Immune-based therapies in ovarian cancer

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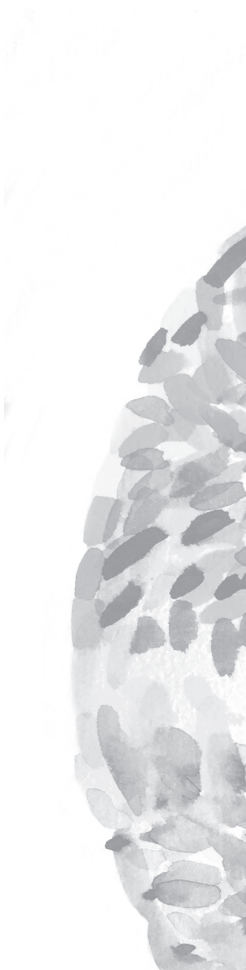
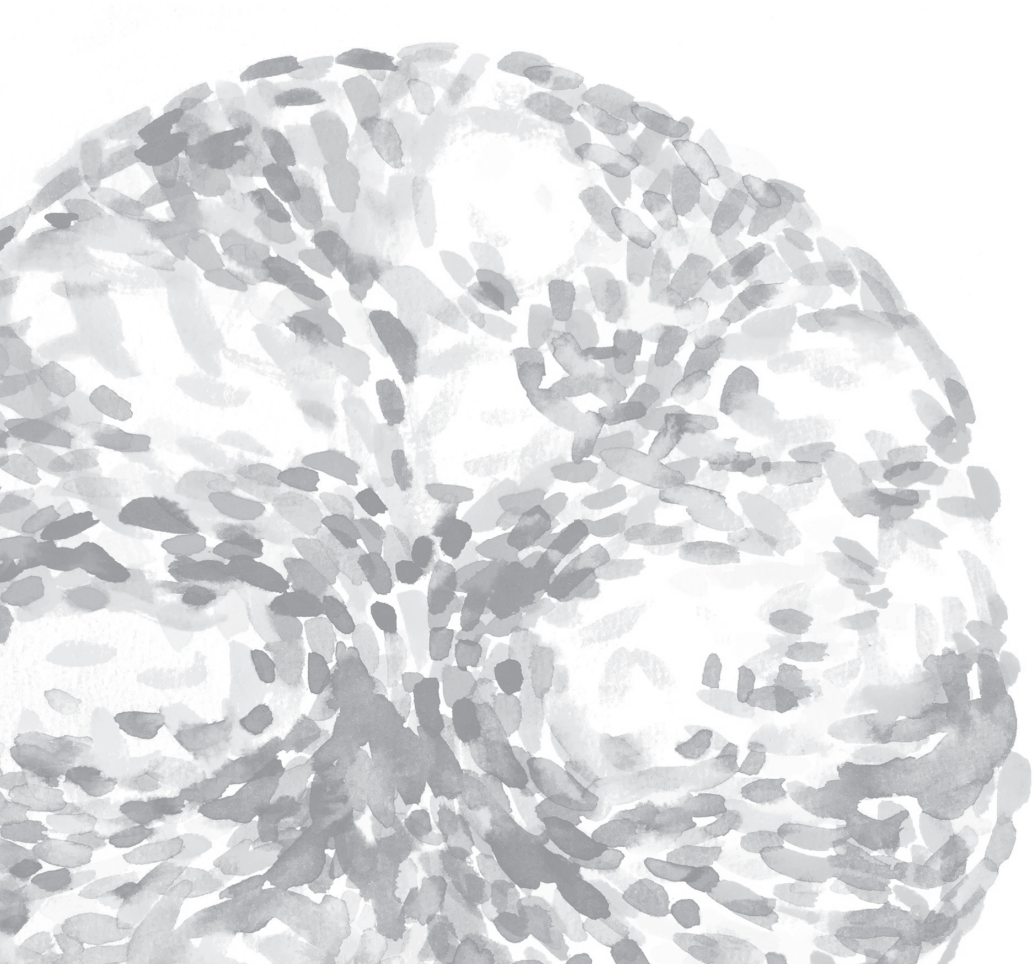


CHAPTER 7

Summary and general discussion



Tumours are characterized by sustained and uncontrolled cell proliferation, evasion of growth suppressors, activation of invasion and metastasis, acquisition of cell immortality, induction of angiogenesis and resistance to cell death. These hallmarks of cancer were proposed to constitute relevant therapy targets by Hanahan and Weinberg in 2000 [1]. In 2011, this overview was updated with two immunological hallmarks: avoiding immune destruction and tumour promoting inflammation [2]. Tumours and tumour stroma can be infiltrated by a high number of immune cells and the balance of effector to regulatory T cell (Treg) mechanisms has a major influence on clinical outcomes. Despite the progress, which was made in the last decade on the knowledge of immunity in ovarian cancer, still the majority of patients with epithelial ovarian cancer ultimately succumb to recurrent disease. How can the immunity to epithelial ovarian cancer be enhanced to curative levels?

In this chapter, the results of the research described in this thesis will be the basis for the discussion on the development of innovative combined chemo-immunotherapeutic strategies for ovarian cancer. Furthermore, novel future therapy combinations for ovarian cancer are provided.

