Immuno-oncology of gynecological malignancies
Komdeur, Fenne Lara

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Citation for published version (APA):

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General introduction
GENERAL INTRODUCTION

The immune system plays a crucial role in recognition and elimination of (pre)malignant cells. Immune T cells in particular are highly skilled at surveying the body and identifying (pre) malignant cells to be eliminated. When T cells infiltrate the tumor micro-environment they are referred to as tumor-infiltrating lymphocytes (TIL). TIL have a pronounced effect on the survival of patients across malignancies. Moreover, it has also become abundantly clear that TIL can be targeted by cancer immunotherapy to induce lasting clinical benefit. In this thesis, we explored several aspects of TIL biology, including localization and differentiation, with the ultimate aim of developing novel therapeutic interventions.

TUMOR-IMMUNOLOGY

The identification of (pre)malignant cells by T cells is based on the recognition of so-called antigens. These antigens are either ‘self’ or ‘non-self’ proteins. Bacterial and viral pathogens are prototypical examples of non-self and are therefore easily recognized as foreign by the T cell. Nevertheless, as discussed in more detail below, tumor antigens can also be recognized by the T cell as non-self. Such tumor antigens are therefore prime targets for the immune system and can serve as a locus of immune activation against (pre)malignant cells.

This immune activation against the tumor starts with capture of tumor antigens by antigen presenting cells (APCs). After uptake of antigens, APCs migrate to draining lymph nodes and process antigens for presentation. Antigens are then presented in the form of small peptides in the context of major histocompatibility complex (MHC) molecules to T cells. When T cells encounter such a MHC-peptide complex, T cells recognize this complex via the T cell receptor. Upon specific recognition via the T cell receptor T cells become activated and undergo proliferation. The antigen-experienced, activated T cells can now exit the lymph node, enter the circulation and migrate towards the tumor bed. The T cell receptor on these T cells subsequently recognizes the specific MHC-peptide complex on tumor cells. After recognition, the T cell secretes perforins and granzymes to form pores in the membrane of the tumor cell and triggers lysis and/or apoptosis.¹

The interaction between the immune system and (pre)malignant cells, also referred to as cancer immunoediting, is a dynamic process which can be divided in three phases.² The first phase, also called the elimination phase, involves the immune system recognizing and successfully eradicating (pre)malignant cells. This recognition and eradication of (pre)malignant cells by the immune system is also called immune surveillance. If eradication of the cancer is incomplete, an equilibrium phase -the second phase- is established. Herein, the immune system is only able
to exert limited control over malignant cells in which cancer cell growth is restricted by the immune cells, but the cancer is not eliminated. The final ‘escape’ phase involves tumor cells completely evading the immune system allowing unrestricted growth and metastasis. At clinical presentation most cancers have already reached this point. Nevertheless, as discussed in this thesis, therapeutic intervention by immunotherapy or even standard treatment can reverse this cancer-induced immune suppression and result in remarkable clinical benefit.

**IMMUNOTHERAPY**

Immunotherapy aims to eradicate cancer by inducing or augmenting anti-tumor immunity. To date, several immunotherapeutic modalities have been approved by the Food and Drug Administration (FDA) and many more are under clinical investigation (>1400 ongoing clinical trials). For the purpose of brevity, the immunotherapeutic modalities that will be discussed within this thesis are immune checkpoint blockade, therapeutic vaccination, and adoptive cellular transfer.

Immune checkpoint blockade (ICB) targets molecules involved in the activation/inhibition pathways of T cells (e.g. cytotoxic T-lymphocyte–associated antigen 4 [CTLA-4] and programmed cell death-1 [PD-1]). ICB leads to durable clinical responses, but only in a fraction of patients. There are ongoing studies to identify predictive biomarkers for the selection of patients for ICB treatment. Major predictive markers for response to ICB include high levels of infiltrating T cells, high neo-antigenic load, high tumor mutational load (e.g. microsatellite instability), and PD-L1 expression. On the contrary, failure to respond to ICB is thought to result from insufficient spontaneous tumor-specific T cell responses. A logical combination approach is therefore to increase the amount of tumor-specific T cells within the tumor. This can be achieved in multiple ways; actively by therapeutic vaccination or passively by adoptive cellular transfer.

