

The Role of Mesothelin Expression in Serous Ovarian Carcinoma: Impact on the Diagnosis, Prognosis, and Therapeutic Target.

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Simple Summary

Ovarian cancer is the most lethal gynaecological malignancy, and serous carcinoma is the most common subtype. The lack of symptoms and sensitive diagnostic tests in the early stage of development may explain why the diagnosis often occurs late when the neoplasm has already spread outside the pelvis. Currently, the standard treatment of ovarian carcinomas requires cytoreductive surgery followed by platinum-based systematic chemotherapy, which does not reduce recurrences or mortality. Despite intense efforts to develop novel therapies that involve the use of new chemotherapeutic agents, such as anti-angiogenesis agents and poly (ADP-ribose) polymerase inhibitors that improve patient outcomes, the five-year survival for this malignancy still remains low. Therefore, it is important to identify new targetable molecules for early diagnosis, disease monitoring, and treatment or early diagnosis, disease monitoring, and treatment of this malignancy.

The aim of this review is to discuss the role of mesothelin in serous ovarian carcinomas, focusing on its diagnostic, prognostic, and therapeutic perspectives.

Abstract

Mesothelin is a protein that is expressed in the mesothelial cell lining in the pleura, peritoneum, and pericardium. The gene of mesothelin encodes a precursor protein that is processed to yield mesothelin, which is attached to the cell membrane by a glycoposphatidylinositol linkage and a shed fragment named the

megakaryocytic-potentiating factor. The biological functions of this substance in normal cells are still unknown. Experimental studies on knockout mice suggest that this substance does not play an important role in development and reproduction.

In contrast, it has been observed that mesothelin is produced in abnormal amounts in several malignant neoplasms, such as mesotheliomas and pancreatic adenocarcinomas.

Given that mesothelin is overexpressed in many solid tumours and has antigenic properties, this molecule could be considered a tumour marker or an antigenic target for many malignancies. Many molecular studies also have demonstrated that mesothelin is overexpressed in serous ovarian carcinomas and may bind to ovarian cancer antigen Ca-125, favouring the spread of the tumour in the abdominal cavity.

Here, we discuss the current knowledge of mesothelin and focus on its role in clinical and pathological diagnoses as well as its impact on the prognosis in serous ovarian carcinomas.

We also briefly discuss the latest progress of mesothelin-targeting therapies for this aggressive and lethal neoplasm.

Keywords: mesothelin; ovarian carcinoma; biomarker; mesothelin-targeting therapy

Introduction

Mesothelin (MSLN) is a glycoprotein that is located on the mesothelial lining of the body cavities and in many neoplasms [1]. It is anchored at the cell membrane by a glycosylphosphatidylinositol linkage. The mesothelin gene was first cloned by Chang and Pastan [1], and it encodes a precursor protein that is processed to yield a 40 kDa mesothelin protein and a 31 kDa soluble fragment. The soluble human fragment, named the megakaryocyte-potentiating factor (MPF), was reported to have a megakaryocyte-potentiating activity in mouse bone marrow [2].

In normal tissue, the physiological/biological function of MSLN is still uncertain. Studies in molecular biology have demonstrated that a lack of MSLN in an MSLN knockout mouse model did not affect development, growth, or reproduction [3].

Conversely, MSLN is considered to be involved in several mechanisms of cancer pathogenesis. In

ovarian carcinomas, it was demonstrated that binding with the partner MUC16 (CA125) could play a role in cell adhesion, facilitating intraperitoneal ovarian cancer metastasis [4-6].

There is evidence that mesothelin can be used as a new cancer biomarker [7] and as a target molecule for gene therapy [8].

Here, we discuss the current knowledge of MSLN, focusing on its role in clinical and pathological diagnoses as well as its impact on the prognosis in serous ovarian carcinomas. We also briefly discuss the latest progress of mesothelin-targeting therapies for this aggressive and lethal neoplasm.