In the last two decades, advances in the understanding of ovarian cancer immunogenicity have opened the door to immunotherapeutic approaches to treat ovarian cancer. A crucial early step in establishing the validity of ovarian cancer immunotherapy was the observation that CD3+ tumour-infiltrating T cells correlated with increased overall survival [3]. Later work confirmed the importance of tumour-infiltrating lymphocytes (TILs) and specifically identified the CD3+CD8+ T cells as important antitumour effectors [4;5]. On the other hand, Tregs and tumour associated macrophages (TAMs) may promote disease progression through multiple mechanisms [6-8]. In our preclinical studies, we attempted to gain more insight in the complex immune response and how and when cancer cells interfere.

Ovarian cancer has the ability to escape the immune system because of its pathological interactions between cancer cells and host immune cells in the tumour microenvironment thereby creating an immunosuppressive network that promotes tumour growth and protects the tumour from immune system [9;10]. When immune suppressive elements like Tregs, M2 macrophages and cytokines such as Intereukin-10 (IL-10), IL-6, tumour necrosis factor alpha (TNF-α), and transforming growth factor beta (TGF-β) are elevated in the tumour microenvironment this is linked to a worse prognosis [7;11-13].

IL-6, A PROMISING TARGET IN OVARIAN CANCER?

In particular, we focussed on the role of IL-6 in the tumour microenvironment as a possible target for (combined chemo-) immunotherapy in ovarian cancer patients. Up-regulation of IL-6 in serum and ascites of patients is associated with disease progression and resistance to chemotherapy in patients with ovarian cancer. IL-6 is a major mediator of cancer-related

inflammation by stimulating inflammatory cytokine production, tumour growth, tumour angiogenesis, and tumour macrophage infiltration in ovarian cancer [14-16]. The knowledge of the molecular biology of IL-6 and its interrelations with human cancer cells as well as their microenvironments have led to the development of novel antibody-based therapy targeting IL-6(R), which is summarized in **Chapter 2**. However, the intricate interactions between IL-6 and tumour infiltration by myeloid cells in ovarian cancer are not well understood. Therefore, we investigated (**Chapter 3**) the differentiation of monocytes towards macrophages, with emphasis on the role of IL-6 and chemotherapy in this process. Ovarian cancer cells but also TAMs have been reported to produce IL-6 [17;18]. However, it is still debatable whether increased IL-6 levels in patients with ovarian cancer are the result of IL-6 produced by the tumour itself or mainly by immune cells. Previously, Heusinkveld *et al*. [19] found that DC differentiation was hampered or even skewed towards M2 macrophages by tumour-derived prostaglandin E2 (PgE2) and IL-6. We now showed that treatment with cisplatin or carboplatin increased IL-6 and PgE2 production by cancer cells. This strengthens the potency of tumour cell lines to induce IL-10–producing M2 macrophages, which displayed increased levels of activated signal transducers and activators of transcription (STAT3), due to tumour-produced IL-6, as well as decreased levels of activated STAT1 and STAT6 related to the PgE2 production of tumour cells.

In **Chapter 4** we assessed the composition of markers of the IL-6 pathway and infiltrating myeloid cells in a cohort of 160 patients with epithelial ovarian cancer. Indeed, patient with tumours that produce high amounts of IL-6 were, in accordance with the literature [15;16;20], associated with a worse survival. A clustering of all immune parameters and IL-6 pathway markers, revealed the existence of two types of tumour environments. The first group comprises tumours having a high expression of IL-6 and a dense infiltration of M2 macrophages as would be expected from our studies in chapter 3. Furthermore, these tumours display a dense infiltration with CD163+ mature myeloid cells and Tregs, while the infiltration with CD8+ T cells was low. These tumours are associated with a worse prognosis. The other group of tumours are defined by a high expression of IL-6R, a high infiltration of cytotoxic CD8+ cells, a low infiltration of immature myeloid cells, less M2 macrophages and less FoxP3 + Tregs.

When tumour cell lines produce IL-6, treatment with platinum-based chemotherapy will enhance the production of IL-6 (**Chapter 3**). This effect should be considered detrimental to patients as it implies that, upon treatment with platinum-based regimens of PgE2 and/or IL-6-producing tumours, the number of local tumour-promoting M2 macrophages may increase, helping the tumour to defy the chemotherapeutic treatment [21;22]. This would fit well with the existing literature showing that chemoresistance of cervical and ovarian cancer is associated with increased levels of PgE2 and IL-6 [16;23;24]. Importantly, our studies showed no correlation between the production of PgE2 or IL-6 by cancer cells and their intrinsic resistance to chemotherapy-induced cell death, supporting the notion that

other mechanisms such as the increase in the number of M2 macrophages may underlie the reported chemoresistance of tumours producing these factors. Resistance to platinum-containing chemotherapy in gynaecological malignancies poses a major problem that limits further treatment options and decreases overall survival.

