

Dendritic cell-based immunotherapy in ovarian cancer

An Coosemans^{1,*}, Ignace Vergote¹, and Stefaan W Van Gool²

¹Department of Gynecologic Oncology; Leuven Cancer Institute, KU Leuven, Belgium; ²Department of Microbiology and Immunology; KU Leuven, Belgium

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Worldwide, 80% of patients with ovarian cancer die of the disease. New treatments for this aggressive disease are therefore being intensively searched. Although dendritic cell-based vaccines against gynecological malignancies are in their infancy, this immunotherapeutic approach holds much promise. Here, we present our view on an optimal dendritic cell-based immunotherapeutic strategy against ovarian cancer.

Ovarian cancer is the second most common type of gynecologic tumor in women, with a worldwide incidence of 12.5/100,000 women. Ovarian cancer presents as a silent killer, usually metastasizing throughout the abdomen before causing symptoms. Consequently, in 63% of patients ovarian cancer is detected as FIGO stage III or IV disease. This is associated with a poor prognosis, median survival being 36–53 and 20 months for patients with FIGO stage III and IV disease, respectively. Surgery in combination with platin-based adjuvant chemotherapy remains the cornerstone therapeutic modality. If the tumor relapses within 6 months of the initial treatment with platin-based combination chemotherapy, the prognosis is very poor.¹ Eventually, 80% of patients that are diagnosed with ovarian cancer will die of the disease.

It is thus clear that new treatment modalities against ovarian cancer are urgently needed. Nowadays, the major focus of targeted anticancer therapies is on molecular changes and genetic alterations, that may offer patients a personalized treatment. However, the heterogeneity of neoplastic cells constituting primary neoplastic lesions and their metastases is an important issue in this respect. In an attempt to target rather universal mechanisms of tumorigenesis, for example vascular endothelial growth factor (VEGF)-dependent

vascularization, passive forms of immunotherapy based on preformed antibodies have been devised and tested in several clinical Phase II trials, mainly resulting in a prolongation of progression-free survival, but associated with a non-negligible toxicity.²

Active immunotherapy as a measure to treat gynecological malignancies has been neglected for a long time and still is in its infancy. This approach relies on anticancer vaccines that are able to elicit an immune response against tumor-associated antigens (TAAs) in the human body. Several technical aspects of this type of immunotherapy should be considered.

First, TAA-based immunotherapeutic strategies can be divided into 2 groups: one, those that rely on products derived from whole cancer cells, including whole cancer-cell lysates, dendritic cell (DC)/cancer cell fusions, total cancer cell RNA or mRNA; and two, those that rely on one or more specific TAAs. Both have specific advantages and disadvantages, though whole cancer-cell approaches are gaining momentum, mostly because they encompass a personalized and broad range of known and unknown tumor antigens (and hence may minimize the development of tumor escape variants) as well as many proteins shared with normal cells, including immunostimulatory cytokines. Moreover, this approach

provides both MHC class I and II TAA-derived epitopes. The same advantages do not all apply when specific TAAs are used. However, side effects should generally be milder with TAA-based vaccines than with whole cancer cell-based approaches. Over 60 ovarian cancer-relevant TAAs have been studied, including Wilms' tumor 1 (WT1), which is overexpressed by a relevant proportion of these tumors.³

Second, TAAs can be administered as pure preparations, i.e., as naked peptides, proteins or DNA molecules, or via a carrier. DCs are one of the most common carriers used in this setting, offering a superior platform for stimulating TAA-specific immune responses *in vivo*.⁴

One of the main obstacles against the use of synthetic peptides is that only patients bearing the HLA type for which the peptide is restricted can obtain benefits from this approach. One strategy that would circumvent this issue is the transfection of TAA-coding mRNA into DCs, resulting in transient TAA expression and subsequent presentation of antigenic TAA-derived epitopes on the DC surface. The work of several laboratories suggests that this approach is an effective, if not superior, method to generate immunostimulatory DCs.⁵ However, in order to present TAA-derived epitopes in the context of both MHC class I and II molecules,

*Correspondence to: An Coosemans; Email: an.coosemans@gmail.com

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Table 1. Overview on DC-based immunotherapy in ovarian cancer