Materials and Methods

Search strategy

We conducted a review of the current literature in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement PRISMA [9] to report the current knowledge of MSLN in normal tissue and in serous ovarian carcinomas, focusing on its role in diagnosis, prognosis, and therapy. The aim of this review was to report on the latest progress of mesothelin-targeting therapies in this neoplasm.

The medical subject heading terms used for the search in PubMed and Scopus were ‘mesothelin’ and ‘ovarian carcinomas’, as well as ‘serous carcinoma’, ‘diagnosis’, and ‘therapy’.

The systematic review process was performed independently by two authors (E.F. and A.T.) and checked by another (G.G.).

Inclusion/exclusion criteria

Articles considered were published from 1994 to 2021 (June).

The duplicates, e.g., articles that were not written in the English language and those evidently not relevant to the topic based on the title and a revision of the abstract, were excluded from the review. All works describing the MSLN expression and therapies in non-ovarian carcinomas were also excluded from our analysis.

Data extraction

We selected studies that focused on the role of MSLN in clinical and pathological diagnoses as well as its impact on the prognosis in serous ovarian carcinomas and data for MSLN-targeting therapies in ovarian carcinomas, especially the serous subtype.

Results

The number of records identified through PubMed and Scopus was, respectively, 220 and 160.

Records after duplicated papers were removed amounted to 90.

Records screened based on title and Abstract were 220.

We excluded 5 articles that were not written in English, 23 studies with a few cases (< 40) and 113 not relevant to the topic. Thus, we considered in the paper a total of 79 articles, and their full texts were read.

We also searched for and considered 5 other useful articles present in the bibliography of those already evaluated.

Analysis of these results has enabled us to report studies on mesothelin as a cancer biomarker for the diagnosis and prognosis of ovarian carcinomas in sera and in other fluids of patients suffering from this severe and lethal neoplasm.

We have also been able to report studies in which the value of MSLN is reported in other diagnostic methods, such as radioimmunoimaging, in immunohistochemical and PCR analysis. Moreover, we reported and discussed papers on mesothelin as a therapeutic target.

Mesothelin as a New Cancer Biomarker for the Diagnosis and Prognosis of Ovarian Carcinomas

Among gynaecological neoplasms, ovarian carcinomas have the highest mortality because the diagnosis of this malignancy is often late, when the neoplasm is already in an advanced stage of development.

The early detection of this neoplasm is difficult due to the absence of physical symptoms and a lack of sensitive screening methods [10].

Mesothelin as a Serological Biomarker

Cancer antigen 125 (CA125) is currently the most common serological biomarker used for the diagnosis and management of patients with epithelial ovarian/fallopian tube or primary serous peritoneal cancers. Many studies suggest that CA125 can also be expressed at high levels in other types of cancers, such as breast cancer [11, 12], mesotheliomas [13, 14], non-Hodgkin's lymphoma [15, 16], and leukaemia [17], as well as leiomyomas and leiomyosarcomas with a gastrointestinal origin [18]. CA125 was also found to be elevated in the sera of patients with benign conditions, such as cirrhosis, ovarian cysts, endometriosis, pregnancy, congestive heart failure, and musculoskeletal inflammatory disorders [19].

Only half the patients with early stage ovarian carcinomas had elevated CA125 levels [20].

Thus, the sensitivity and specificity of CA125 for the detection of early stage ovarian carcinomas are unfortunately low [21]. Therefore, it is extremely important to identify new molecules for the early diagnosis and disease monitoring of this lethal neoplasm.

Concerning the use of MSLN as a biomarker for the diagnosis of ovarian carcinomas, a significant amount of data in the literature suggests that this substance is expressed in different subtypes of ovarian carcinomas, especially serous [22]. A splice variant of soluble mesothelin, named the soluble megakaryocyte-potentiating factor (SMRP), was found in the sera of patients with ovarian carcinomas [23].