If M2 macrophages are the 'bad guys', should we put all our effort in developing a strategy to deplete them? Although several mouse studies [25-27] would support this idea by showing a possible beneficial effect, there is also evidence that macrophages are necessary for a T-cell mediated tumour response. Van der Sluijs *et al.* [28] recently showed that therapeutic peptide vaccination could induce cytokine-producing T cells with strong macrophage-skewing capacity necessary for tumour shrinkage, and suggest that the development of macrophage-polarizing, rather than macrophage-depleting, agents is warranted. As M2 differentiation is mainly induced by IL-6, blocking this differentiation is an attractive possibility. Several IL-6 targeting antibodies have been developed in the recent years; one of them was evaluated in clinical trials. Siltuximab (CNTO 238), a chimeric anti IL-6 antibody was found to be well tolerated in patients having different types of cancers, including ovarian cancer [29]. In a phase 2 single arm study, 18 women with advanced platinum-resistant ovarian cancer siltuximab showed some clinical activity in recurrent, platinum-resistant ovarian cancer. Siltuximab 5.4 mg/kg every 2 weeks was well tolerated. In 8 patients, stable disease (SD) was achieved and 10 patients had progressive disease (PD). Siltuximab blocks IL-6 itself, which could be a difficult way of treatment, considering the high levels of IL-6 in the tumour microenvironment and serum.

In our preclinical study we, therefore, choose tocilizumab; a monoclonal antibody against IL-6R, which inhibits the IL-6 induced pathway by blocking the receptor and is effectively used to treat IL-6 driven auto-immune diseases [30-32] and the macrophage activation syndrome when it occurs in CAR-therapy [33]. This antibody was capable to fully block the effects of IL-6 produced by untreated or chemotherapy-treated tumour cells (**Chapter 3**). Pilot experiments revealed that this antibody to some extent also blocked IL-6 signaling in the tumour cells themselves.

Successively, we conducted a phase I/II trial, the PITCH trial, for patients with recurrent platinum-sensitive epithelial ovarian cancer, in which we combined standard chemotherapy (carboplatin and (pegylated liposomal) doxorubicin) with tocilizumab, as well as immune enhancer interferon-α 2b (Peg-Intron). We determined its safety, feasibility, the effect of chemo-immunotherapy on the immune system, and its relation with clinical outcome. The addition of tocilizumab to standard chemotherapy had an acceptable safety profile and potential immunological benefit. Main (manageable) toxicity consisted of fatigue, nausea and neutropenia. Addition of Pegintron in the last cohort showed an enhancement of the neutropenia. Serum IL-6 and sIL-6R levels both were significantly increased after treatment with tocilizumab. This, and the fact that the CRP-levels (inflammation marker which reflects the blockade of IL-6 pathway) were completely normalized after treatment, indicate the

functional blocking of the IL-6R pathway in patients with ovarian cancer. The effective blocking of IL-6 signalling during the first three cycles of chemotherapy resulted in a relief of immune suppression as evidenced by myeloid cells producing more IL-12 and IL-1β while T cells were more activated and secreted higher amounts of the effector cytokines IFN-γ and TNF-α. Additionally, there was an effect on the downstream pathway of IL-6/IL-6R. A decrease in phosphorylation of STAT3 was observed in myeloid cells and CD4+ and CD8+ cells. We were not able to test the STAT regulation in the cancer cells themselves, because at evaluation after 3 months, there was hardly any tumour bulk left to draw any sample of. Pegintron was added to the combination of carboplatin/doxorubicin and tocilizumab but this did not result in additive changes in the immune profile. Therefore, its use should be carefully considered in each possible combination separately.

Obviously in this trial we did not stimulate the tumour-specific T cell response. It is highly likely that stimulation of such T cells in addition to the relief of myeloid cell-mediated immune suppression is required as Tumeh et al [34] recently showed that alleviating immune suppression by checkpoint blockade was dependent on pre-existing tumour-specific responses, and since these are not always present, especially not in patients with end-stage disease, there is a need for vaccines.

P53-VACCINATION IN COMBINATION WITH PEGINTRON

Vaccination strategies are designed to activate tumour-specific T cells increasing both the number of cells and the avidity of receptors sufficient to generate a effective clinical response. Most cancer vaccines aim to deliver tumour antigen in a context which it can be recognised, captured and processed by antigen presenting cells (APCs), and subsequently stimulate tumour-specific responses in resting T cells [35]. The concept behind vaccine combinations is to first prime the immune response to tumour antigens, and then boost the response with a second vaccination to install memory T cell responses.

In the CHIP trial, we combined gemcitabine, which in mouse models eliminates MDSCs, with p53-vaccination and immune-enhancer Pegintron, in patients with platinum-resistant epithelial ovarian cancer. Our goal was to test the feasibility to combine these therapies. P53-antigen is over-expressed in 50% of the ovarian cancers and known to activate spontaneous T cell responses in these patients [36]. Previous studies of our group showed that vaccination with p53 synthetic long peptides (p53 SLP) was safe and able to induce p53-specific T cell responses [37-39]. The first important finding was that gemcitabine treatment decreases MDSC and Tregs also in humans. Moreover, we showed that this regimen resulted in an increase in both M1 macrophages and activated T cells. All vaccinated patients showed a strong vaccine-induced p53-specific T-cell response.

