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Review Article

Harnessing natural killer cells for the treatment of ovarian cancer

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HIGHLIGHTS

- Part of patients receiving NK cell therapy against ovarian cancer reached stable disease.
- NK cell adoptive transfer against ovarian cancer has mild side effects.
- Intraperitoneal infusion might be the future of NK cell therapy in ovarian cancer.
- Combination with checkpoint inhibitors and intraperitoneal NK cells for better OC outcome

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ABSTRACT

Introduction. Adoptive cellular immunotherapy could be an interesting new treatment option for ovarian carcinoma (OC), as research has demonstrated that OC is an immunogenic disease. In particular, natural killer (NK) cells have attracted attention due to their ability to kill tumor cells without prior sensitization. The therapeutic value of allogeneic NK cells has been first observed in hematological cancers and is increasingly being explored in solid tumors.

Methods. To substantiate the rationale for NK cell therapy in OC we performed a literature search in the Pubmed database and in the international trial register *clinicaltrials.gov* with attention for the effect of OC on NK cell function, the effect of current treatment on NK cell biology and the evidence on the therapeutic value of NK cell therapy against OC.

Results. In six clinical trials only 31 OC patients have been reported that received NK cell adoptive transfer. The majority of patients reached stable disease after NK cell therapy, with a mild pattern of side effects. In patients who received repeated infusions, more complete responses are described. All reported studies investigated the intravenous infusion of NK cells. Whereas the studies that are currently recruiting, investigate intraperitoneal infusion of allogeneic NK cells.

Conclusion. In this review the pre-clinical evidence and current trials on NK cell immunotherapy in OC patients are summarized. Furthermore, challenges that have to be overcome for NK cell adoptive therapy to have a significant impact on disease outcome are discussed.

Contents

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1. Introduction

Ovarian carcinoma (OC) is the most lethal gynecological malignancy and despite therapeutic advances the 5-year survival for women with advanced stage disease is only 29% [1]. Because OC is generally asymptomatic until ascites or metastases beyond the pelvis have developed, patients are often diagnosed in advanced stage. Current therapy consists of debulking surgery combined with platinum and taxane based chemotherapy, but the majority of patients develop a recurrence within 3 years [2]. Immunotherapy could be an interesting option, since OC is an immunogenic disease with the presence of T and natural killer (NK) cell infiltration in the tumor [3]. Interestingly, the presence of tumor-infiltrating CD3+ T cells positively correlates with survival in OC patients [4–6]. Furthermore, it was reported that CD103+ tumor-infiltrating NK cells often co-infiltrate with CD8+CD103+ T cells, but the contribution of NK cells to improving outcome is difficult to assess [4]. Recently, we described that the frequency of NK cells within the lymphocyte fraction in ascites is positively related to overall survival suggesting that NK cells are involved in anti-OC immune responses [7]. Numerous preclinical studies showed that OC is susceptible to NK cell attack [7–12]. But tumor cells develop various strategies to inhibit proper anti-tumor immune responses, and it has been demonstrated that patient's NK cells in the tumor microenvironment are less cytotoxic and have an 'exhausted' phenotype [13]. Therefore, various strategies are being explored to develop highly effective NK cell products for adoptive transfer in cancer patients. Allogeneic NK cell therapy has shown to be safe and well tolerated in clinical trials in hematological cancers [14]. In particular, complete remission in refractory acute myeloid leukemia has been reported in small scale clinical trials [15,16]. In this review, we discuss the current published evidence on harnessing NK cell therapy towards OC as well as the most promising strategies for maximizing the results of NK cell therapy in OC patients.