Studies have reported that SMRP serum was significantly higher in ovarian carcinomas than in benign ovarian lesions and healthy subjects. It was also observed that serum SMRP levels were related to FIGO pathological staging (International Federation of Gynecology and Obstetrics system) and grading of the neoplasms, demonstrating that high serum levels of mesothelin may be indicative of tumour progression and poor survival [24-26].

Mesothelin as a Biomarker in Other Fluids

Okla et al. observed that peritoneal fluid mesothelin levels did not differ significantly in patients with benign and malignant ovarian epithelial neoplasms. They also did not observe any differences in peritoneal fluid MSLN levels in different FIGO stages and histological types of neoplasms.

Thus, in contrast to the serum levels of MSLN, low levels of MSLN in the peritoneal fluid were not associated with a better prognosis [25].

Studies in the literature have reported that MSLN can also be detected in the urine samples of patients affected by ovarian carcinomas [27-30].

In particular, Badgwell et al. observed, for the first time, that the urinary levels of MSLN could be considered to have a greater sensitivity than the serum levels in the early stages of ovarian carcinomas [27]. Similarly, Hellstrom et al. demonstrated that, in women with a pelvic mass, assaying urine for human epididymis protein 4 (HE4) or mesothelin may detect early ovarian carcinomas more often than assaying the serum [28].

In their study, Hollevoet K et al. demonstrated that mesothelin levels in the urine depended on an impaired glomerular and tubular function, which could influence the interpretation of the mesothelin measurements and cause false-positive results [30]. Wu et al. considered SMRP serum to be a promising marker for the diagnosis and monitoring of ovarian carcinomas, especially in combination with CA125, showing a sensitivity of 98.4% and specificity of 88.4% [24].

Mesothelin as a Biomarker in Radioimmunoimaging

As mesothelin is a membrane antigen overexpressed in a variety of solid neoplasms, including ovarian carcinomas, there are many studies in the literature that prove that analyses of radioimmunoimaging can be used for the non-invasive detection of mesothelin-overexpressing tumours [31-36].

Radioimmunoimaging is a type of molecular nuclear medicine imaging that applies specific antibodies of tumour-specific antigens labelled with radionuclides for imaging [37]. Thus, this molecular imaging allows the assessment of the tumour uptake and distribution in the primary and secondary tumour sites as well as the response to therapy. Different anti-mesothelin antibodies have been used in animal models.

In several studies, anti-mesothelin antibodies were used, and these could be detected by fluorescence imaging or magnetic resonance [32,33]. For detection by positron emission tomography (PET), other authors have demonstrated in ovarian models that ^{89}Zr -labelled antibodies could be used to target MSLN, an antibody–drug conjugate (ADC) that can provide information regarding both the organ distribution and drug dosing [33-36].

Mesothelin Detection in Neoplastic Tissue

In diagnostic pathology, the immunohistochemical expression of MSLN, in several instances, is a useful marker to distinguish between primary and metastatic ovarian carcinomas. In their paper, Kanner et al. demonstrated that MSLN expression could assist in differentiating Müllerian serous carcinomas from metastatic breast carcinomas (particularly those with a papillary morphology) and documented that none of the breast carcinomas was stained for mesothelin [38].

Ordóñez demonstrated that the carcinomas that most frequently exhibited a strong MSLN reactivity were non-mucinous carcinomas of the ovary; however, they observed that this marker was also expressed in other non-mucinous carcinomas, such as clear-cell carcinomas of the ovary, endodermal sinus tumours, or renal cell carcinomas, as well as clear-cell type and transitional-cell carcinomas of the ovary [22]. Weidemann et al., to identify tumours that might benefit from targeted cancer therapies, observed that the highest prevalence of MSLN positivity was present in ovarian carcinomas (serous 97%) by the analysis of tissue microarrays for the MSLN expression of 122 different tumour types. Conversely, MSLN was rare in the cancers of the breast, kidney, thyroid gland, soft tissues, and prostate [39].

The immunohistochemical expression of MSLN in the neoplastic section of serous ovarian carcinomas was also investigated to establish its impact on the prognosis.

