Sperm protein 17 targeting for epithelial ovarian cancer treatment in the era of modern immunoengineering

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Due to the vague symptomatology of the disease and a lack of effective screening methods, most patients with epithelial ovarian cancer (EOC) present late in their disease. Despite advances in chemotherapeutic agents, the prognosis of these patients has uniformly been extremely poor. Although cisplatin-based chemotherapy regimens induce responses in most of these patients, the patients invariably experience disease progression or relapses. In an attempt to improve the treatment outcome using a different therapeutic approach, immunotherapy was investigated nearly 20 years ago. Many tumor antigens that are potentially suitable for specific immunotherapy were identified, and many immunotherapeutic approaches were attempted. However, although some responses were observed, the results from clinical studies were generally disappointing. Recent advances in immunoengineering and successes observed among patients treated for refractory/relapsed hematologic malignancies have rekindled the interest of modern immunoengineering technology.

INTRODUCTION
Epithelial ovarian cancer (EOC) remains one of the commonest causes of cancer-related deaths in women in the 21st century. In the United States, there were approximately 235,000 EOC patients in 2018.1 It is estimated that there will be 21,410 new cases, with 13,770 deaths, in the United States in 2021.1 Although EOC is potentially a curable disease in its early stages, due to the generally vague and nonspecific symptoms and lack of effective screening strategies, most patients present late in their disease when metastasis has already occurred. Despite the advances in modern chemotherapeutic agents and personalized medicine, a significant inroad into the treatment of EOC has not been made. Cytoreduction through primary surgery followed by platinum-based chemotherapy has remained the first-line treatment for women with newly diagnosed EOC,2 and this has not changed significantly in the last three decades. While a high proportion of patients will respond to the combination of surgical debulking followed by adjuvant chemotherapy with carboplatin and paclitaxel, disease recurrence/progression invariably occurs.2

New approaches for advanced EOC in the past decade include anti-angiogenic agents3–5 and poly(ADP-ribose) polymerase (PARP) inhibitors.6,7 The most commonly used and well-studied antiangiogenic agent in the treatment of EOC is bevacizumab. However, as reported in ICON-75 and GOG 281,9 bevacizumab added to platinum-based chemotherapy did not increase overall survival (OS) in the study populations as a whole but did show a significant difference in OS in a predefined subgroup of patients with poor-prognosis disease in ICON-7 and stage IV disease in GOG 218. PARP inhibitors, on the other hand, have shown meaningfully prolonged progression-free survival (PFS) for the first time after decades of studying different chemotherapy approaches.10 The well-studied PARP inhibitors for the treatment of high-grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancer include olaparib, rucaparib, niraparib, and veliparib. Most of them are approved by the Food and Drug Administration to be used for first- or second-line maintenance therapy after cytotoxic chemotherapy or as the single agent for third- or fourth-line therapy. Optimal use of these newer agents remains to be determined, but at present the clinical benefits in OS are modest. The lack of successes with the currently available chemotherapeutic agents suggests that other approaches are much needed.

In this review, we discuss briefly the principles and history of immunotherapy in EOC, dissect Sperm protein 17 (Sp17) as a target for immunoengineering, and examine how the current advances in immunoengineering might be exploited to utilize Sp17 for cell-based therapy of EOC.

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Both extracellular and intracellular tumor antigens have been demonstrated in ovarian cancer. Examples of these antigens are shown in Table 1.11–27 Most of these antigens arise due to either overexpression or aberrant expression of normal cellular proteins. Extracellular antigens include Sp17,18 MUC1,11 and mesothelin.14 Intracellular tumor antigens in ovarian cancer cells have also been demonstrated in various studies, including the MAGE family of antigens,16 NY-ESO1,12 and LACE-1.17 Other antigens including neoantigens14,24–27 may have arisen from random mutations associated with neoplastic process, although genomic analysis of EOC has found the tumor cells to harbor low mutational burden.28

EOC cells are susceptible to the cytotoxic machinery of T cells. Peripheral blood lymphocytes primed to MUC1,27 MAGE,30,31 Sp17,13 and mesothelin13 have demonstrated the ability to lyse EOC cells. Similarly, susceptibility of EOC cells to killing by T cells has been demonstrated in mouse models of EOC.24,25 Clinically, the correlation between the intensity of tumor-infiltrating lymphocytes (TILs) and clinical outcomes36–38 further supports this notion. Clinical responses were observed in EOC patients treated with TILs expanded ex vivo.39–42

Since ovarian tumor cells harbor immunogenic antigens and tumor-reactive T cells are present in the immune repertoire of the autologous hosts, harnessing the host de novo antitumor immune responses through immunotherapy is an attractive option. Unfortunately, the results from cell-based immunotherapy in EOC have thus far been uniformly disappointing (Table 2).39–46 One of the major reasons for the lack of successes is an immune repertoire that has been contracted severely by chemotherapy, rendering the host response to be unable to mount an efficient effector/target ratio in vivo. This notion is clearly seen in the clinical use of immune checkpoint inhibitors in EOC patients. Due to low tumor mutational burden (17) and thus a low number of neoantigen-reactive T cells, the response rate has been uniformly disappointing. Advances in immunoengineering provide a means to increase the effector/target ratio. However, this approach is applicable only for known antigens and not for unidentified neoantigens that arise from random genomic mutation in EOC.

