A view on dendritic cell immunotherapy in ovarian cancer: how far have we come?

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Abstract

Ovarian cancer is the second most important pelvic gynaecologic malignancy and nowadays still kills 80% of patients. New treatment options are mandatory. Although it has been shown that ovarian cancer is an immunogenic tumor, the possibility of developing immunotherapy has been neglected for a long time. This article focuses on the importance of the immune system in the development and progression of cancer and the possibilities and problems of dendritic cell-based immunotherapy to influence the immune system.

Key words: Ovarian cancer, immunotherapy, DC, dendritic cell, targeted therapy, cancer.

Introduction

Ovarian cancer is the second most important pelvic gynaecologic tumor. According to the National Cancer Institute, the number of new cases is 12.3 per 100000 women per year. There are multiple histological subtypes of ovarian tumors, but 85% is epithelial in origin of which high-grade serous ovarian carcinomas are the most frequent ones. The major issue with ovarian cancer is that it can spread inside the abdomen before causing symptoms. Patients start showing symptoms once the tumor is metastasized over the peritoneum and causes the rise of ascites. As a silent killer, ovarian cancer is consequently most often diagnosed at an advanced stage with poor prognosis: 36-53 months at FIGO stage III and 20 months at stage IV. This is already a substantial increase compared to the situation 15 years ago. The reason is the improved surgical insights and combination with chemotherapy. Surgery is the cornerstone and should be radical to resect all macroscopic tumor burdens. Chemotherapy in the form of carboplatin-paclitaxel completes the primary treatment. In 2010, Vergote et al. (2010) could demonstrate that the overall survival was equally beneficial if chemotherapy was given first and then followed by surgery or vice versa. This important finding caused an enormous reduction in comorbidity due to radical surgery in the ovarian cancer patients with widespread disease. However, in case of relapse, therapeutic options are limited, especially if the relapse occurs within 6 months after completion of primary treatment. Consequently, nowadays, 80% of patients will still die of their disease.

It is clear that new treatments are necessary for ovarian cancer. The discovery of chemotherapy has certainly increased the survival of many patients in the past. However, adverse effects are inevitable and most tumors will reach a point of complete chemo-resistance. New treatments therefore have to be oriented differently. More and more attention is being paid to the targeted therapies. In these treatments, there is a focus present that is specific for the tumor or even for the tumor in a specific patient (personalized treatment). This focus can be variable: molecular, genetic, immunological ... For ovarian cancer, some international interest has already been shown, with for example an EU (European Commission)-approval for the use of
Dendritic cell immunotherapy is an attempt to increase the number of efficient DCm (and consequently tumor-specific T cells) in order to shift the balance from immunosuppression towards immune surveillance or to reprogram the immune system away from the ‘escape’ phase towards the equilibrium or elimination phase (Fig. 1) (Gilboa, 2007). Although some reports are now being published on augmenting the already existing DC in the body, ex vivo DC culturing is nowadays still the state-of-the-art. Ex vivo DC immunotherapy can schematically be presented as shown in Figure 2. It is a laboratory process, starting from the patient’s own white blood cells. However, several variations are possible at
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It might seem contradictory that chemotherapy will beneficially influence the immune system. For a long period of time, it was believed that chemotherapy only destroyed the white blood cells and therefore could certainly not contribute to the immune system in a positive way, until literature reports appeared showing a beneficial effect of chemotherapy (Antonia et al., 2006; Chu et al., 2012; Coosemans et al., 2013). The explanation is most probably multifactorial. The direct cytotoxic effect of chemotherapy destroys the tumor cells, which will lead to an increase in TAA. Moreover, some cytotoxic agents will also cause an Immunogenic Cell Death (ICD) of tumor cells, like etoposide and doxorubicin for example, leading to an increased secretion of DAMPs (Damage-Associated Molecular Patterns) (Vacchelli et al., 2014). Due to their immunostimulatory effects and their ability to lead to a better antigen presentation to DC, they increase the visibility of the massively released TAA to the immune system. On top of that, chemotherapy can render tumor cells more sensitive to granzyme, which is released by cytotoxic T cells and is able to start the process of programmed cell death in cancer cells. Chemotherapy can also increase the expression of Fas on tumor cells which makes them also visible for cytotoxic T cell mediated killing (Kadam and Abhang, 2015).