The literature suggests limited and conflicting immunohistochemical data regarding MSLN expression and the prognostic impact on ovarian cancers.

According to the study of Chang et al., the immunohistochemical MSLN expression was related to

the survival outcomes in patients with ovarian carcinomas. They observed that the neoplasms with a high expression of mesothelin showed a statistically worse prognosis than those with a low immunoreactivity [40].

Similarly, Yildiz et al. observed that a high expression of MSLN in advanced serous ovarian cancers was associated with a poor prognosis and with worse platinum sensitivity in the advanced stage [41]. Cheng et al. observed that a high MSLN expression, investigated by a molecular study using real-time quantitative reverse transcription-polymerase chain reaction (PCR), was associated with chemoresistance and poor survival in ovarian carcinomas [42].

In contrast, Yen et al., separating neoplasms with diffuse immunoreactivity from neoplasms with focal positivity, observed that a diffused MSLN expression correlated with prolonged patient survival in serous ovarian carcinomas [43]. According to these authors, this finding could indicate that immune response to mesothelin-expressing ovarian carcinoma cells may result in a reduction of tumour load and contribute to a patient's prolonged overall survival. Conversely, neoplasms with focal MSLN expression can progress, as neoplastic cells cannot be detected by the immune system and continue to progress.

To validate immunohistochemical results, in eight frozen representative cases, Yen et al., using reverse transcriptase-PCR, observed that the PCR product of mesothelin was strongly representative in tumours with diffuse mesothelin immunoreactivity (*4+ and 3+ positivity*) (FIGS 1a and b), but it was scarcely detectable in negative tumours (*score: 0*).

The results of Yen et al. were not in accordance with those of other studies, in which a high expression of MSLN was associated with poor survival in other malignant epithelial neoplasms, such as lung adenocarcinomas and pancreatic ductal adenocarcinomas [44, 45].

The conflicting data on MSLN expression and its prognostic impact on patients with ovarian carcinomas may be due to many factors, such as the different antibodies, protocols, and criteria used to evaluate the immunoreactivity. Magalhaes et al., by immunohistochemical analysis, demonstrated that MSLN expression in patients with high-grade serous carcinomas did not predict the clinical outcome but correlated with the CD11c+ positive-immune infiltrate in neoplasms. The MSLN expression also significantly correlated with CD11c+ in the metastatic sites and in the perivascular areas of the primary neoplasm. Thus, they concluded that these data could also have an important impact on the outcome of immune-related therapies [46].

Mesothelin as a therapeutic target

Currently, ovarian cancer treatment consists of surgical tumour debulking complemented by taxane- and platinum-based chemotherapy [47], occasionally associated with Avastin (bevacizumab, an anti-vascular endothelial growth factor therapy) [48]. In advanced or recurrent diseases, in patients with a BRCA mutation, maintenance therapy with a poly adenosine diphosphate (ADP-ribose) polymerase (PARP) inhibitor treatment represented an effective treatment option [49].

However, radical treatment regimens and multiple chemotherapeutic treatments do not reduce the recurrences of the disease or the death of the patients.

Given that MSLN is over-expressed in many solid tumours and has antigenic properties, this molecule could be considered to be the antigenic target for immunotherapeutic strategies in ovarian carcinomas. The main immunotherapeutic strategies using different therapeutic agents include anti-mesothelin immunotoxin SS1P, MORAb-009 (chimeric anti-mesothelin mAb) and anti-mesothelin antibody–drug conjugate (BAY-94 9343). Chimeric antigen receptor T cell (CAR T) therapy and vaccines were also evaluated.

In Figure 2, the main immunotherapeutic strategies for ovarian cancer are summarized (FIG. 2).