**SPERM PROTEIN 17 AS A TARGET FOR IMMUNOTHERAPY**

**Sp17 gene and protein**

Human Sp17 gene, located on chromosome 11q24.2, encodes a highly conserved antigenic protein of 17.4 kDa molecular weight expressed in spermatozoa and is involved in acrosome reactions during fertilization.47 Immunohistochemistry on tissue arrays and RT-PCR of a panel of tissue RNA48 showed Sp17 to be restricted in its normal tissue distributions, being expressed primarily in normal testes. Immunotherapy targeting Sp17 will, therefore, be more specific and less toxic.

Sp17 is a highly immunogenic protein. Many vasectomized normal males develop autoantibodies against Sp17.49 It was, therefore, the target of investigation as an immunococntceptive.50 Sp17 is primarily an extracellular protein and promotes heparan sulfate-mediated cell-cell adhesion.50,51 Although it is an extracellular protein, Sp17 undergoes modulation and is shed into the serum of cancer patients.50

**Sp17 as a tumor antigen**

Sp17 was first identified in our laboratory as an aberrantly expressed tumor antigen in multiple myeloma.50 It has since been found to be expressed in non-Hodgkin’s lymphoma51 and non-small cell lung cancer.50 Interestingly, despite being a normal testicular protein,

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**Table 1. Tumor antigens in epithelial ovarian cancer**

| Class                        | Antigens               | Comments                                                                 |
|------------------------------|------------------------|--------------------------------------------------------------------------|
| Overexpressed normal cellular antigens |                        |                                                                          |
| MUC1                         |                        | these are normal cellular proteins that are overexpressed on EOC cells.   |
| Wilms’ tumor 1 (WT1)         |                        | T cells within the host immune repertoire are expected to be of low       |
| Telomerase                   |                        | affinity for the tumor cells, since any high-affinity T cells would have  |
| Mesothelin                   |                        | been deleted from the immune repertoire during development to avoid       |
| Erb                          |                        |                                                                          |
| MAGE family                  |                        | these are normal testicular proteins that are aberrantly expressed by EOC  |
| NY-ESO1                      |                        | cells. They are generally highly immunogenic because testes are immune    |
| LACE-1                       |                        | sanctuary sites, hence high-affinity antigen-reactive T cells are not     |
| Sp17                         |                        | deleted by the thymus during development. Furthermore, being whole        |
| AKAP3                        |                        | proteins, immune responses tend to be polyclonal, directed at more than   |
| AKAP4                        |                        | one epitope within the antigens, providing high in vivo effector/target    |
| GAGE-1/2                     |                        | ratios. Even though CTA are testicular-restricted proteins, highly        |
| PRAME                        |                        | sensitive qPCR has detected leakage of expression of some of these genes,  |
| TRAG-3                       |                        | at low levels, in normal tissues. Their expression in tumor cells is       |
| mutared KRas                  |                        | is believed to be mediated, in most cases, by changes in the methylation  |
| mutated p53                   |                        | status of the gene during carcinogenesis                                  |
| mutated Erb                  |                        |                                                                          |
| mutated ARID1a               |                        |                                                                          |

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**PRINCIPLES AND HISTORY OF IMMUNOTHERAPY IN EOC**

Immunotherapy has always been an attractive cancer therapeutic option, since it is not only more specific but also kills tumor cells via molecular mechanisms different from those induced by chemotherapeutic agents. It may, therefore, be used to either circumvent chemoresistance or in conjunction with chemotherapy to augment the cytotoxicity. Various clinical and laboratory data support the applicability of immunotherapy in EOC.
Sp17 expression on tumor cells does not appear to be gender biased. In fact, Sp17 is expressed in high proportions of patients with gynecologic cancers such as ovarian cancer, endometrial cancer, and cervical cancer (Table 3). In these conditions, Sp17 was not only expressed at the mRNA level but also at the protein level, arguing for a possible function of the protein in tumor cells. Sp17 expression is correlated with chemoresistance in clear-cell adenocarcinoma. It also has roles in metastasis and drug resistance; for example, EOC with high Sp17 expression shows resistance to paclitaxel, the first line of EOC treatment. EOC cells with enhanced Sp17 expression demonstrate increased migration. Progressive tumor growth by ID8 cells in mice is dependent on Sp17 expression. Immunotherapy targeting Sp17 is, therefore, attractive and may be used to eradicate the chemoresistance clones to augment the efficacy of chemotherapy.