As a consequence, DC immunotherapy should not only evoke a positive immune response, but also overcome these immunosuppressive effects and this appears to be a problem. The sole use of DC immunotherapy does not seem to be sufficient to create this answer to the escape. Large clinical effects are consequently still lacking when using DC immunotherapy as a targeted treatment. The last years, more and more attention is being paid to the concept of chemo-immunotherapy.

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**Fig. 2.** — Schematic picture of *ex vivo* dendritic cell immunotherapy

DCi: Immature DC; DCm: mature DC; TAA: tumor-associated antigen
It is clear that the ideas and prejudices on chemotherapy have to be abandoned. However, it is not yet clear how, at what dose and at what time point chemotherapy should be integrated in the new immunotherapeutic options to create a powerful answer. This is the subject of further research.

On the other hand, experiments have shown that the T cells that will experience a certain decrease and have to recover, will preferentially be the T cells that recognized the release TAA. Moreover, it has been shown that the decrease of white blood cells will also include a decrease in immunosuppressive cells. Every chemotherapy has its own specifications in this respect. For example, cyclophosphamide would be able to reduce Treg and gemcitabine is most probably able to reduce MDSC. Recently, a nice overview has been published (Galluzzi et al., 2012). Finally, the use of chemotherapy can also lead to an increased tumor infiltration of T cells (Matarollo et al., 2011).

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| Step in the culturing process | Possibilities |
|------------------------------|--------------|
| Initiation of the culture    | From bone marrow CD34 precursor cells | Plastic adherence |
|                              | From monocytes | CD14 bead selection |
|                              |               | Counterflow centrifugal elutration |
| Culturing material           | Plastic bags  | Adherent surface |
|                              |               | Flasks or cell factories |
| Non-adherent surface         | IL-4 and GM-CSF | IL-15 and GM-CSF |
| Differentiation into DCi     | IFNα and GM-CSF | + addition of AA to the culturing medium |
| TAA                          | One specific TAA | Peptide or RNA |
|                              | A few specific TAA | Peptide or RNA |
|                              | Total tumoral mRNA | |
|                              | Whole tumor cell lysate | obtained by irradiation, freeze-thaw cycles, ICD inducing agents |
|                              | Fusion cells composed of autologous DCs and tumor cells | |
|                              | Synthetic long peptides | |
| Maturation DCi               | TriMix electroporation | One or more pro-inflammatory cytokines |
|                              |               | TNFα, IL-1b, IL-6, IRX-2, CD137, IFNg |
|                              |               | + PGE2 |
|                              |               | + TLR |
|                              |               | + LPS |
|                              |               | + PolyI:C |
|                              |               | + addition of Rapamycin to the culturing medium |
| Culuring time                | Ranging from 3-10 days | |
| Injection of DC              | Intradermal | |
|                              | Intravenous | |
|                              | Intranodal | |
|                              | Subcutaneous | |
|                              | Intratumoural | |

AA: arachidonic acid; CD: cluster of differentiation; DC: dendritic cell; DCi: immature dendritic cell; GM-CSF: granulocyte macrophage colony-stimulating factor; ICD: immunogenic cell death; IL: interleukin; IFN: interferon; IRX-2: mix of cytokines; LPS: lipopolysaccharide; mRNA: messenger ribonucleic acid; PGE2: prostaglandin E2; TAA: tumor-associated antigen; TLR: toll-like receptor; TNF: tumor necrosis factor; TriMix: mRNA encoding CD70, CD40L and a constitutively active TLR4.
### Table II. — Overview on DC immunotherapy in ovarian cancer