Although we did not observe a positive effect of Pegintron in the PITCH trial, we detected that Pegintron in combination with vaccination stimulated higher frequencies of circulating proliferating CD4+ and CD8+ T cells but not Tregs. Previous studies have shown that p53 SLP vaccination induced p53-specific T cell responses in ovarian cancer patients [37;38] and that the combination with Pegintron resulted in stronger immune responses [40]. Also in our trial the combination of Pegintron and p53 SLP resulted in a strong immune response reflected by the local vaccine site reactions and the T-cell response against the vaccine peptides as measured by IFN-γ ELISPOT assay. Moreover we showed that concurrent administration of at least two cycles of gemcitabine does not affect p53-specific T-cell reactivity.

FUTURE PERSPECTIVES

Combination immunotherapies appear more promising than individual immunotherapy agents. Immunotherapy may be combined with other standard of care treatments, including cytotoxic agents, small molecule inhibitors, radiation therapy or surgery, when based on rational concepts aiming to capitalize on therapeutic synergy.

Vaccines, immune checkpoint blockade, immune stimulatory antibodies, and adoptive T cell therapy have been associated with clinical activity in patients, and these strategies provide a platform for future progress. The potential of combined therapeutic possibilities is high but they should be critically evaluated in the clinic. First, timing should be well evaluated; at which point should immunotherapy be incorporated in the treatment of ovarian cancer? Wu *et al.* investigated by measuring cytotoxic T lymphocyte (CTL) responses at different time-points, that it is probable that the "window" period of days 12-14 after chemotherapy provides the best opportunity for immunotherapy [41]. Our laboratory evaluated this question in cervical cancer patients, confirming this time lapse [42]. Notably, current studies all investigated patients with advanced disease, but efficacy of immunotherapeutic strategies will be higher in immuno-competent early-stage patients, with tumours enriched for clonal neoantigens with a low tumour burden than in immunodeficient end-stage patients [43;44].

Second, despite the positive effects of the treatments, there will always be various adverse events depending on the therapy: that makes that in combined therapies these adverse events for the single therapies now accumulate and might become a limiting factor. Our clinical studies showed that in triple combination with carboplatin/doxorubicin and tocilizumab, Pegintron enhanced neutropenia, while this was not observed in the CHIP trial. Therefore, careful consideration of the beneficial as well as disadvantages is warranted.

Last but certainly not least, patient and tumour characteristics remain the cornerstones for improved outcome. We need to know which immune related hurdles are present in the patient to be treated. Does the tumour express tumour antigens that can form targets for

the immune system? Is there a spontaneous anti-tumour T cell response or does it needs to be induced? What types of immune suppressing pathways are most dominant in the tumour, and what is the basis for their induction? Is there sufficient immune cell attraction towards the tumour? And also, is the tumour platinum sensitive and how does a patient metabolize the treatment?

Tailored combination therapy to enhance outcome of EOC

Chemotherapy has shown to have several positive and negative interactions with the immune system. The challenge of combining chemotherapy and immunotherapy may be to let the benefits of both treatments be synergistic and not counteractive. Current first line treatment of ovarian cancer consists of cytoreductive surgery combined with chemotherapy, a combination of platinum-based therapy with paclitaxel. In patients with recurrent disease (platinum-sensitive), platinum-based therapy is a first choice. Apoptosis by massive cell-death caused by platinum-based chemotherapy can be a priming event for anti-tumour immunity, in which strategies to augment antigen presentation (e.g. interferon) and T cell expansion (e.g. vaccination or adoptive T cell therapy) are needed [45;46].

As we demonstrated in our clinical trials, carboplatin, as well as gemcitabine, does not interfere with effector T cells and are therefore suitable for combinatory therapies. In ovarian cancer there is often leukocytosis, which comprise mainly M2 macrophages and MDSCs. It therefore depends on the characteristics of the patient which chemotherapy might be most applicable. For instance, as gemcitabine eliminates immunosuppressive MDSCs and Tregs and increases M1 macrophages, it is an attractive combinational drug and one could argue that, for instance, in patients having a high amount of MDSC Gemcitabine can be used in combination with T-cell stimulatory based immunotherapeutic strategies, for its ability to decrease the number of immune suppressive cells. This makes it an attractive combination of drugs. Carboplatin combined with paclitaxel normalizes the numbers of circulating myeloid cells but has no negative effect on the number and function of lymphocytes [42] .Thus far, bevacizumab, a monoclonal antibody that targets vascular endothelial growth factor (VEGF), is the only immune therapy now approved for clinical use in patients with EOC in addition to single-agent chemotherapy and as maintenance therapy. Although the therapy has entered routine clinical practice (54), the exact effect on the immune responses are unknown. Current open questions are (i) whether there are biomarkers that predict response to bevacizumab, (ii) whether bevacizumab can be used beyond progressive disease, (iii) what is the specific dose of bevacizumab (2.5 or 5 mg/kg/week), and (iv) what is the optimal method of combining cytotoxic agents. As we discussed, TAMs play an important role in enhancing the vasculature of tumours, by secreting the VEGF. Bevacizumab can stop this process of angiogenesis that a tumour mass needs in order to sustain its viability and therefore may decrease macrophage infiltration in the tumour microenvironment [47;48]. VEGF can be a potential therapeutic target, as captivation of it by for instance bevacizumab

not only would work by its anti-angiogenic activity, but also by decreasing the macrophage recruitment which is the source of VEGF secretion and further angiogenesis. The addition of a VEGF inhibitor to chemotherapy has been shown to increase progression free survival for platinum-resistant ovarian cancer patients, but not overall survival [49]. Clearly, there appears to be a subset of patients who benefit from the addition of anti-angiogenesis therapy to chemotherapy, and macrophage density and type of macrophages in such tumours may be the guide to the subset of patients who benefit most from this therapy.