We and others have previously demonstrated the ability to generate Sp17-specific human leukocyte antigen (HLA) class I-restricted

| Authors            | No. of patients (n) | Intervention                                                                 | Outcome                                                                                      | Comments                                                                                   |
|--------------------|---------------------|-------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| Kershaw et al.43    | 8 in cohort 1; 6 in cohort 2 | T cells with reactivity against the ovarian cancer-associated antigen α-folate receptor (FR) were generated from autologous T cells with a chimeric gene incorporating an anti-FR single-chain antibody linked to the signaling domain of the Fc receptor γ chain. Eight patients in cohort 1 received a dose escalation of T cells in combination with high-dose interleukin-2, and six patients in cohort 2 received dual-specific T cells (reactive with both FR and allogeneic cells) followed by immunization with allogeneic peripheral blood mononuclear cells | no response was observed. In-labeled adoptively transferred T cells in cohort 1 did not show any tumor localization except in one patient where some signals were detected in the peritoneal deposit | phase 1 study in which all 14 patients had metastatic disease and had undergone surgical debulking and combination chemotherapy |
| Haas et al.64       | 15 patients in total: 6 in cohort 1; 3 each in cohorts 2, 3, and 4 | CAR was directed at mesothelin and contained CD3z and 4-1BB in the lentiviral construct. Cohorts 1 and 2 patients received $1\times10^6$ TILs and cohorts 3 and 4 patients received $3\times10^6$ TILs CAR-T cells. Patients in cohorts 2 and 4 also received lymphodepletion with cyclophosphamide, 1.5 mg/m$^2$ day prior to CAR-T infusion | stable disease was observed in 11/15 patients on day 28 | phase 1 study that included 5 patients each, with ovarian cancer, malignant pleural mesothelioma, and pancreatic cancer |
| Aoki et al.39       | 17 patients with advanced/recurrent EOC | 7 patients received TILs following one dose of cyclophosphamide (group A) and 10 received alternating cisplatin-containing regimen and TILs (group B) | 1 CR and 4 PR in group A, and 7 CR and 2 PR in group B | responses in both primary and metastatic lesions lasted 3–5 months in group A |
| Fujita et al.60     | 13                  | all patients were treated in the adjuvant setting after surgical debulking and cisplatin regimen. Each received $1\times10^7$ TILs | 3-year DFS was 81.2% and OS 100% | compared with a control group of 11 patients, DFS and OS were significantly prolonged |
| Freedman et al.51   | 8 patients with advanced EOC refractory to platinum-based chemotherapy | all patients received $10^{10}$–$10^{11}$ TILs intraperitoneally and recombinant interleukin-2. One patient received two doses of TILs | no measurable response was observed in any of the patients | pilot study to evaluate feasibility and clinical response of intraperitoneal TILs |
| Liu et al.65        | 92                  | all patients were treated in the adjuvant setting following surgical debulking and 6–8 cycles of carboplatin/paclitaxel; 46 patients were treated with monthly CIK cells and 46 in the control group with observation only | PFS was 37.7 months in the treatment group compared with 22.2 months in the control group (p = 0.004). OS was not improved | paired study of monthly maintenance CIK cells |
| Pedersen et al.52   | 6                   | all patients had platinum-resistant progressive metastatic EOC. They all received TILs followed by rIL-2 | 4 patients showed stable disease for 3 months and 2 for 5 months | high number of infused TILs expressed LAG-3 and PD-1 |
| Wright et al.66     | 7                   | all patients had recurrent EOC confined to the peritoneal cavity. They received peripheral blood lymphocytes stimulated with MUC1 intraperitoneally | 1 patient attained CR | no significant reduction in serum CA125 |

CR, complete response; PR, partial response; DFS, disease-free survival; OS, overall survival; PFS, progression-free survival; TILs, tumor-infiltrating lymphocytes; CIK, cytokine-induced killer; rIL-2, recombinant interleukin-2; MUC1, Mucin 1; LAG-3, lymphocyte activation gene 3; PD-1, programmed cell death protein 1; CA125, carbohydrate antigen 125.
cytotoxic T cells from patients with various cancer types, including ovarian cancer. These cytotoxic T cells were able to kill fresh autologous ovarian tumor cells. T cell epitopes restricted by HLA-A1 and HLA-A2 have been identified. These works suggest that, despite being a surface antigen, Sp17 protein is also processed and presented in conjunction with the major histocompatibility complex (MHC). Furthermore, they also clearly demonstrate that despite the presence of active cancer and previous chemotherapy, Sp17-reactive T cells were still present in the immune repertoire of these patients. However, while molecules of MHC I expression are retained, Sp17-positive tumor cells have lower expression of MHC II molecules along with an increase in STAT3. STAT3 overexpression is associated with invasive and metastatic properties of EOC cells.

**Regulation of Sp17 expression**

Unless the tumor antigen is a driver protein needed for tumor cell survival, progressive downregulation of a tumor antigen gene often occurs. This is a form of immune tumor escape mechanism and poses one of the biggest obstacles to successful immunotherapy. Although there is currently no evidence supporting progressive downregulation of Sp17 expression, Sp17 is heterogeneously expressed within tumor specimens.