| Author         | Tumorantigen | Type of immunotherapy                                | Number of patients | Clinical outcome                                           |
|----------------|--------------|-------------------------------------------------------|--------------------|------------------------------------------------------------|
| Brossart 2000  | MUC-1 or HER-2 | DC + peptide                                          | 3                  | 1 : SD > 8m; 1 SD during 8 w                              |
| Hernando 2002  | Lysate + KLH  | DC + lysate and KLH                                   | 6                  | 3 SD                                                       |
| Loveland 2006  | mannan-MUC1 fusionprotein | DC + peptide                                         | 1                  | SD                                                         |
| Homma 2006     | Tumor cells   | DC/tumor-fusie vaccin + rhIL-12                        | 4                  | Only available in 1 patient: PD with transient decease of CA125 |
| Hernando 2007  | α-FR          | DC + mRNA-α-FR                                        | 1                  | PR                                                        |
| Peethambaram 2009 | HER-2     | Mix of PBMC and DC + a recombinant fusion antigen of HER2 | 4                  | 2 SD                                                       |
| Chu 2012       | HER2-neu + hTERT + PADRE | DC + peptide +/- cyclophosphamide 2 days prior to vaccination + pneumococcal vaccination | 11                 | 2 PD during vaccination, 3 PD between 6-26 m follow up, 6 CR |
| Rahma 2012     | p53          | Peptide + IL2 SC vs DC + peptide + IL2 IV              | 21                 | 4 NED after 2y, 16 PD                                     |
| Kandalaft 2013 | Lysate       | A/ In 6 patients: IV bevacizumab + metronomic cyclophosphamide PO, followed by bevacizumab + lysate loaded DC B/ In 3/6 patients this was followed by l/ lymphodepletion + 2/ transfer of autologous T cells in combination with the vaccin | 6                  | A/ 2 PR, 2 SD, 2 PD B/ 1 PR, 1 SD, 1 PD                  |
| Coosemans 2013 | WT1          | DC + mRNA-WT1                                         | 2                  | PD with prolonged OS if chemotherapy was administered after immunotherapy stop |
| Mitchell 2014  | MUC-1        | DC + peptide                                          | 26                 | Decrease in CA125 in 5 patients, of which 2 PD, 2 PR, 1 CR |
| Kobayashi 2014 | WT1, MUC-1, CA125 | DC + peptide                                         | 56                 | After 3m: 32 PD, 14 SD, 2 PR, 8 not evaluable             |
| Bapsy 2014     | Lysate       | DC + lysate IV                                        | 7                  | 4 PD, 2 SD, 1 PR                                          |

CR, complete remission; CA125, cancer antigen 125; DC, dendritic cell; IL, interleukin; KLH, keyhole limpet hemocyanin; MUC-1, mucin 1; mRNA, messenger ribonucleic acid; NED, no evidence of disease; PBMC, peripheral blood mononuclear cell; OS, overall survival; PD, progressive disease; PR, partial remission; rh, recombinant human; SC, subcutaneous; SD, stable disease; TAA, tumor-associated antigen; TERT, telomerase reverse transcriptase; WT1, Wilms’ tumor gene 1; y, year; m, month; w, weeks; SC, subcutaneous; IV, intravenous; PO, orally; HER-2, human epidermal growth factor 2; PADRE, DR-restricted Th helper epitope.
Clinical studies on DC immunotherapy in ovarian cancer

Though still limited, there is some interest in the development of DC immunotherapy in ovarian cancer. Table II gives an overview on existing studies. A total of 148 patients have been included in 13 studies over the past 14 years. As mentioned in table 1, there is an enormous variety in the culturing process of the DC, which is confirmed when looking to these clinical data. Results vary tremendously. So far, only two groups have undertaken additional steps to influence the immunosuppressive cells (Chu et al., 2012; Kandalaft et al., 2013).

Conclusion

Nowadays, 80% of ovarian cancer patients still die of their disease. There is an urgent need for new therapies. Immunotherapy has been neglected for a long time as a substantial candidate in ovarian cancer. Today, 13 clinical studies on DC immunotherapy are available. However, at the same time it also becomes clear that DC immunotherapy alone will not be able to induce an adequate immune response and shift the immune balance again towards immune surveillance. Immunosuppressive cells hampering this immune response appear to be very important players. The association of chemotherapy with DC immunotherapy could offer a possibility in overcoming this immunosuppression. Further studies will be needed to explore what chemotherapy at what dose and time point in the conventional treatment of the patient will be most beneficial.

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