Anti-Mesothelin Immunotoxin SS1P

SS1P is an anti-MSLN immunotoxin, which was obtained from immunized mice fused to a truncated form of pseudomonas exotoxin A (PE38) (FIG 2A). SS1P binding to MSLN formed a complex that was internalized by endocytosis and PE, translocated in cytosol and killed cells catalyzing protein synthesis, thus initiating programmed cell death [50]. Studies in vitro have demonstrated the cytotoxic effect of SS1P on the neoplastic cells of patients affected by ovarian carcinomas [51]. In a phase I clinical trial (ClinicalTrials.gov Identifier: NCT00066651), patients with ovarian carcinomas presented with stable disease. The side effects of treatment are dose-related and include capillary leak syndrome and pleuritis due to SS1P binding to normal mesothelial cells and inflammation. The association with prednisone reduces the risk of toxicity, allowing increased dosage [52]. Moreover, in line with cases of mesothelioma, SS1P could be used in combination with chemotherapy to obtain a major response [53]. However, as observed in treatments for mesotheliomas, it must be kept in mind that the efficacy of SS1P is limited by anti-drug antibody formation.

Thus, SS1P is being administrated in association with pentostatin and cyclophosphamide, which are lymphocyte-depleting drugs that allow patients to receive multiple cycles of treatments [54].

MORAb-009 (Chimeric Anti-Mesothelin mAb)

MORAb-009 (chimeric anti-mesothelin mAb), also named amatuximab, represents the heavy and light chain variable regions of a mouse anti-mesothelin single-chain Fv grafted onto a human IgG1 and k constant region (FIG 2B). MORAb-009 has a high affinity with mesothelin, and, in a preclinical evaluation, it was demonstrated that it could inhibit the adhesion between the cell lines expressing mesothelin and MUC 16 (Ca 125), as well as cause cell-mediated cytotoxicity on mesothelin-bearing tumour cells [55].

In clinical trials, it has been observed that patients treated with MORAb-009 showed a marked increase in CA125 serum, suggesting that it could block the binding between mesothelin and Ca-125. It was demonstrated that MORAb-009 could inhibit cellular adhesion during metastasis in cases of both ovarian carcinomas and mesotheliomas [56, 57]. Studies in vivo on animal models demonstrated that these effects were markedly increased in combination with chemotherapy agents such as gemcitabine and Taxol [57] or, in a phase II clinical trial, with other chemotherapeutic substances for cases of mesotheliomas (ClinicalTrials.gov Identifier: NCT00738582) [58]. The reduction in the MPF level in serum, after treatment, demonstrated a correlation with a good prognosis [58]. However, the combination with chemotherapy agents caused adverse events, such as hypersensitivity reactions, neutropenia, and atrial fibrillation [58].

Although most studies (ClinicalTrials.gov Identifiers: NCT01521325, NCT01413451) on ovarian carcinomas focused on the efficacy of monotherapy with MORAb-009, these data suggest that a combination with different chemotherapeutic agents could provide satisfactory results, with prolonged overall survival.

Anti-Mesothelin Antibody Drug Conjugate (BAY-94 9343)

Anti-mesothelin antibody–drug conjugate (BAY-94 9343), known as anetumab ravtansine, is an

antibody–drug conjugate (ADC) that is a complex consisting of a fully human immunoglobulin G1 anti-mesothelin monoclonal antibody conjugated to the maytansine derivative tubulin inhibitor DM4 through a reducible disulphide linker (FIG 2C) [59]. BAY-94 9343 has an anti-proliferative activity because, after binding to mesothelin on tumour cells, it is internalized, and the disulphide linker is cleaved, releasing DM4. Subsequently, DM4 binding to tubulin disrupts the microtubule polymerization, causing cell cycle arrest and apoptosis and consequently killing the dividing cells [60, 61].

Preclinical studies have shown that anetumab ravtansine was highly cytotoxic in MSLN-expressing mesotheliomas as well as pancreatic, non-small-cell lung and ovarian cancer cell lines [58].

In vivo, anetumab ravtansine had antitumor activity in mesotheliomas as well as pancreatic and ovarian xenograft models [59].