These observations implicate that it is important to select the right therapy for the right patient. As different chemotherapeutic agents have different modes of action, one could select more than one chemotherapeutic drug for different reasons; e.g. gemcitabine to eliminate MDSCs and Tregs, platinum-based therapy and/or paclitaxel to sensitize tumour cells to CTLs [50]. However, combination of these therapies can increase the number and severity of adverse events as well. A possible way to prevent this might be to decrease the dose of chemotherapy to eliminate immunosuppressive cells, and an addition of another immunotherapy approach such as monoclonal antibodies, which also have the qualities to inhibit the immunosuppressive actions of myeloid cells.

Our study showed that the combination of carboplatin/(liposomal pegylated)doxorubicin with tocilizumab is safe and has possible beneficial immunological effects. Studies in more patients are needed to test the effects on the tumour microenvironment and survival.

In ovarian cancer, PD-1 and its ligand PD-L1 have shown to be promising targets. PD-(L)1 serves as immune checkpoints, which refer to a series of pathways that can regulate T cell activity as either co-inhibitory or co-stimulatory signals by APC [51]. Tumours use many of these pathways as important mechanisms to escape antitumour immune responses. PD-L1 overexpression on murine ovarian cancer cells inhibited CTL and PD-L1 blockade reversed this effect. A recent clinical trial in patients with platinum-resistant ovarian cancer showed a 20% partial response and 26% of all patients had stable disease [51]. Recently, in a mouse model of ovarian cancer, treatment with paclitaxel and a PD-1/PD-L1 signal blockade increased CD8+ T-cell infiltration into the tumour site, upregulated PD-L1 expression, and activated NF-kB signalling [52]. These mice survived longer than mice treated with paclitaxel alone. Here, chemotherapy induces local immune suppression in ovarian cancer through NF-kB–mediated PD-L1 upregulation. Therefore, a combination of chemotherapy and immunotherapy targeting the PD-L1/PD-1 signalling axis may improve the antitumour response and offers a promising new treatment modality against ovarian cancer than the use of either therapy alone. These combinations are currently under investigation. Even combinations of two checkpoint inhibitors are another potential approach. CTLA-4 inhibitors enhance early T cell activation and increase the frequencies of tumour-specific T cells, whereas inhibition of the PD-1/PD-L1 axis modulates the T cell effector phase to overcome T cell anergy in the tumour microenvironment. In ID8-VEGF model of ovarian carcinoma mice the co-administration of anti-PD-1 and anti-CTLA4 antibodies reversed the TIL dysfunction

and induced tumour regression in 50% of the mice relative to 25% with either agent as monotherapy [53]. In melanoma patients, however, increased toxicity was observed in the combined therapy arm [54]; the incidence of grade 3 or 4 toxicity with the combination was increased compared with either single agent (55 versus 16 and 27 percent, respectively, for nivolumab and ipilimumab). Treatment-related adverse events were more common with the combination (36 versus 8 and 15 percent, respectively), but there were no treatment-related deaths with the combination in this study.

Other possible checkpoint blockades in ovarian cancer patients suitable for treatment options are HLA-E and NKG2A. HLA-E is frequently overexpressed in ovarian cancer and positively associated with expression patterns of antigen processing components, classical HLA molecules, and immune cell infiltrate [55;56]. In ovarian cancer, in situ analysis of the interacting receptors of HLA-E, e.g., the inhibitory CD94/NKG2A and the activating CD94/NKG2C, revealed a frequent expression of the inhibitory receptor on intraepithelial $CD8^+$ T cells. The presence of HLA-E is able to neutralize the protective role of the relatively scarce intratumoural CTLs [57], indicating that HLA-E hampers activity of antitumour CTLs in the tumour microenvironment.

The clinical effect of checkpoint blockade is dependent on the presence of clonal neo-antigen burden and tumour-specific T cells. In a substantial amount of patients, there will be a lack of these T cells. Therefore, it is important to select the patients who benefit or alternatively to induce specific T cells by T cell vaccination or oncolytical viruses. However, the presence of suppressive MDSCs and/or M2 macrophages may form a condition in which checkpoint blockade or vaccination could fail. Additional strategies to improve the effectiveness of these therapies, such as combinations with chemotherapy that depletes MDSC and/or Tregs or Treg depletion by using CD25 monoclonal antibodies are worth investigating. In breast cancer and melanoma patients, first trials show that daclizumab depleted the CD4(+) FoxP3(+)CD25(high) Tregs from the peripheral circulation, but did not enhance the efficacy of the dendritic cell vaccination. Timing and dosing should be evaluated [58;59].