2. NK cell recognition of cancer cells

One of the strengths of NK cells to exploit in cancer immunotherapy is their ability to kill tumor cells without prior sensitization [17]. This capacity of these large granular lymphocytes was first described by Herberman in 1975 [18]. Furthermore, a seminal epidemiological study showed that low NK cell activity is associated with increased cancer risk in humans. Moreover, numerous preclinical studies have indicated that NK cells target and kill cancer cells and spheroids [14]. This cytotoxic response against neoplastic cells is regulated through a fine balance of activating and inhibitory receptors [19]. Unlike cytotoxic T cells, NK cell recognition of tumor cells is not antigen-dependent but is dependent of the balance of triggering by activating and inhibitory receptors. For preventing autoreactivity, NK cells are tolerant to healthy cells due to the expression of killer immunoglobulin-like receptors (KIRs) and the C-type lectin-like receptor NKG2A, which interact with classical and non-classical major histocompatibility complex (MHC) molecules, respectively. Upon engagement of these inhibitory receptors, NK cells transmit intracellular signals preventing activation and cytolytic activity. Thereby, loss of MHC I expression on tumor cells triggers NK cell activation and tumor killing, the so-called 'missing-self recognition'. Later studies showed that lack of MHC expression was not sufficient to induce NK cell activation, but signaling from activating receptors is required [20].

It has been revealed that upon activation, NK cells can upregulate expression of various activating receptors including DNAX Accessory Molecule-1 (DNAM-1), the natural killer group 2 member D (NKG2D) and natural cytotoxicity receptors (NCRs; NKp30, NKp44 and NKp46). These receptors provide activating signals upon binding to stress-induced ligands on tumor cells, which is referred as 'induced-self recognition' [20]. In addition, cytotoxic NK cells express CD16 that interacts with the Fc portion of IgG antibodies, allowing NK cells to be activated by tumor cells coated with tumor-targeting antibodies (*i.e.* antibody-dependent cellular cytotoxicity; ADCC). In case of triggering more activating signals on the NK cell surface than inhibitory signals, the cytolytic machinery towards tumor cells will be effectuated by several mechanisms. These mechanisms include release of cytotoxic granules containing perforin and granzymes, and the induction of tumor cell apoptosis through TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand on the NK cell. Lastly activated NK cells produce pro-inflammatory cytokines, including interferon gamma (IFN-γ), promoting innate and adaptive anti-tumor immune responses.

3. NK immunobiology in ovarian cancer

3.1. NK cells in the OC tumor environment

While the importance of CD8+ T cell infiltration in ovarian cancer tumors has been clearly demonstrated, the role of infiltrating innate NK cells remains unclear [4]. Garzetti et al. reported that the amount of NK cells in peripheral blood was significantly lower in patients at time of disease progression [21]. But it has been found that CD103+ (which is marker for tissue-resident immune effector cells) tumor-infiltrating NK cells often co-infiltrate with CD8 + CD103+ T cells [4,22].

Furthermore, we recently found that the frequency of NK cells within the lymphocyte fraction in ascites at diagnosis is related to better overall survival in OC patients [7]. However, most support comes from *in vitro* studies showing susceptibility of OC to NK cell mediated killing [8–11,21,23–25], and live-imaging confocal microscopy demonstrates that NK cells efficiently infiltrate and kill OC cells in 3D tumor spheroids [23]. A better understanding of tissue immunity is necessary to be able to manipulate this therapeutically.

3.2. Effects of ovarian cancer on NK cells

The immune defense against cancer may be weakened by secretion of immunosuppressive cytokines by tumors or infiltrating suppressive immune cells, augmenting the expression of inhibitory receptors on immune cells and inducing downregulation of MHC I molecules on malignant cells. All these principles also apply to OC. In ascites high

concentrations of the immunosuppressive cytokines TGF-β and IL-8 have been described [26,27]. TGF-β can effectuate immunosuppression in two ways: first it induces a strong downregulation of CD16 on NK cells, thereby impairing antibody dependent cytotoxicity. Second, it contributes to downmodulation of NKp30 and NKG2D, both important NK cell activating receptors. Also expression of the inhibitory ligand B7-H6 on tumor cells impairs NK cell effector function, and OC cells can excrete soluble B7-H6 leading to downmodulation of NKp30 [28,29]. Gubbels described that MUC16 expressing ovarian cancer cells are protected from recognition by NK cells. The immune protection provided by MUC16 may lead to selective survival of ovarian cancer cells that are more efficient in metastasizing within the peritoneal cavity and also at overcoming anti-tumor innate immune responses [24,30]. Another activating NK cell receptor that is downregulated by factors (such as CD155 and MUC16) in the tumor microenvironment is DNAM-1, thereby diminishing NK cell activation towards OC [12]. Furthermore, NK cells are equipped with several NK cell specific inhibitory receptors (KIRs and NKG2A), and in addition can express non-MHC binding inhibitory receptors like programmed death-1 (PD-1) and T cell immunoreceptor with Ig and ITIM domains (TIGIT). It has been found that PD-1 positive NK cells exhibit a strongly reduced cytotoxic capacity against OC [31] and that NK cell function could be boosted by TIGIT blockade in a subset of patients [32]. Antibody blockade of these inhibitory factors of NK cell functionality can be used for future combination therapy with adoptive transfer strategies.