The study of Quanz et al. demonstrated that in ovarian cancer cell lines and patient-derived xenografts, the combination of anetumab ravtansine with pegylated liposomal doxorubicin (PLD) or with carboplatin, copanlisib, or bevacizumab showed an additive anti-proliferative activity both in vitro and in vivo compared with either agent used as a monotherapy [62].

Chimeric Antigen Receptor T cell (CAR T) Therapy

MSLN has also been regarded as an attractive target for chimeric antigen receptor T cell (CAR T) therapy because of its abundant expression in tumour cells and a limited expression in normal cells.

CAR T therapy is a type of treatment in which the T cells of a patient, obtained by apheresis, are changed in the laboratory by inserting a gene for a special receptor called a chimeric antigen receptor (CAR) into them. CAR T cells can target cell surface antigens without MHC (Major histocompatibility complex) restriction. Thus, CAR T cells can use for broad HLA-diverse allogenic recipients.

The CAR usually is complex with an extra-cellular antigen recognition domain, which corresponds to a single chain variable fragment (scFv) of a specific antibody, a transmembrane domain, anchored at the cell membrane of T cell and an intra-cellular domain that transmits to T cell activation signals. To amplify the activation signals in Cars, MSLN can be used in two costimulators domains which allow obtaining major activation in terms of proliferation, cytotoxicity, and, consequently, major anti-tumour efficacy. The major effectiveness of this subtype of CAR, known as 'third-generation MSLN', was proven in many neoplasms and in ovarian carcinoma [63].

In the laboratory, CAR-T cells can be produced using a lentivirus vector, which is integrated into the

genome of the host T cell. This method is widely used in phase I studies in advanced solid cancer with MSLN expression [64].

The CAR T cells are grown in the laboratory and then given to the patient by infusion. The CAR T cells are able to bind to the antigens on the cancer cells and kill them. Once attached to the antigens present on the neoplastic cells, the CAR T cells become activated and stimulate the host immunosystem, which in

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turn attacks the MSLN-expressing cells [65]. The effectiveness of CAR T therapy has been observed in mouse models of different solid neoplasms, including ovarian carcinomas and mesotheliomas, in which the chimeric receptors recognize human MSLN, and the inflammatory cytokines secreted by the T cells (including IL-2, IL-6, tumour necrosis factor alpha, and Interferon- γ) that produce cytotoxic effects for the cancer cells (FIG 2 D) [66, 67].

For high-grade serous ovarian cancer (HGSC), investigating the co-expression of CA125, MSLN and folate receptor alpha (FOLRA) on individual tumour cells and their relationship with tumour-infiltrating T cells (TIL), Banville et al. provided insights into the design of logic-gated CAR T cell strategies with a greater number of antigens. They demonstrated that the most promising pairwise combination was CA125 and/or MSLN. Thus, a CAR T cell strategy against CA125 and MSLN would target most tumour cells in most cases. The antigen expression and T cell infiltration demonstrated that this strategy was effective both in primary and recurrent diseases [68].

However, as observed in treatments for other neoplasms, it must be kept in mind that the immunosuppressive tumour microenvironment of neoplasms plays an important role in response to CAR T therapy in vivo. Many authors have demonstrated that a transmembrane protein named programmed death-ligand 1 (PD-L1) has an important role in regulating T cell response. The binding of this substance to an inhibitor programmed cell death protein 1 (PD-1) or the binding of PD-1 to the immune co-inhibitory receptors lymphocyte activation gene-3 (LAG3) transmit an inhibitory signal, causing a reduction in the proliferation of antigen-specific T-cells, and, consequently, reduction in the infiltration of T cells into the tumour lesion [69].

For ovarian carcinoma, recent preclinical studies in vivo showed that it is possible to restore the functions of tumour-specific checkpoint blockade in MSLN-directed CAR T cells using different substances [70-72].

The side effects of treatment observed during CAR T therapy are related to excessive immune activation, which causes cytokine release syndrome (CRS) and neurotoxicity. These adverse effects are probably due to non-specific T cell activation.