Finally, the strong proliferation of T cells after chemotherapy-induced lymphodepletion is an opportunity for post-chemotherapy vaccination and/or adoptive T cell therapy [41]. Lymphodepletion with chemotherapy (or radiotherapy) creates the space allowing T cells to expand to large numbers as well as ensures that the homeostatic cytokines are available mostly for the activated T cells. In addition, it will eliminate Tregs [60]. Currently, one trial is recruiting patients combining chemotherapy (cyclofosfamide or fludarabine, known to deplete Tregs) with adoptive T cell therapy in ovarian cancer (NCT02482090). In the LUMC a trial is initiated in which ACT will be scheduled starting in patients with recurrent EOC after the second cycle of carboplatin paclitaxel chemotherapy. The rationale and timing of this trial is based on previous observation by us and others that the tumour-induced immune suppressive leukocytosis is reversed after 2 cycles of this chemotherapy and associated with

increased T cell reactivity [42]. In the future, a combination with vaccination or immune checkpoint inhibitors to sustain immune reactivity can be envisaged.

CONCLUSION

An advance in understanding the role of immune system in the pathogenesis of ovarian cancer has led to the rapid evolvement of immunotherapy, aiming to establish a sustained immune system response against cancer cells. In ovarian cancer, only bevacizumab is currently registered. Successful tumour control by immunotherapy requires activation of the immune system, alleviation of immune suppression, the expansion of effector cells, infiltration of activated effector cells into the tumour tissue, and destruction of the tumour cells. Cytotoxic chemotherapy can alleviate immune suppression installed by Tregs, M2 macrophages and MDSC and work synergistic with the immunotherapy. Other agents will likely be needed to abrogate overlapping mechanisms of immune suppression within the tumour microenvironment and unleash the full force of the immune system to fight cancer or to prevent extra adverse events.

This thesis has explored new strategies, immune-modulation of the IL-6 pathway and a vaccine against p53, to enhance immune surveillance and to disable tumour immune evasion in ovarian cancer patients. The future challenge for immunotherapy against ovarian cancer is a tailored combinatorial approach to test the rationale of potentially synergistic therapies that can induce efficient antitumour immunity and prolong patients' survival.

REFERENCE LIST

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100(1):57-70.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144(5):646-674.
3. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003; 348(3):203-213.
4. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A 2005; 102(51):18538-18543.
5. Tomsova M, Melichar B, Sedlakova I, Steiner I. Prognostic significance of CD3+ tumor-infiltrating lymphocytes in ovarian carcinoma. Gynecol Oncol 2008; 108(2):415-420.
6. Preston CC, Maurer MJ, Oberg AL, Visscher DW, Kalli KR, Hartmann LC et al. The ratios of CD8+ T cells to CD4+CD25+ FOXP3+ and F. PLoS One 2013; 8(11):e80063.
7. Wolf D, Wolf AM, Rumpold H, Fiegl H, Zeimet AG, Muller-Holzner E et al. The expression of the regulatory T cell-specific forkhead box transcription factor FoxP3 is associated with poor prognosis in ovarian cancer. Clin Cancer Res 2005; 11(23):8326-8331.
8. Barnett B, Kryczek I, Cheng P, Zou W, Curiel TJ. Regulatory T cells in ovarian cancer: biology and therapeutic potential. Am J Reprod Immunol 2005; 54(6):369-377.
9. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013; 19(11):1423-1437.
10. Lavoue V, Thedrez A, Leveque J, Foucher F, Henno S, Jauffret V et al. Immunity of human epithelial ovarian carcinoma: the paradigm of immune suppression in cancer. J Transl Med 2013; 11:147.
11. Colvin EK. Tumor-associated macrophages contribute to tumor progression in ovarian cancer. Front Oncol 2014; 4:137.
12. Knutson KL, Karyampudi L, Lamichhane P, Preston C. Targeted immune therapy of ovarian cancer. Cancer Metastasis Rev 2015; 34(1):53-74.
13. Latha TS, Panati K, Gowd DS, Reddy MC, Lomada D. Ovarian cancer biology and immunotherapy. Int Rev Immunol 2014; 33(5):428-440.
14. Coward J, Kulbe H, Leader D, Quigley R, Thompson R, Leinster A et al. Interleukin-6 as a therapeutic target in advanced ovarian cancer. J.Clin.Oncol. J Clin Oncol 28:15s, 2010 (suppl; abstr 5089)[15s]. 2010. Ref Type: Abstract
15. Scambia G, Testa U, Benedetti PP, Foti E, Martucci R, Gadducci A et al. Prognostic significance of interleukin 6 serum levels in patients with ovarian cancer. Br J Cancer 1995; 71(2):354-356.
16. Berek JS, Chung C, Kaldi K, Watson JM, Knox RM, Martinez-Maza O. Serum interleukin-6 levels correlate with disease status in patients with epithelial ovarian cancer. Am J Obstet Gynecol 1991; 164(4):1038-1042.
17. Duluc D, Delneste Y, Tan F, Moles MP, Grimaud L, Lenoir J et al. Tumor-associated leukemia inhibitory factor and IL-6 skew monocyte differentiation into tumor-associated macrophage-like cells. Blood 2007; 110(13):4319-4330.
18. Hagemann T, Robinson SC, Thompson RG, Charles K, Kulbe H, Balkwill FR. Ovarian cancer cell-derived migration inhibitory factor enhances tumor growth, progression, and angiogenesis. Mol Cancer Ther 2007; 6(7):1993-2002.
19. Heusinkveld M, de Vos van Steenwijk PJ, Goedemans R, Ramwadhdoebe TH, Gorter A, Welters MJ et al. M2 macrophages induced by prostaglandin E2 and IL-6 from cervical carcinoma are switched to activated M1 macrophages by CD4+ Th1 cells. J Immunol 2011; 187(3):1157-1165.