3.3. Pre-clinical murine studies of NK cell therapy in OC

Pre-activated or expanded NK cells recognize and destroy cancer cells *in vitro* [9,10,15,33–40]. Moreover, *ex vivo*-expanded NK cells from peripheral blood and ascites of ovarian cancer patients are cytotoxic against autologous primary OC cells [25]. Adoptive transfer studies performed with different allogeneic NK cell products in human OC xenograft models have shown promising results. Hermanson et al. demonstrated in a mouse model with the OC cell line MA148 that intraperitoneal (IP) delivery of induced pluripotent stem cell (iPSC)-derived NK cells inhibits tumor growth [10]. Similarly, hematopoietic stem and progenitor cell (HSPC)-derived NK cells generated by a combined SR1/IL-15/IL-12 based culture protocol mediate an anti-OC effect and significantly prolong the survival of SKOV-3 bearing mice. To further boost these NK cell mediated anti-OC responses, adoptive transfer can be combined with NK cell stimulating cytokines including IL-2 and IL-15 [41]. Mostly IL-2 has been used with allogeneic NK cell transfer but IL-15 might be a more effective stimulator and does not stimulate regulatory T cells that impair NK cell functionality. In this regard, the IL-15 superagonist ALT-803, which is a hybrid molecule of a mutated IL-15, IL-15 receptor α and IgG1 Fc, is very promising. In the presence of ALT-803 higher NK cell mediated anti-tumor effects were reported [8]. Another strategy to circumvent the immunosuppressive nature of OC could be the cytokine induced memory like (CIML) NK cells. These CIML NK cells have enhanced functionality and persistence against ovarian cancer *in vitro* and *in vivo*, even when exposed to ascites fluid [42,43]. Finally, in a murine model with patient-derived OC tumors, allogeneic *ex vivo*-expanded NK cell therapy has shown reduced tumor growth, improved survival and prevention of systemic metastasis [44].

3.4. Effect of current standard of care in OC on NK cell functionality

Current standard of care in OC comprises counseling, chemotherapy and surgery, combined with targeted therapy. Surgery by itself might have impact on NK cell functionality. In other cancers, Iannone et al. described that surgical resection of the tumor has an effect on NK cell count in the peripheral blood and that a higher number of NK cells on day 30 after surgery is related to better survival [45]. In addition, the used technique of anesthesia at surgery can have impact on the cytolytic activity of NK cells. Propofol anesthesia demonstrated a favorable impact on immune function in breast cancer surgery by preserving NK cell cytotoxicity, compared with sevoflurane anesthesia [46].

In OC patients, carboplatin-paclitaxel is the main chemotherapeutic regimen. It is known that paclitaxel inhibits NK cell-mediated killing without affecting the viability of NK cells, while carboplatin has no effect on killing capacity or on viability of NK cells *in vitro* [47]. In recurrent OC other chemotherapeutic regimens are applied, such as a combination with gemcitabine. Lin et al. described that gemcitabine treatment impaired ULBP2 shedding in pancreatic cancer, thereby potentially enhancing NK cell activation [48]. Another new agent in OC treatment is the poly ADPribose polymerase (PARP) inhibitors. Recently, it was shown in prostate cancer that the PARP inhibitor olaparib significantly increased tumor cell sensitivity to NK cell mediated killing [49]. Future research should focus on finding the best selection of treatment agents to be combined with adoptive NK cell therapy.