### Table 3. Expression of Sperm protein 17 in tumor specimens

| Authors                  | Tumor type                        | Comments                                                                 |
|--------------------------|-----------------------------------|--------------------------------------------------------------------------|
| Lim et al.54             | multiple myeloma                  | detected by RT-PCR and western blot analysis in 12/47 (26%) of specimens |
| Straughn et al.18        | ovarian cancer                     | detected by RT-PCR, northern blot analysis, and immunohistochemistry in 7/11 (64%) of papillary serous or mixed, 4/8 (50%) endometrioid carcinoma, 2/2 clear cell, 3/3 ovarian unspecified. Overall, 17/25 (68%) specimens |
| Liggin et al.19          | diffuse large B cell lymphoma      | detected by RT-PCR, western blot analysis, and immunohistochemistry in 6/11 (55%) specimens |
| Zhou et al.17            | invasive breast cancer             | detected by RT-PCR and immunohistochemistry in 27/100 (27%) specimens. Sp17 expression correlated with lymph node metastasis |
| Mirandola et al.16       | invasive breast cancer             | detected by immunohistochemistry in 10/22 (45%) invasive breast cancers and 17/36 (47%) triple-negative breast cancers |
| Li et al.19              | endometrial and cervical cancer    | detected by immunohistochemistry in 33/50 (66%) endometrial cancers and 19/31 (61%) cervical cancers |
| Grizzi et al.12          | neuroectodermal tumors             | detected by immunohistochemistry in 6/28 (21%) tumors                     |
| Gupta et al.13           | esophageal squamous cell cancer    | detected by RT-PCR in 26/30 (86%) and by immunohistochemistry in 60/80 (75%) specimens |
| Xia et al.14             | hepatocellular cancer              | detected by immunohistochemistry in 36/45 (80%) specimens                 |
| Mirandola et al.15       | non-small cell lung cancer         | detected by RT-PCR and immunocytochemistry in 16/40 (40%) specimens      |

**Figure 1. Schematic illustration of immunoengineering of CAR-T or TCR-T cells for treating patients with epithelial ovarian cancer**

1. Autologous CD3+ T cells first are procured by apheresis from the patient. 2. T cells are genetically modified, most commonly using a lentiviral construct, to express chimeric antigen receptors (CARs) or engineered T cell receptors (TCRs). 3. CAR-T or TCR-T cells are expanded ex vivo to obtain required number. 4. Engineered T cells are infused into the same EOC patient to lyse EOC cells. Patients are commonly given chemotherapy via fludarabine and/or cyclophosphamide to lymphodeplete the immune repertoire prior to infusion of the engineered T cells. Lymphodepletion facilitates the homeostatic expansion of the infused engineered T cells.
SP17-TARGETED IMMUNOTHERAPY IN THE ERA OF MODERN IMMUNOENGINEERING

Sp17 appears to be an ideal tumor target for modern immunotherapy of EOC for the following reasons: (1) it is expressed on the surface of a high proportion of EOC cells; (2) it is processed and presented by the MHC molecules on the surface of EOC cells; (3) it shows restricted normal tissue distribution; and (4) its expression may be regulated pharmacologically. Being a surface antigen, Sp17 may be amenable to specific antibody targeting. However, Sp17 monoclonal antibodies have not been tried in the clinic and the feasibility of this approach remains to be determined. Anti-Sp17 monoclonal antibody was found to have some antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity in vitro against Sp17-expressing ovarian cancer cells. Potential obstacles to its successful use include the presence of shed Sp17 protein in the serum of these patients. However, it remains to be determined whether the functions of ADCC remain intact and efficient in patients with advanced EOC.

On the other hand, data showing the susceptibility of ovarian tumor cells to T cells would support the use of T cell immunotherapy for EOC. In mice injected with ID8 ovarian cancer cells, coadministration of recombinant Sp17 and CpG oligonucleotides every 30 days for a total of ten doses prevented ovarian tumor formation for up to 300 days with a survival rate of 77% and 80% in prophylactic and therapeutic groups, respectively. It generated strong immune response evident by an increase in T-helper 17 cells, increases in tumor necrosis factor α, interferon-γ, and granulocyte-macrophage colony-stimulating factor in serum, and a decrease in regulatory T cells. Oral coadministration of M cell-targeted microparticles loaded with T and B cell immune-dominant region of recombinant Sp17 and CpG oligonucleotide triggered humoral and cellular immune responses in mice injected with ID8 ovarian cancer cells and retarded ovarian tumor growth. Human anti-Sp17 monoclonal cytotoxic T lymphocytes, obtained either from advanced ovarian cancer patients or from healthy donors, directly migrated to tumor sites and eradicated the tumor in mice injected with human ovarian cancer cells. Clinically, dendritic cells pulsed with the Sp17 recombinant protein were administered to one patient with EOC. Although no obvious clinical benefits were observed in this patient, there were transient drops in serum CA125. The transient drop in the tumor marker most likely reflects the low in vivo effector/target ratio generated by the dendritic cells within a very restricted immune repertoire. A modern immunoengineering approach would be an ideal platform for a cell-based immunotherapy for EOC targeting Sp17. Two approaches are currently available, chimeric antigen receptor T (CAR-T) cells or T cell receptor T (TCR-T) cells. Both approaches involve the procurement of autologous T cells by apheresis, followed by ex vivo expansion and genetic modification of the T cells in the laboratory to become either CAR-T or TCR-T, before the T cells are reinfused back into the patients (Figure 1). The ex vivo expansion will provide a means to increase the effector/target ratio following infusion of the genetically modified T cells.