20. Plante M, Rubin SC, Wong GY, Federici MG, Finstad CL, Gastl GA. Interleukin-6 level in serum and ascites as a prognostic factor in patients with epithelial ovarian cancer. Cancer 1994; 73(7):1882-1888.
21. Sugimura K, Miyata H, Tanaka K, Takahashi T, Kurokawa Y, Yamasaki M et al. High infiltration of tumor-associated macrophages is associated with a poor response to chemotherapy and poor prognosis of patients undergoing neoadjuvant chemotherapy for esophageal cancer. J Surg Oncol 2015; 111(6):752-759.
22. Yang C, He L, He P, Liu Y, Wang W, He Y et al. Increased drug resistance in breast cancer by tumor-associated macrophages through IL-10/STAT3/bcl-2 signaling pathway. Med Oncol 2015; 32(2):352-0352.
23. Ferrandina G, Lauriola L, Zannoni GF, Fagotti A, Fanfani F, Legge F et al. Increased cyclooxygenase-2 (COX-2) expression is associated with chemotherapy resistance and outcome in ovarian cancer patients. Ann Oncol 2002; 13(8):1205-1211.
24. Gastl G, Plante M. Bioactive interleukin-6 levels in serum and ascites as a prognostic factor in patients with epithelial ovarian cancer. Methods Mol Med 2001; 39:121-123.
25. Fritz JM, Tennis MA, Orlicky DJ, Lin H, Ju C, Redente EF et al. Depletion of tumor-associated macrophages slows the growth of chemically induced mouse lung adenocarcinomas. Front Immunol 2014; 5:587.
26. Cannarile MA, Ries CH, Hoves S, Ruttinger D. Targeting tumor-associated macrophages in cancer therapy and understanding their complexity. Oncoimmunology 2014; 3(9):e955356.
27. Kiyota T, Takahashi Y, Watcharanurak K, Nishikawa M, Ohara S, Ando M et al. Enhancement of anticancer effect of interferon-gamma gene transfer against interferon-gamma-resistant tumor by depletion of tumor-associated macrophages. Mol Pharm 2014; 11(5):1542-1549.
28. van der Sluis TC, Sluijter M, van DS, West BL, Melief CJ, Arens R et al. Therapeutic Peptide Vaccine-Induced CD8 T Cells Strongly Modulate Intratumoral Macrophages Required for Tumor Regression. Cancer Immunol Res 2015; 3(9):1042-1051.
29. Coward J, Kulbe H, Chakravarty P, Leader D, Vassileva V, Leinster DA et al. Interleukin-6 as a Therapeutic Target in Human Ovarian Cancer. Clin Cancer Res 2011; 17(18):6083-6096.
30. Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N, Kakehi T. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. Blood 2008; 112(10):3959-3964.
31. Shirota Y, Yarboro C, Fischer R, Pham TH, Lipsky P, Illei GG. Impact of anti-interleukin-6 receptor blockade on circulating T and B cell subsets in patients with systemic lupus erythematosus. Ann Rheum Dis 2013; 72(1):118-128.
32. Illei GG, Shirota Y, Yarboro CH, Daruwalla J, Tackey E, Takada K et al. Tocilizumab in systemic lupus erythematosus: data on safety, preliminary efficacy, and impact on circulating plasma cells from an open-label phase I dosage-escalation study. Arthritis Rheum 2010; 62(2):542-552.
33. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 2013; 368(16):1509-1518.
34. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014; 515(7528):568-571.
35. Melief CJ, van HT, Arens R, Ossendorp F, van der Burg SH. Therapeutic cancer vaccines. J Clin Invest 2015; 125(9):3401-3412.
36. Lambeck A, Leffers N, Hoogeboom BN, Sluiter W, Hamming I, Klip H et al. P53-specific T cell responses in patients with malignant and benign ovarian tumors: implications for p53 based immunotherapy. Int J Cancer 2007; 121(3):606-614.