The social support of patients may also be an important factor in treatment of patients with OC. This is highlighted by the research of Lutgendorf et al. [50]. In this study, patients with greater social support had higher levels of NK cell activity both in peripheral blood as in tumor infiltrating lymphocytes (TILs), whereas patients with higher levels of distress had lower cytotoxicity activity in TILs. A multivariate model indicated independent associations of both distress and social support with NK cell activity in TILs [50]. In breast cancer, psychosocial interventions have also shown to have a positive effect on NK cell cytotoxicity, and these changes could be explained by adrenergic immunomodulating mechanisms [51]. Together these findings show that current standard of care has an effect on NK cell cytotoxicity and that it is important to further explore these interactions in OC to be able to develop the best strategy and the best timing for combining adoptive NK cell therapy with current care.

4. NK cell-based immunotherapy in cancer

In 2003, Ruggeri et al. showed that after haplo-identical hematopoietic stem cell transplantation, allogeneic NK cells (generated from the donor graft in the recipient) support graft-*versus*-tumor effects and reduce leukemia recurrence in patients with acute myeloid leukemia (AML) [52]. In this setting, NK cells are thought to become reactive to the tumor cells through the 'missing-self hypothesis', where the NK cell becomes reactive against the tumor cells that lack donor-specific self MHC I molecules [17]. This finding paved the way for NK cell adoptive transfer studies in hematological cancers. Autologous NK cells from peripheral blood have been investigated but have not proven to induce clinical benefit in solid tumors, potentially because their functional status is often poor and effectivity is inhibited by the autologous MHC I expression on tumor cells. Allogeneic NK cell therapy is well tolerated in hematological as well as in solid cancers, but various challenges need to be overcome in order to make NK cell therapy more efficient. After intravenous (IV) infusion of allogeneic NK cells, there is effective expansion in a minority of patients and limited persistence of NK cells in the long term. Besides, due to the combination with IL-2, regulatory T cells may suppress NK cell activity [15]. Interestingly, allogeneic NK cells seem to be more effective in consolidation therapy than in refractory patients. As a consolidation therapy in both pediatric and adult AML patients allogeneic NK cell adoptive transfer showed prolonged remission [53,54]. Furthermore, complete remission in refractory disease has been reported in small scale clinical trials [15,16]. Moreover, as a bridge to allogeneic stem cell transplantation in AML patients, NK cell therapy might have contributed to a durable response [15,16]. NK cell therapy is often co-administered with a preparative chemotherapy regimen of cyclophosphamide and fludarabine, based on comparative studies comparing different preparative regimens [55]. NK cell therapy is increasingly being explored for solid tumors such as neuroblastoma, pancreatic cancer, hepatocellular carcinoma, breast cancer and melanoma [56]. A promising sign is, in all clinical trials in solid tumors, allogeneic NK cells are well tolerated and no graft-*versus*-host-disease

(GVHD) is reported [14]. Due to the findings that allogeneic NK cell therapy has mild side effects and does not induce GVHD, as they selectively attack malignant transformed or virus-infected cells, they are an excellent candidate to use in cancer immunotherapy.

Although allogeneic NK cell therapy showed promise, an easily accessible NK cell product that is available as an 'off-the-shelf' product in large quantities, has yet to be found. Different NK cell products from different sources have been used in clinical trials. Donor peripheral blood apheresis is the most common source, from which an activated NK cell product is produced through enrichment by CD56 positive selection and/or CD3 T cell depletion followed by short stimulation with activating cytokines (IL-2, IL-12, IL-15, IL-18). Other cell sources that are used for production of NK cell therapeutics are umbilical cord blood (UCB) and bone marrow [14]. Rezvani et al. isolated and expanded resident CD56+ NK cells from UCB for clinical application [57]. Furthermore, UCB provides a rich source of HSPCs from which high numbers of therapeutic cells, including NK cells can be generated with potent immune effector functions [53]. Exploiting UCB has the advantage of non-invasive collection and off-the-shelf availability of large number of units from UCB banks worldwide. Interestingly, NK cells can also be efficiently generated *ex vivo* from induced pluripotent stem cells (iPSC), which have shown to have high cytotoxicity against OC cells [10]. These HPSC- and iPSC-derived NK cell products can be utilized as an off-the-shelf NK cell therapeutic, and clinical trials have commenced to explore their potential. Another off-the-shelf possibility is use of the cell line NK92, which is being investigated as an allogeneic NK cell therapeutic. The major advantages of this lymphoma derived cell line, with of-the-shelve availability are counterbalanced with the risk of tumor engraftement. Because of this risk NK92 cells need to be irradiated before application. This radiation affects the survival and engraftement of the cells.