Chimeric antigen receptor T cells
CAR-T cells are autologous peripheral blood T cells engineered to display antigen-binding fragments of a specific antibody fused to intracellular T cell signaling domains. Human antibody variable fragments directed at Sp17 may be obtained through immunizing transgenic mice carrying the human immunoglobulin genes. CAR-T has produced encouraging clinical results in those with refractory B cell acute lymphoblastic leukemia, B cell non-Hodgkin’s lymphoma, and multiple myeloma. CAR-T in hematologic malignancies have thus far relied primarily on targeting differentiation antigens to avoid potential antigen downregulation as a form of tumor escape from immunsurveillance.
In EOC, there are no suitable differentiation antigens that have been identified so far. Sp17 is, therefore, a suitable alternative. CAR-T cells targeting Sp17 may be engineered to bind either the intact surface Sp17 or the HLA class I/Sp17 peptide complex (Figure 2) on EOC cells. Unlike other tumor antigens, Sp17 expression can be upregulated using DNA-hypomethylating agents. Intermittent upregulation of Sp17 expression may be sufficient to prevent tumor immunosurveillance escape and provide comparable efficacy with CAR-T that is directed at a differentiation antigen. The presence of both surface antigens and HLA class I/tumor peptide complex on the surface provides the opportunity for dual CAR-T therapy using a combination of CAR-T directed at the intact surface Sp17 protein and the HLA class I/tumor peptide complex to improve the efficacy of tumor cell killing and heterogeneity of antigen expression.

**Figure 3. Schematic diagram of TCR T cell structure**

(A) The TCR of an autologous T cell is a heterodimer surrounded by CD3 chains. It recognizes intracellular antigens presented by the MHC molecules to initiate cell death. (B) The autologous TCR-T is genetically engineered by cloning of the TCR variable genes (Va and Vb) expressed by Sp17-reactive T cells to a viral vector expressing the TCR C genes (Ca and Cb) linked to the transmembrane CD3ζ and transduced into autologous T cells. (C) Sp17 immunogenic peptides presented on the EOC cell surface in association with the MHC I molecules is recognized by TCR-T cells to initiate cell death.

**Figure 4. To enhance the efficacy of the TCR-T cell and CAR-T cell mediated immune response, the costimulatory domains of CD27 or CD28 are incorporated into the viral construct for either TCR-T cells or second-generation CAR-T cells**
various laboratories. To generate cytotoxic T cells directed against Sp17 suggest that this could be readily accomplished by cloning TCR from the cytotoxic T cell lines generated. TCR-T cells are currently being explored in various tumor types. However, since TCR does not undergo hypermutation and affinity maturation like an antibody, the affinity of TCR-T cells for their targets will invariably be considerably lower than those of CAR-T.

**Enhancing the efficacy of cell-based immunotherapy targeting Sp17**

The efficacy of CAR-T cells and TCR-T cells may also be enhanced if the antigen-binding fragment is linked to the T cell costimulatory domains such as CD28 or CD27 (Figure 4). It may also be possible to improve the efficacy of the infused CAR-T or TCR-T cells by using them in combination with immune checkpoint inhibitors or sequential administration of chemotherapy. Other approaches to enhance the efficacy of cell-based immunotherapy for solid tumors include enhancing tumor cell apoptosis via iCaspase-9 release and strategies to overcome the adverse immunologic conditions induced by cytokines, such as transforming growth factors that impair T cell functions in the tumor microenvironment.

**CONCLUSIONS**

Recent advances in immunoengineering have rekindled interest in cell-based immunotherapy in EOC. It is expected that increasing efforts will be made to revisit the tumor antigens previously identified in solid tumors, especially cancer-testis antigens. However, although cell-based immunotherapy has transformed the treatment paradigm in a number of hematologic malignancies, it has so far not made significant inroads into solid tumors. Tumor microenvironments associated with solid tumors, such as malignant asciites and various immunosuppressive cytokines associated with EOC, will likely be the main obstacles impairing the efficacy of cell-based immunotherapy. Although there are many tumor antigens that may be suitable for targeting, in addition to enhancing T cell activation and upregulating antigen expression pharmacologically, successful cell-based immunotherapy for EOC may need to incorporate strategies that include sequential chemotheraphy and methods to overcome the deleterious tumor microenvironmental cytokines.

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**AUTHOR CONTRIBUTIONS**

M.P.: initial data collection and writing of the first draft of the manuscript. D.D.: initial data collection, editing of the drafts, and drawing of the figures. Y.L.: editing of the drafts and initial conceptualization of the review. S.H.L.: overall responsibilities, conceptualization of the review, supervision, and writing of final draft.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

**REFERENCES**

1. Howlader, N., Noone, A.M., Krapcho, M., Miller, D., Brest, A., Yu, M., Ruhl, J., Tatalovich, Z., Mariotto, A., Lewis, D.R., et al. (2021). SEER Cancer Statistics Review, 1975-2018 (National Cancer Institute), based on November 2020 SEER data submission, posted to the SEER web site, https://seercancer.gov/csr/1975_2018/

2. Matulonis, U.A., Sood, A.K., Fallowsfield, L., Howitt, B.E., Sehoulji, J., and Karlan, B.Y. (2016). Ovarian cancer. Nat. Rev. Dis. Primers 2, 16061.

3. Gadducci, A., Lanfredini, N., and Sergiampietri, C. (2015). Antiangiogenic agents in gynecological cancer: state of art and perspectives of clinical research. Crit. Rev. Oncol. 95, 113–128.

4. Jackson, A.L., Eisenhauer, E.L., and Herzog, T.J. (2015). Emerging therapies: angiogenesis inhibitors for ovarian cancer. Expert Opin. Emerg. Drugs 20, 331–346.

