- and nectin-like binding molecules and above all NKG2D. Also the TME plays a major role in the reduced cytotoxicity of NK cells. It is therefore of the outmost importance that interactions with this immunosuppressive TME are taken into account in future research for new treatment options. Today, 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, many questions on interactions remain unanswered and need to be explored. Several new compounds are currently being tested in (pre)clinical settings. Here, the potential of cytokine based treatments looks very appealing, especially with the rising of new superagonist compounds like ALT-803. Also the use of oncolytic viruses and 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.

8. Conflict of interest

The authors declare to have no conflict of interest. Jonas RM Van Audenaerde is a research fellow of the Research Foundation Flanders (fellowship number 1S32316N). He received a travel grant from the Research Foundation Flanders (V4.090.17N) to visit the PeterMacCallum Cancer Centre. We also want to express our special gratitude to Mr. Willy Floren and the Vereycken family for their kind gifts which enabled us to perform this work.

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