A clinical trial involving advanced lung cancer patients showed encouraging results, and additional trials are ongoing to validate the safety profile and clinical value of NK92 cells [58].

5. NK cell-based immunotherapy in ovarian cancer

5.1. Literature search

We conducted a search in the Pubmed database using the combination of the following items.

-NK cell, NK cells, natural killer cell, natural killer cells, ovarian cancer.

To find all current and future trials on natural killer cells and ovarian cancer we searched at clinical trials.gov with the following search terms: ovarian and natural killer. In total 21 trials were screened for relevance. Doubles were excluded. This resulted in the inclusion of 6 trials in this review (Table 1).

5.2. Pioneering studies

Although NK cell therapy is broadly investigated in hematological malignancies, there is yet limited data in ovarian cancer. In six clinical studies only 31 patients are described that received NK cell adoptive transfer. The largest study on allogeneic NK cell adoptive transfer in OC is the study of Geller et al., which included 14 OC patients [59–61], while the other reports are case series. Patients were pretreated with cyclophosphamide and fludarabine (Cy-Flu) and in 5 patients the Cy-Flu was combined with total body irradiation to improve *in vivo* NK cell expansion. Included patients were heavily pretreated with median seven lines of prior chemotherapy. Patients were infused with an average of 2.2×10^7 NK cells/kg and 6 subcutaneous dosages of 6 million units IL-2. Overall this regimen was well tolerated, but a severe adverse event occurred leading to death after tumor lysis syndrome. Although no sustained NK cell expansion in the periphery was noted, the evaluation after three months by computed tomography (CT) showed that 4 patients had partial response, 8 had stable disease and 1 progressive disease. The response on CT might be attributed to the NK cells or the cyclophosphamide containing preparative chemotherapy. Long term follow-up of patients is not available.

The study by Yang et al. investigated allogeneic NK cells against various cancers, included two OC patients, who were treated with healthy donor-derived allogeneic NK cells from peripheral blood mononuclear cells that were *ex vivo*-expanded after CD3-based T cell depletion. This *ex vivo*-expanded NK cell product was administered in a single dose in the first cohorts and repeated infusions were administered in the later cohorts. In one patient with advanced recurrent OC, stable disease was reached with one NK cell infusion of 10^6 cells without prior immunodepleting chemotherapy, whereas the other OC patient had progressive disease after 3 weekly infusions of 10^6 cells [61]. The latter patient was heavily pretreated with chemotherapy in the 139 months before NK cell therapy was given.

Table 1
Review.

Trial status	Year	No of patients	Population	Phase	Treatment	Pretreatment	NCT/hospital/ref	Clinical response	NK cell response
Terminated	2010	12	Refractory disease	I	Allogeneic IV NK + IL2	Cy/flu	NCT00652899 Masonic Cancer Center	3 PR, 8SD, 1PD	
Completed	2011	20 (14 OC)	Refractory disease >3 prior therapies	II	Allogeneic IV NK+ IL2	Cy/flu 4× with TBI	NCT01105650 Geller et al	Well tolerated 2 SAE including 1 death.	No sustained *in vivo* expansion of NK cells
Completed	2016	20 (2 OC)	Advanced disease	I	Allogeneic IV NK	None	Yang et al		
Completed	2017	1	Primary treatment	Case report	Allogeneic IV NK	None	Xie et al	Partial response	Expanded NK cells in culture?
Completed	2017	2	Refractory disease	I	Allogeneic IP NK+ IL2+ IDO	Cy/flu	NCT02118285 Masonic Cancer center Unpublished	na	na
APOLLO, recruiting	2018	4	Refractory disease >4 prior therapies	I	Allogeneic IP NK+ IL2		NCT03213964 FATE press release, unpublished Masonic Cancer center	1 progressive disease 1 stable disease 6.2 months, other 2 na	Well tolerated NK Persistence after 2 weeks
Recruiting	2018	200 (40 OC)	Refractory disease >3 prior therapies	I/II	Autologous IV NK repeated	None	NCT03634501 Xuanwu Hospital		
INTRO, recruiting	2019	12	Recurrent OC <2 prior therapies	I	UCB IP NK+ IL2	Cy/flu or none	NCT03539406 Radboudumc		