The (CRS) is an acute systemic inflammatory disorder characterized by fever and, sometimes, fatal dysfunction of many organs [73, 74]. Patients with severe CRS symptoms can culminate in delirium, seizures, and encephalopathy caused by high levels of IL-6, IFN gamma, and CAR T cells in the cerebrospinal fluid [75].

Compartmental CRS (C-CRS) has been reported in a patient with advanced ovarian cancer treated with mesothelin-targeted CAR T cells characterized by the elevation of IL-6 and accumulation in the pleural fluid [74].

The treatment used against this serious side effect sometimes involves using an anti-IL-6R antibody, tocilizumab [76]. In cases with involvement of the nervous system and unresponsive cases to tocilizumab, corticosteroids were often used [75, 77, 78], or suicide genes were introduced within T cells to reduce their number and activity (ClinicalTrials.gov Identifiers: NTC0374965).

Vaccine

The cancer vaccine is an immunotherapy that induces a tumour-specific immunoresponse in the host, which is capable of recognizing and eliminating neoplastic cells. The ability of T cells to recognize antigens present on neoplastic cells and to produce an immune response capable of destroying them has long been known.

In fact, as early as 1891, Dr William Coley observed the regression of a sarcoma by injecting inactivated *Streptococcus pyogenes* and *Serratia marcescens* into the neoplasm [79].

Currently, *Listeria monocytogenes*, a Gram-positive bacterium, can be used as a vector for this type of immunotherapy for MSLN-positive cancers. In humans, this bacterium causes infections with gastroenteritis, meningitis, and encephalitis, but generally, the human immune system is capable of controlling the disease [80, 81].

The CRS-207 vaccine uses attenuated *Listeria monocytogenes* (Lm) (Lm Δ actA/ Δ inlB) that are engineered to express human MSLN and can be used to treat MSLN-positive neoplasms (FIG 2E) [82].

The methods used to attenuate the virulence of Lm are mostly based on the deletion of certain genes that allow for sufficient infectivity and antigen production but have the potential for severe infection. Therefore, this treatment should be used with caution for patients who have immunodeficiency [83].

Treatment with CRS-207 with *Listeria*-expressing human MSLN allows for stimulating the immune system with a robust response against neoplastic cells by different mechanisms. After the fusion with a lysosome in the cytoplasm of an antigen-presenting cell, Lm can be killed; the secretions of its antigens into the cytosol as well as prior to the degradation in the phagosome can be loaded onto (Major

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Histocompatibility Complex) MHC I and MCH II, causing the activation of potent CD 4 helper lymphocytes and CD8 cytotoxic lymphocytes.

In addition, during its entry in the antigen-presenting cell, Lm, by Toll-like receptors, can activate pro-inflammatory genes, which can amplify, by inflammatory cytokines, the response against neoplastic cells [84].

After phase I testing, the safety of CRS-207 was demonstrated in patients with ovarian cancers as well as pancreatic, mesothelioma, and lung carcinoma (ClinicalTrials.gov identifier NCT00585845) and in platinum-resistant ovarian, fallopian or peritoneal serous carcinomas (ClinicalTrials.gov identifier NCT02575807).

CONCLUSIONS

In conclusion, a typical expressing pattern of MSLN in normal and cancer tissues makes it a promising target for diagnosis and therapeutic applications. Although many clinical trials regarding MSLN-targeting therapies in ovarian carcinomas are underway, further studies are necessary to establish the effects on the health of patients and behavioural outcomes.

Author Contributions

Conceptualization, G.G. and E.F.; methodology, G.G. and A.T.; formal analysis, G.G. and E.F.; writing-original draft preparation, G.G. and E.F.; writing-review and editing, G.G. and A.T.

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Conflicts of Interest

The authors declare no conflict of interest

Figure Legend

FIG 1: Examples of high-grade serous carcinoma, with diffuse mesothelin immunoreactivity. (*a*: score 4+, *x100*; *b*: score 3+, *x100*).

FIG 2: Schematic and simplified representation of the main therapeutic strategies, using mesothelin as a target.

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