37. Leffers N, Lambeck AJ, Gooden MJ, Hoogeboom BN, Wolf R, Hamming IE et al. Immunization with a P53 synthetic long peptide vaccine induces P53-specific immune responses in ovarian cancer patients, a phase II trial. Int J Cancer 2009; 125(9):2104-2113.
38. Vermeij R, Leffers N, Hoogeboom BN, Hamming IL, Wolf R, Reyners AK et al. Potentiation of a p53-SLP vaccine by cyclophosphamide in ovarian cancer: a single-arm phase II study. Int J Cancer 2012; 131(5):E670-E680.
39. Speetjens FM, Kuppen PJ, Welters MJ, Essahsah F, Voet van den Brink AM, Lantrua MG et al. Induction of p53-specific immunity by a p53 synthetic long peptide vaccine in patients treated for metastatic colorectal cancer. Clin Cancer Res 2009; 15(3):1086-1095.
40. Zeestraten EC, Speetjens FM, Welters MJ, Saadatmand S, Stynenbosch LF, Jongen R et al. Addition of interferon-alpha to the p53-SLP(R) vaccine results in increased production of interferon-gamma in vaccinated colorectal cancer patients: a phase I/II clinical trial. Int J Cancer 2013; 132(7):1581-1591.
41. Wu X, Feng QM, Wang Y, Shi J, Ge HL, DI W. The immunologic aspects in advanced ovarian cancer patients treated with paclitaxel and carboplatin chemotherapy. Cancer Immunol Immunother 2010; 59(2):279-291.
42. Welters MJ, van der Sluis TC, van MH, Loof NM, van Ham VJ, van DS et al. Vaccination during myeloid cell depletion by cancer chemotherapy fosters robust T cell responses. Sci Transl Med 2016; 8(334):334ra52.
43. Yi Q. Novel immunotherapies. Cancer J 2009; 15(6):502-510.
44. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 2016; 351(6280):1463-1469.
45. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol 2008; 8(1):59-73.
46. Zitvogel L, Apetoh L, Ghiringhelli F, Andre F, Tesniere A, Kroemer G. The anticancer immune response: indispensable for therapeutic success? J Clin Invest 2008; 118(6):1991-2001.
47. Colombo N, Conte PF, Pignata S, Raspagliesi F, Scambia G. Bevacizumab in ovarian cancer: Focus on clinical data and future perspectives. Crit Rev Oncol Hematol 2016; 97:335-348.
48. Dineen SP, Lynn KD, Holloway SE, Miller AF, Sullivan JP, Shames DS et al. Vascular endothelial growth factor receptor 2 mediates macrophage infiltration into orthotopic pancreatic tumors in mice. Cancer Res 2008; 68(11):4340-4346.
49. Pujade-Lauraine E, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G et al. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial. J Clin Oncol 2014; 32(13):1302-1308.
50. van MH, Kenter GG, Burggraaf J, Kroep JR, Welters MJ, Melief CJ et al. The need for improvement of the treatment of advanced and metastatic cervical cancer, the rationale for combined chemo-immunotherapy. Anticancer Agents Med Chem 2014; 14(2):190-203.
51. Mandai M, Hamanishi J, Abiko K, Matsumura N, Baba T, Konishi I. Anti-PD-L1/PD-1 immune therapies in ovarian cancer: basic mechanism and future clinical application. Int J Clin Oncol 2016.
52. Peng J, Hamanishi J, Matsumura N, Abiko K, Murat K, Baba T et al. Chemotherapy Induces Programmed Cell Death-Ligand 1 Overexpression via the Nuclear Factor-kappaB to Foster an Immunosuppressive Tumor Microenvironment in Ovarian Cancer. Cancer Res 2015; 75(23):5034-5045.
53. Duraiswamy J, Kaluza KM, Freeman GJ, Coukos G. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. Cancer Res 2013; 73(12):3591-3603.

54. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 2015; 373(1):23-34.
55. Braud VM, Allan DS, O'Callaghan CA, Soderstrom K, D'Andrea A, Ogg GS et al. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. Nature 1998; %19;391(6669):795-799.
56. Speiser DE, Valmori D, Rimoldi D, Pittet MJ, Lienard D, Cerundolo V et al. CD28-negative cytolytic effector T cells frequently express NK receptors and are present at variable proportions in circulating lymphocytes from healthy donors and melanoma patients. Eur J Immunol 1999; 29(6):1990-1999.
57. Gooden M, Lampen M, Jordanova ES, Leffers N, Trimbos JB, van der Burg SH et al. HLA-E expression by gynecological cancers restrains tumor-infiltrating CD8(+) T lymphocytes. Proc Natl Acad Sci U S A 2011; 108(26):10656-10661.
58. Rech AJ, Mick R, Martin S, Recio A, Aqui NA, Powell DJ, Jr. et al. CD25 blockade depletes and selectively reprograms regulatory T cells in concert with immunotherapy in cancer patients. Sci Transl Med 2012; 4(134):134ra62.
59. Jacobs JF, Punt CJ, Lesterhuis WJ, Sutmuller RP, Brouwer HM, Scharenborg NM et al. Dendritic cell vaccination in combination with anti-CD25 monoclonal antibody treatment: a phase I/II study in metastatic melanoma patients. Clin Cancer Res 2010; 16(20):5067-5078.
60. Muranski P, Boni A, Wrzesinski C, Citrin DE, Rosenberg SA, Childs R et al. Increased intensity lymphodepletion and adoptive immunotherapy--how far can we go? Nat Clin Pract Oncol 2006; 3(12):668-681.
Chapter 8