5. Matulonis, U.A., Berlin, S., Ivey, P., Tyburski, K., Krasner, C., Zarwan, C., Berkenblit, A., Campos, S., Horowitz, N., Cannistra, S.A., et al. (2009). Cediranib, an oral inhibitor of vascular endothelial growth factor receptor kinases, is an active drug in recurrent epithelial ovarian, fallopian tube, and peritoneal cancer. J. Clin. Oncol. 27, 5601.

6. Coleman, R.L., Sill, M.W., Bell-McGuinn, K., Aghajanian, C., Gray, H.J., Tewari, K.S., Rubin, S.C., Rutherford, T.J., Chan, J.K., Chen, A., et al. (2015). A phase II evaluation of the potency, highly selective PARP inhibitor veliparib in the treatment of persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer in patients who carry a germline BRCA1 or BRCA2 mutation—an NRG oncology/gynecoologic oncology group study. Gynecol. Oncol. 137, 386–391.

7. Sandbu, S.K., Scholman, W.R., Wilding, G., Moreno, V., Baird, R.D., Miranda, S., Hylands, L., Ruijnae, R., Forster, M., Onlin, A., et al. (2013). The poly (ADP-ribose) polymerase inhibitor niraparib (MK4827) in BRCA mutation carriers and patients with sporadic cancer: a phase 1 dose-escalation trial. Lancet Oncol. 14, 882–892.

8. Perren, T.J., Swart, A.M., Pfisterer, J., Ledermann, J.A., Pujade-Lauraine, E., Kristensen, G., Carey, M.S., Beale, P., Cervantes, A., Kuzred, C., et al.; ICON7 Investigators (2011). A phase 3 trial of bevacizumab in ovarian cancer. N. Engl. J. Med. 365, 2484–2496.

9. Tewari, K.S., Burger, R.A., Enserro, D., Norquist, B.M., Swisher, E.M., Brady, M.F., Bookman, M.A., Fleming, G.F., Huang, H., Homesley, H.D., et al. (2019). Final overall survival of a randomized trial of bevacizumab for primary treatment of ovarian cancer. J. Clin. Oncol. 37, 2317–2328.

10. Mirza, M.R., Coleman, R.L., Gonzalez-Martín, A., Moore, K.N., Colombo, N., Ray-Coquard, I., and Pignata, S. (2020). The forefront of ovarian cancer therapy: update on PARP inhibitors. Ann. Oncol. 31, 1148–1159.

11. Engelstaedter, V., Heublein, S., Schumacher, A.L., Lenhard, M., Engelstaedter, H., Andergassen, U., Goemethner-Biller, M., Kuhn, C., Back, B., Kupka, M., et al. (2012). Mucin-1 and its relation to grade, stage and survival in ovarian carcinoma patients. BMC Cancer 12, 600.

12. Zhang, W., Lu, X., Cui, P., Piao, C., Xiao, M., Liu, X., Wang, Y., Wu, X., Liu, J., and Yang, L. (2019). Phase II/I clinical trial of a Wilms’ tumor 1-targeted dendritic cell vaccination-based immunotherapy in patients with advanced cancer. Cancer Immunol. Immunother. 68, 121–130.

13. Zheng, P.S., Iwasaka, T., Yamashita, F., Ouchi, M., Yoshikawa, M., Nakao, Y., Fukuda, K., Matsuyama, T., and Sugimori, H. (1997). Telomerase activity in gynecologic tumors. Gynecol. Oncol. 64, 171–175.

14. Hassan, R., and Ho, M. (2008). Mesothelin targeted cancer immunotherapy. Eur. J. Cancer 44, 46–53.

15. Baxevanis, C.N., Sotiropoulou, P.A., Sotiriadou, N.N., and Papanicolaou, M. (2004). Immunobiology of HER-2/neu oncoprotein and its potential application in cancer immunotherapy. Cancer Immunol. Immunother. 53, 166–175.

16. Yamada, A., Kataoka, A., Shichijo, S., Kamura, T., Imai, Y., Nishida, T., and Itoh, K. (1995). Expression of MAGE-1, MAGE-2, MAGE-3/-6 and MAGE-4a/4b genes in ovarian tumors. Int. J. Cancer 64, 388–393.

17. Ödunjı, K., Junghubl, A.A., Stockert, E., Qian, F., Gnjatic, S., Tamemela, J., Intengan, M., Beck, A., Keitz, B., Santiago, D., et al. (2003). NY-ESO-1 and LAGE-1 cancer-testis antigens are potential targets for immunotherapy in epithelial ovarian cancer. Cancer Res. 63, 6076–6083.
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36. Hwang, W.-T., Adams, S.F., Tahirovic, E., Hagemann, I.S., and Coukos, G. (2012). Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. Gynecol Oncol. 124, 192–198.

37. Nielsen, I.S., Sahota, R.A., Milne, K., Kost, S.E., Nesslinger, N.J., Watson, P.H., and Nelson, B.H. (2012). CD20+ tumor-infiltrating lymphocytes have an atypical CD27 memory phenotype and together with CD8+ T cells promote favorable prognosis in ovarian cancer. Clin Cancer Res. 18, 3281–3292.