Therapeutic vaccination aims to stimulate the immune system against tumor peptides. The main advantage of active therapeutic vaccination is the potential to initiate durable effects by the induction of immunological memory. Adoptive cellular transfer consists of the transfusion of *ex-vivo* stimulated or generated T cells, directed against a specific antigen. Initially, adoptive cellular transfer led to transient increases in tumor-specific T cells, for which multiple infusions were needed to initiate a long-lasting response. Currently, new T cell engineering techniques (e.g. second and third generation chimeric antigen receptor T cells [CAR T cells]) for adoptive cellular transfer are being developed and under clinical investigation and are also able to induce long-lasting immune responses.
Most of these modalities have proven effective across cancer types, with mainly differences in the type of antigen targeted for effective therapy. Although many of the lessons learned from the studies described in this thesis can also be applied in a pan-cancer manner, the focus will remain on immunity in gynecological malignancies, and the possibilities for immunotherapy in these diseases.

**IMMUNITY IN GYNECOLOGICAL MALIGNANCIES**

Gynecological malignancies account for approximately 16% of the cancer cases among women worldwide (GLOBOCAN 2012) and include ovarian, uterine, cervical, vaginal, and vulvar cancers. Although the initial primary treatment for most gynecological malignancies is effective, cancer recurrences frequently occur, and are often therapy resistant. As gynecological cancer burden and mortality remain high, new therapeutic strategies urgently need to be explored. In this thesis, we focus on the 3 most prevalent gynecological malignancies; cervical, endometrial and ovarian cancer.

**Cervical cancer**

Of all gynecological malignancies, cervical cancer is the most prevalent, and the fourth most common cancer among women, with an estimated 528,000 new cases worldwide. Further, approximately 266,000 women die from cervical cancer each year, accounting for 7.5% of all female cancer deaths. A large majority of the global cervical cancer burden occurs in the less developed regions, where it accounts for almost 12% of all female cancers. In addition, almost 9/10 cervical cancer deaths occur in the less developed regions. This large geographic variation in cervical cancer rates reflects differences in the availability of screening, and human papillomavirus (HPV) infection prevalence. Screening in particular allows for effective treatment by early detection and removal of precancerous lesions. Indeed, in countries where screening programs have been established, cervical cancer rates have decreased by as much as 65% over the past 40 years. In addition, prognosis for patients treated in an early stage of this disease is good.

Persistent infection with HPV has been established as the causative agent for cervical cancer. Upon infection, HPV integrates into the genome of the host cells. This integration leads to the production and overexpression of the early viral proteins E6 and E7. The viral proteins E6 and E7 block the function of important tumor suppressor pathways, Rb and P53 respectively, leading to malignant transformation of the host cells. Persistent expression of the viral proteins E6 and E7 is needed to maintain the transformed phenotype. Therefore, the viral proteins E6 and E7 represent ideal tumor-specific antigens for the immune system in HPV-associated cancer.
Endometrial cancer

The second most common gynecological malignancy is endometrial cancer. Worldwide, endometrial cancer is the sixth most common cancer in women, with an estimated 320,000 new cases and 50,000 deaths. In contrast to cervical cancer, the majority of endometrial cancer cases occur in developed countries where it accounts for 6% of all cancers in women, whereas in developing countries, endometrial cancer accounts for only 2% of cancers in women. The increased incidence and prevalence of endometrial cancer in developed countries can be explained by the increase in life expectancy, increased obesity rates, and reproductive factors such as younger age at menarche and late age at menopause, null parity, age of the first child, and long-term use of unopposed estrogens for hormone replacement therapy.\(^\text{21}\) As endometrial cancer often presents at an early stage of disease and with clear symptoms, prognosis is fairly good.

In endometrial cancer, 17% of endometrial tumors are microsatellite unstable (MSI), which is caused by defects in the DNA- mismatch repair (dMMR) mechanisms. MSI/dMMR-deficient tumors have a high mutational load leading to the formation of neo-antigens expressed by the tumor cells. Neo-antigens can be recognized by the immune system as non-self and form a locus of immune attraction against the cancer cells. Therefore, MSI/dMMR-deficient tumors are characterized by high levels of infiltrated immune cells (e.g. CD8-positive cytotoxic T lymphocytes). For these neo-antigen rich tumors, treatment with ICB has proven to be highly effective in initial trials.\(^\text{22,23}\) As such, the FDA has approved ICB for the treatment of this subset of endometrial cancer patients.\(^\text{24}\)