The case report of Xie et al. described the first OC patient treated with NK cells as primary treatment. A 60 year old patient, diagnosed in advanced stage OC with massive ascites and a sizeable tumor was treated with *ex-vivo* expanded, highly activated allogeneic NK cells [60]. These infusions of *circa* 1.5×10^9 NK cells were administered every 2 weeks and a total of 6 infusions were given. Patient experienced no side effects and showed clinical recovery. Her CA125 level dropped from 11,270 to 580, and all ascites disappeared. Furthermore, the masses on CT scan reduced in volume.

5.3. Intraperitoneal NK cell therapy

Since OC is confined to the peritoneal cavity for the majority of patients till the last stage, IP treatment is a rationalistic approach. Recently, ovarian hyperthermic intraperitoneal chemotherapy (OVHIPEC) treatment has shown to prolong median overall survival with 12 months and combined IP and IV chemotherapy increased the overall survival with 15 months and progression free survival with 6 months [62,63]. Because the GOG252 trial produced no significant differences in PFS after IP chemotherapy combined with bevacuzimab in OC patients, we are still waiting for the randomized controlled Asian trial on IP chemotherapy (iPocc NCT01506856) [64]. However, studies have shown that IP delivery can achieve a 20-fold increase in cisplatin concentration and a 1000-fold increase in local concentration of paclitaxel, with ensuing gains in OS [62,63]. Therefore, direct delivery of new therapeutic agents including cellular immunotherapy could be very effective approach. Intraperitoneal NK cell therapy could potentially bypass the depletion of transferred cells by the liver and spleen, giving the cells the opportunity to encounter the cancer cells directly in the abdominal cavity. Therefore, IP infusion of NK cells could enhance the effectiveness of NK cell transfer against OC in the same compartment as the disease.

The APOLLO trial investigating the feasibility of IP FATE-NK100 (*ex-vivo* expanded terminally differentiated adaptive NK cells) infusion following outpatient chemotherapy in platinum resistant OC patients is currently recruiting (NCT03213964). The preliminary results presented on the Innate killer Summit 2018 showed the results of the first 2 patients, wherein the first patient in the first dose level showed progressive disease on day 28, but the second patient who had repeated NK cell infusion, showed stable disease with evidence of tumor reduction on the first time point. In China, a trial is being conducted investigating autologous *ex vivo*-expanded NK cell therapy in 200 cancer patients of whom 40 with OC. Repeated IV infusions will be given in a three-weekly regimen, and the NK therapy will be combined with chemotherapy and targeted therapies (NCT03634501). We recently opened the INTRO trial, which investigates the safety and feasibility of a single IP infusion of highly activated NK cells, generated *ex vivo* from CD34+ umbilical cord blood HSPCs in recurrent OC patients (NCT03539406). Next to feasibility, safety and toxicity, we will address the question whether a lymphodepleting chemotherapy regimen is needed or not for peritoneal expansion and persistence of allogeneic NK cells.

So far the completed trials and case series showed that allogeneic NK cell therapy is overall well tolerated, might have a beneficial effect on clinical outcome but needs to be investigated further for sustainable NK cell expansion and clinical effects *in vivo*.

6. Concluding remarks and future perspectives

In this review, the current status of NK cell research in harnessing OC and early results of NK cell therapy trials in OC are summarized. Substantial pre-clinical evidence has been provided for the role of NK cells in OC and their potential therapeutic impact. However, besides the promising results there are significant challenges to address. The immune suppressive microenvironment of ovarian carcinoma should be investigated and manipulated to give NK cell therapy more opportunity. The question of limited NK cell expansion *in vivo* should be addressed, in order to maximize the anti-OC effect. Current trials on IP NK cell therapy might bring these answers. Furthermore, different sources to produce an easily available allogeneic NK cell product are being developed. Ultimately, a standardized protocol for highly active NK cell preparation off-the-shelf would be required to bring NK cell therapy to the clinic. Lastly, NK cell based immunotherapy could be further maximized by combination with other agents.