38. Sato, E., Olson, S.H., Ahn, J., Bundy, B., Nishikawa, H., Qian, F., Jungbluth, A.A., Frosina, D., Gnjatic, S., Ambrosone, C., et al. (2005). 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 102, 18538–18543.

39. Aoki, Y., Takakuwa, K., Kodama, S., Tanaka, K., Takahashi, M., Tokunaga, A., and Takahashi, T. (1991). Use of adoptive transfer of tumor-infiltrating lymphocytes alone or in combination with cisplatin-containing chemotherapy in patients with epithelial ovarian cancer. Cancer Res. 51, 1934–1939.

40. Fujita, K., Ikarashi, H., Takakuwa, K., Kodama, S., Tokunaga, A., Takahashi, T., and Tanaka, K. (1995). Prolonged disease-free period in patients with advanced epithelial ovarian cancer after adoptive transfer of tumor-infiltrating lymphocytes. Clin Cancer Res. 1, 501–507.

41. Freedman, R.S., Edwards, C.L., Kavanagh, J.J., Kudelka, A.P., Katz, R.L., Carrasco, C.H., Atkinson, E.N., Scott, W., Tomasovic, B., Templin, S., et al. (1994). Intraperitoneal adoptive immunotherapy of ovarian carcinoma with tumor-infiltrating lymphocytes and low-dose recombinant interleukin-2: a pilot trial. J Immunother. 16, 198–210.

42. Pedersen, M., Westergaard, M.C.W., Milne, K., Nielsen, M., Borch, T.H., Poulsen, L.G., Hendel, H.W., Kennedy, M., Briggs, G., Ledoux, S., et al. (2018). Adoptive cell therapy with tumor-infiltrating lymphocytes in patients with metastatic ovarian cancer: a pilot study. Oncoimmunology 7, e1290295.

43. Kershaw, M.H., Westwood, J.A., Parker, L.L., Wang, G., Eshhar, Z., Mavroukakis, S.A., White, D.E., Wunderlich, J.R., Canevari, S., Rogers-Freewy, L., et al. (2006). A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res. 12, 6106–6115.

44. Haas, A.R., Tanyi, J.L., O’Hara, M.H., Gladney, W.L., Lacey, S.F., Torrigan, D.A., Soulen, M.C., Tian, L., McGarvey, M., Nelson, A.M., et al. (2019). Phase I study of lentiviral-transduced chimeric antigen receptor-modified T cells recognizing mesothelin in advanced solid cancers. Mol Ther. 27, 1919–1929.

45. Liu, J., Li, H., Cao, S., Zhang, X., Yu, J., Qi, J., An, X., Yu, W., Ren, X., and Hao, X. (2014). Maintenance therapy with autologous cytotoxic-induced killer cells in patients with advanced epithelial ovarian cancer after first-line treatment. J Immunother. 37, 115–122.

46. Wright, S.E., Revers, F.-E., Quinlin, I.S., Phillips, C.A., Townsend, M., Philip, R., Dobrzenski, M.J., Lockwood-Cooke, P.R., and Robinson, W. (2012). Cytotoxic T-lymphocyte immunotherapy for ovarian cancer: a pilot study. J Immunother. 35, 196–204.

47. Freyne, J., and Hall, L. (2002). A re-evaluation of sperm protein 17 (Sp17) indicates a regulatory role in an A-kinase anchoring protein complex, rather than a unique role in sperm-zona pellucida binding. Reproduction 124, 767–774.

48. Zhang, Y., Wang, Z., Robinson, W.R., and Lim, S.H. (2004). Combined real time PCR and immunohistochemical evaluation of sperm protein 17 as a cancer-testis antigen. Eur J Haematol. 73, 280–284.

49. Lea, I.A., Adoyo, P., and Michaeli, G.O. (1997). Autoimmunogenicity of the human sperm protein Sp17 in vasectomized men and identification of linear B cell epitopes. Fertil Steril 67, 355–361.

50. Diekmann, A.B., and Herr, J.C. (1997). Sperm antigens and their use in the development of an immunocontraceptive. Am J Reprod Immunol. 37, 111–117.

51. Lucy, H.M., and Sanderson, R.D. (2001). Sperm protein 17 is expressed on normal and malignant lymphocytes and promotes heparin sulfate-mediated cell-cell adhesion. Blood 98, 2160–2165.

52. Wen, Y., Richardson, R.T., Widgren, E.E., and O’Rand, M.G. (2001). Characterization of Sp17: a ubiquitous three domain protein that binds heparin. Biochem. J. 357, 25–31.
53. Brunette, L.L., Mhaweuc-Fauceglia, P.Y., Ji, L., Skeate, J.G., Brand, H.E., Lawrenson, K., Walia, S., Chiriva-Internati, M., Groschen, S., Roman, L.D., et al. (2018). Validity and prognostic significance of sperm protein 17 as a tumor biomarker for epithelial ovarian cancer: a retrospective study. BMC Cancer 18, 970.

54. Lim, S.H., Wang, Z., Chiriva-Internati, M., and Xue, Y. (2001). Sperm protein 17 is a novel cancer-tests antigen in multiple myeloma. Blood 97, 1508–1510.

55. Liggins, A.P., Lim, S.H., Soilleux, E.J., Pulford, K., and Banham, A.H. (2010). A panel of cancer-tests genes exhibiting broad-spectrum expression in haematological malignancies. Cancer Immun. 10, 8.