Ovarian cancer

The third most common gynecological cancer in woman is ovarian cancer. While not the most prevalent gynecological cancer, ovarian cancer is the most lethal gynecological malignancy. Indeed, ovarian cancer is only the seventh most common cancer among women worldwide, with an estimated 239,000 new cases, but results in 152,000 deaths annually. The poor prognosis of the disease is largely due to diagnosis at advanced stage and therapy-resistant disease relapses. In contrast to cervical and endometrial cancer, there are neither sufficient screening methods nor early symptoms of disease that could lead to early detection of ovarian cancer.\(^\text{25–27}\) Ovarian cancer can be divided into different histological subtypes of which epithelial ovarian cancer is the most common subtype (95%). Epithelial ovarian cancers derive from malignant transformation of the epithelium of the ovarian or fallopian tube surface. The remaining ovarian cancers arise from other ovarian cell types such as germ cell tumors, sex cord stromal tumors and mixed cell types.\(^\text{28}\) Serous ovarian cancer is the most common subtype of epithelial ovarian cancer.
cancers (75%), whereas mucinous, endometrioid, clear cell, transitional cell, and undifferentiated tumors are less common. The studies described within this thesis mainly focus on high-grade serous ovarian cancer (HGSC), as this is the most common and aggressive subtype.

HGSCs are characterized by TP53 mutations (~95%) and chromosomal instability leading to frequent DNA losses or gains. Despite this chromosomal instability, mutational load and neo-antigen load in HGSC are relatively low. With the exception of TP53 mutations, other somatic mutations in oncogenes are relatively uncommon with the exception of BRCA1 and BRCA2 mutations. Approximately 13% of HGSCs are attributable to germline mutations in BRCA1/2. Additionally, 50% of HGSCs are defective in the homologous recombination (HR) DNA repair pathway. These HR-defects arise mostly from somatic and epigenetic mutations in BRCA1/2, but also other genes in the BRCA pathway can be involved. Interestingly, patients with a germline BRCA1/2 mutation have an improved survival and these tumors have higher levels of infiltrating T cells. Nevertheless, in clinical trials only a subset of HGSC patients (~10%) responded to ICB.

To improve the response rate of ovarian cancer patients to ICB, T cell infiltration likely needs to be increased, e.g. by therapeutic vaccination against tumor-associated antigens as reviewed by Leffers et al. or by adoptive cellular transfer.

Taken together, although the above described malignancies are different in origin and interaction with the immune system, their immunity eventually depends on recognition of antigens and subsequent infiltration of tumor-reactive T cells (e.g., CD8-positive cytotoxic T lymphocytes).
OUTLINE OF THE THESIS

As CD8-positive TIL appear crucial across malignancies, we first defined the CD8-positive immune infiltrate and its prognostic value in gynecological malignancies; in high-grade serous ovarian cancer in chapter 2; endometrial cancer in chapter 3; and cervical cancer in chapter 4. We observed a strong prognostic benefit for patients with TIL that localize within the epithelial cancer islets of gynecological tumors, but not from TIL localized within the connective tissue of tumor, the stroma. We identified a distinguishing cell surface marker, CD103, for these cancer cell-associated epithelial TIL that performed consistently across gynecological malignancies (chapters 2, 3 and 4). In addition, in chapters 5 and 6 we identified that less differentiated "younger" TIL in the cancer cell epithelium were of greater prognostic benefit to patients than "older" more differentiated TIL, and could even compensate in part for incomplete surgical removal of the tumor in high-grade serous ovarian cancer. Interestingly, while we did not observe immune modulating effects of chemotherapy on the tumor microenvironment, in chapter 7 we identified a pronounced depletion of circulating myeloid cells during treatment with carboplatin and paclitaxel in epithelial ovarian cancer patients. Finally, in chapter 4 we demonstrate that, a Semliki forest virus-based vaccine (Vvax001) targeting the cervical cancer oncogenes E6 and E7 was highly effective at inducing the prognostically beneficial intraepithelial CD103-positive TIL in cervical cancer in vivo associated with significant tumor reduction. Currently, we are therefore evaluating Vvax001 in a first-in-human phase I clinical study, as described in chapter 8.
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