There are several pre-clinical studies that describe ways to maximize the effect of NK cell adoptive transfer, which can be roughly divided in 5 strategies: 1) genetic modification, 2) combination with more efficient cytokines, 3) construction of chimeric antigen receptor (CAR)-NK cells, 4) combination with checkpoint inhibitors or 5) combination with targeted therapies. Since the widely co-administered cytokines have side effects, it is an attractive option to genetically modify NK cells to express exogenous cytokines. An example of these is genetically modified NK92 cells carrying high affinity CD16 receptors and expressing IL-2. Currently a clinical trial on these modified NK92 cells is including patients with Merkel cell carcinoma [65]. Moreover genetic modification with IL-15 can be a promising approach for cancer immunotherapy [66,67]. Expanded NK cells expressing membrane bound (mb)IL-15 showed increased cytotoxicity *in vitro* and *in vivo* [67]. Another way to make NK cells more effective is to combine them with a more efficient cytokine, the IL-15 superagonist: ALT-803. A trial investigating ALT803 in ovarian carcinoma patients is conducted now in the USA (NCT03054909).

Improvement of functionality in T cell therapy has been reached by the chimeric antigen receptor T cells (CAR-T). Currently, CD19 CAR-T cells are FDA and EMA approved for treatment of certain hematological cancers (*i.e.* acute lymphoblastic leukemia and diffuse large B cell lymphoma), whereas CAR-NK cells are still in the early clinical phase [57]. Li et al. investigated recently a CAR-iSPC-NK product containing the receptor NKG2D, the 2B4 co-stimulatory domain, and the CD3ζ signaling domain in an OC xenograft. They showed that these CAR-iPSC-NK cells significantly inhibited tumor growth and prolonged survival in an OC xenograft, with comparable activity to that of CAR-T cells [68]. Antigen specificity of NK cell responses can also be improved by better engagement of NK cells to the tumor. Constructs are generated wherein the CD16 targeting scFv domain is fused with a tumor antigen targeting scFv domain such as CD19, CD20, CD33, CD133 or EpCAM (*i.e.* bispecific killer cell engagers or BiKEs) [69]. Additionally, IL-15 can be added in the construct, named TriKEs, to further potentiate the NK cell mediated anti-tumor effect [69]. These BiKEs and TriKEs efficiently stimulate NK cell responses *in vitro* in hematological cancers and solid tumors [69,70].

Another combination therapy that originates in T cell research is the checkpoint inhibitors. Despite the huge success of checkpoint inhibitors in melanoma and lung cancer, OC patients don't experience the success and there is also rarely but extensive toxicity associated with these therapies [71]. A combination with NK cell adoptive therapy has yet to be explored. However, promising results are shown for PD-L1 blockade in combination with NK cell therapy in an OC mouse model [72]. Preclinical work in other solid cancers shows that blocking of TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity [73]. In addition, the combination with current standard therapies for recurrent disease including gemcitabine, PARP-inhibitors and bevacizumab has yet to be explored.

Already being investigated is the step to peritoneal infusion of allogeneic NK cell adoptive transfer in the current clinical trials. Peritoneal infusion might make NK cell therapy more valuable in ovarian cancer treatment. All together it is encouraging that there is sufficient preclinical evidence on the susceptibility of OC to NK cell cytotoxicity, the first clinical trials on NK cell adoptive transfer have been performed and current research progresses on maximizing the effect of NK cell adoptive transfer. In the near future more clinical evidence on the applicability of NK cell adoptive transfer against ovarian carcinoma is expected.

Authorship contributions

JHE performed the review process, and wrote the manuscript; RB, NO, JJ and LM provided advice and reviewed manuscript; HD supervised research and revised the manuscript.

Declaration of competing interest

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