56. Mirandola, L., Figueroa, J.A., Phan, T.T., Grizzi, F., Kim, M., Rahman, R.L., Jenkins, M.R., Cobos, E., Jumper, C., Alalawi, R., et al. (2015). Novel antigens in non-small cell lung cancer: Sp17, Atpkap4, and Pttg1 are potential immunotherapeutic targets. Oncotarget 6, 2812.

57. Zhou, Y., Qiu, J., Wang, Y., Liu, P., Lv, Q., and DU, Z. (2019). Sperm protein antigen 17 expression correlates with lymph node metastasis and worse overall survival in patients with breast cancer. Front Oncol. 9, 710.

58. Mirandola, L., Pedretti, E., Figueroa, J.A., Chiaramonte, R., Colombo, M., Chapman, C., Grizzi, F., Patrinicola, F., Kast, W.M., Nguyen, D.D., et al. (2017). Cancer tests antigen sperm protein 17 as a new target for triple negative breast cancer immunotherapy. Oncotarget 8, 14758.

59. Li, F., Liu, Q., Han, Y., Wu, B., and Yin, H. (2010). Sperm protein 17 is highly expressed in endometrial and cervical cancers. BMC Cancer 10, 429.

60. Grizzi, F., Gaetani, P., Franceschini, B., Di Ieva, A., Colombo, P., Ceva-Grimaldi, G., Bollati, A., Frezza, E.E., Cobos, E., Rodriguez-y Baena, R., et al. (2006). Sperm protein 17 is expressed in human nervous system tumours. BMC Cancer 6, 23.

61. Gupta, G., Sharma, R., Chattopadhyay, T.K., Gupta, S.D., and Ralhan, R. (2007). Cancer immunotherapy targeting Sp17: when should the laboratory findings be translated to the clinics? Am. J. Hematol. 80, 6–11.

62. Brentjens, R.J., Rivière, I., Park, J.H., Davila, M.L., Wang, X., Stefanski, J., Taylor, C., Yeh, R., Bartido, S., Borquez-Ojeda, O., et al. (2011). Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood 118, 4817–4828.

63. Nastoupil, L.J., Jain, M.D., Feng, L., Spiegel, J.Y., Ghobadi, A., Lin, Y., Daihya, S., Lunning, M., Lekakis, L., Reagan, P., et al. (2020). Standard-of-care axicabtagene ciloleucel for relapsed or refractory large B-cell lymphoma: results from the US lymphoma CAR T consortium. J. Clin. Oncol. 38, 3119–3128.

64. Raje, N., Berdeja, J., Lin, Y., Siegel, D., Jagannath, S., Madduri, D., Liedtke, M., Rosenblatt, J., Maus, M.V., Turka, A., et al. (2019). Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N. Engl. J. Med. 380, 1726–1737.

65. Wei, T., Leisegang, M., Xia, K., Kiyotani, K., Li, N., Zeng, C., Deng, C., Jiang, J., Harada, M., Agrawal, N., et al. (2012). Generation of neoantigen-specific T cells for adoptive cell transfer for treating head and neck squamous cell carcinoma. Oncoimmunology 10, 1929726.

66. Lu, F., Ma, X.J., Lin, Y.W., Luo, Y., and Li, X. (2021). Neoantigen specific T cells derived from T cell-derived induced pluripotent stem cells for the treatment of hepatocellular carcinoma: potential and challenges. Front Immunol. 12, 690565.

67. Orlando, D., Miele, E., De Angelis, B., Guercio, M., Boiff, I., Simbaldi, M., Po, A., Caruana, I., Abballe, L., Carai, A., et al. (2018). Adoptive immunotherapy using PRAME-specific T cells in Medulloblastoma. Cancer Res. 78, 3337–3349.

68. Tawara, I., Kageyama, S., Miyahara, Y., Fujisawa, H., Nishida, T., Akatsuka, Y., Ikeeda, H., Tanimoto, K., Terakura, S., Murata, M., et al. (2017). Safety and persistence of WT1-specific T-cell receptor gene-transduced lymphocytes in patients with AML and MDS. Blood 130, 1895–1994.

69. Chen, H., Wei, F., Yin, M., Zhao, Q., Liu, Y., and Huang, Z. (2021). CD27 enhances the killing effect of CAR T cells targeting trophoblast cell surface antigen 2 in the treatment of solid tumors. Cancer Immunol. Immunother. 70, 2059–2071.

70. Long, A.H., Hao, W.M., Shern, J.F., Wanhainen, K.M., Murgai, M., Ingaramo, M., Smith, J.P., Walker, A.J., Kohler, M.E., Venkateshwar, V.R., et al. (2015). 4-IBB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. Nat. Med. 21, 581–590.

71. Hoyos, V., Savoldo, B., Quintarelli, C., Mahendravada, A., Zhang, M., Vera, J., Heslop, H.E., Rooney, C.M., Brenner, M.K., and Dotti, G. (2010). Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia 24, 1160–1170.

72. Andersen, K.G., Strommes, L.M., and Greenberg, P.D. (2017). Obstacles posed by the tumor microenvironment to T cell activity: a case for synergistic therapies. Cancer Cell 31, 311–325.