Author and year	TAA	Type of immunotherapy	N°	Clinical outcome
Brossart 2000	MUC1 or HER2	DCs + peptide	3	1/3 SD > 8 mo; 1/3 SD during 8 weeks
Hernando 2002	Tumor cell lysate	DCs + tumor cell lysates and KLH	6	3/6 SD
Loveland 2006	Mannan-MUC1 fusion protein	DCs + peptide	1	SD
Homma 2006	Tumor cells	DC/tumor cell fusions + rhIL-12	4	1 PD with temporary decrease of CA125
Hernando 2007	α -FR	DCs + α -FR-coding mRNA	1	PRs
Peethambaram 2009	HER2	Mix of PBMCs and DCs + recombinant HER2-based fusion protein	4	2/4 SD
Chu 2012	HER2 + TERT + PADRE	DCs + peptides +/- cyclophosphamide 2 d prior to vaccination + pneumococcal vaccine	11	2/11 PD during vaccination, 3/11 PD between 6–26 mo, 6/11 CR
Rhama 2012	p53	Peptide + IL-2 s.c. vs. DCs + peptide + IL-2 i.v.	21	4/20 NED after 2 y, 16/20 PD (mean 7 mo)
Kandalaf 2013	Tumor cell lysate	(A) In 6 patients: bevacizumab i.v. + metronomic cyclophosphamide p.o., followed by bevacizumab plus vaccination with DCs pulsed with tumor cell lysates (B) In 3/6 patients, this was continued with lymphodepletion followed by the transfer of autologous vaccine-primed T-cells in combination with the vaccine	6	A. 2/6: PR, 2/6: SD, 2/6: PD B. 1/3: PR, 1/3: SD, 1/3: 1/3: PD
Coosemans 2013	WT1	DCs + WT1-coding mRNA	2	PD, but prolonged OS after subsequent chemotherapy

Abbreviations: CR, complete response; DC, dendritic cell; IL, interleukin; KHL, keyhole limpet hemocyanin; MUC1, mucin 1; NED, no evidence of disease; PBMC, peripheral blood mononuclear cell; OS, overall survival; PD, progressive disease; PR, partial remission; rh, recombinant human; SD, stable disease; TAA, tumor-associated antigen; TERT, telomerase reverse transcriptase; WT1, Wilms' tumor gene 1; y, years; mo, months; s.c., subcutaneous; i.v., intravenous; p.o., per oral; HER-2, human epidermal growth factor 2; PADRE, DR-restricted Th helper epitope.

TAA-coding mRNAs should be modified with a lysosome-associated membrane protein (LAMP) signal.⁶ Table 1 gives an overview of all clinical studies testing DC-based immunotherapy in ovarian cancer patients till now. Although DC-based immunotherapeutic strategies hold great promise, significant clinical responses have yet to be achieved. Several studies are ongoing to optimize this approach.

Our research group focuses on two types of tumor: high grade glioblastoma

(HGG)⁷ and pelvic gynecological malignancies.^{8,9} In both these settings, DCs were chosen as carriers of TAAs for vaccination. Since HGG has not yet been associated with a specific TAA, we chose to employ cancer cell lysates. Conversely, in uterine and ovarian cancer we chose to work with a specific TAA, namely, WT1. DCs were loaded with TAA-coding mRNA. Both the use of total tumor lysate and WT1-coding mRNA offered different insights in the tumor biology, behavior

and immune monitoring. In contrast to the rather low clinical responses previously reported for DC-based immunotherapy in this setting, we achieved a significant improvement in the clinical outcome of HGG patients receiving DCs loaded with tumor lysate. Our studies with gynecological cancer patients receiving DCs loaded with WT1-coding mRNA generated profound insights into the immune responses elicited by the vaccine. Therefore, we believe that new studies testing DC-based immunotherapy in ovarian cancer patients should combine the whole tumor cell approach with the use 1 or 2 defined TAAs.

However, besides focusing on the development of immunotherapeutic strategies that elicit improved anticancer immune responses, it is crucial not to neglect the immunosuppressive mechanisms within the tumor microenvironment, which normally hamper antitumor immunity. Therefore, combinatorial approaches are likely to induce therapeutically relevant immune responses, as recently demonstrated by Kandalaf et al. (Table 1). Specific agents have been developed to limit immunosuppression by targeting for example regulatory T cells and myeloid-derived suppressor cells. In our opinion, it is more interesting to harness chemotherapeutic agents that are currently employed against metastatic ovarian cancer. Several of them have indeed been shown to exert immunomodulatory effects and to induce the immunogenic demise of cancer cells.¹⁰ The doses and combination schedules of these immunotherapeutic regimens will however be crucial to achieve optimal anticancer immune responses.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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