Mesothelin as a biomarker for targeted therapy

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Abstract

CAR-T cell therapy targeting CD19 has achieved remarkable success in the treatment of B cell malignancies, while various solid malignancies are still refractory for lack of suitable target. In recent years, a large number of studies have sought to find suitable targets with low “on target, off tumor” concern for the treatment of solid tumors. Mesothelin (MSLN), a tumor-associated antigen broadly overexpressed on various malignant tumor cells, while its expression is generally limited to normal mesothelial cells, is an attractive candidate for targeted therapy. Strategies targeting MSLN, including antibody-based drugs, vaccines and CAR-T therapies, have been assessed in a large number of preclinical investigations and clinical trials. In particular, the development of CAR-T therapy has shown great promise as a treatment for various types of cancers. The safety, efficacy, doses, and pharmacokinetics of relevant strategies have been evaluated in many clinical trials. This review is intended to provide a brief overview of the characteristics of mesothelin and the development of strategies targeting MSLN for solid tumors. Further, we discussed the challenges and proposed potential strategies to improve the efficacy of MSLN targeted immunotherapy.

Keywords: Mesothelin, Biomarker, Targeted therapy, Immunotherapy, CAR-T

Background

The discovery and function of MSLN

The MSLN gene encodes a 71-KD precursor, which is a glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein that is cleaved into two products at arginine 295 (Arg295): a soluble 31-KD N-terminal protein called megakaryocyte potentiating factor (MPF) and a 40-KD membrane-bound fragment called MSLN (mesothelin). Both MPF and MSLN are bioactive, but their exact functions remain unclear. MPF was initially reported to stimulate megakaryocyte colony formation in the presence of interleukin-3 in mice but not alone [1], while its activity is unknown in humans. MSLN was first described as a membrane protein expressed on mesothelioma and ovarian cancer cells [2] and normal mesothelial cells [2, 3]. A previous study showed that MSLN seemed to be a nonessential component in normal cells, as MSLN knockout mice did not present with abnormal development or reproduction [4]. In contrast, preclinical and clinical studies showed that aberrant MSLN expression on tumor cells plays an important role in promoting proliferation and invasion [5]. MSLN has also been identified as a receptor of CA125 that mediates cell adhesion [6]. The interaction of CA125 and MSLN play an important role in ovarian cancer cell peritoneal implantation and increase the motility and invasion of pancreatic carcinoma cells [7–9]. The overexpression of MSLN could activate the NFκB, MAPK, and PI3K pathways and subsequently induce resistance to apoptosis [10] or promote cell proliferation, migration, and metastasis by inducing the activation and expression of MMP7 [9] and MMP9 [5]. An increase in tumor burden and poor overall survival are associated with elevated MSLN expression according to clinical observations [11, 12]. Structural prediction revealed that a superhelical structure with armadillo-type repeats constitutes a part of its three-dimensional structure [13], and the structure of an N-terminal fragment that binds to the Fab SS1 antibody...
has been clarified [14], but the structure of the whole protein is still unclear.

**Expression of MSLN in malignant cells and prognosis**

Generally, MSLN is expressed on normal mesothelial cells in the pleura, pericardium, and peritoneum and in epithelial cells on the surface of the ovary, tunica vaginalis, rete testis, and fallopian tubes in trace amounts [3]. In contrast, the aberrant overexpression of MSLN is observed in various cancer cells. MSLN was initially characterized in mesothelioma and ovarian cancer by Chang et al. with the mAb K1 [15]. Chang and colleagues found that MSLN was present in 10 of 15 non-mucinous ovarian cancers and absent in all 4 mucinous ovarian cancers examined [2]. In addition, all 15 cases of epithelial mesothelioma, but none of the 4 cases of sarcomatous mesothelioma, expressed MSLN [16]. This was in line with the results of another independent study that confirmed MSLN reactivity in all 44 epithelioid mesotheliomas and in the epithelial components of 3 biphasic mesotheliomas, but not in any of 8 sarcomatous mesotheliomas examined [17]. According to the statistics in this study, MSLN was present in 15 of 48 (31%) lung cancers (adenocarcinomas (12/31) and squamous carcinomas (limited, 3/17)) and in 42 of 86 (49%) non-pulmonary adenocarcinomas (ovary (14/14), peritoneum (5/5), endometrium (6/9), pancreas (10/11), stomach (2/4), and colon (5/16); none of 12 breast, 9 kidney, 4 thyroid, and 2 prostate cancers showed evidence of MSLN) according to assays with the 5B2 anti-MSLN monoclonal antibody. MSLN was immunohistochemically evaluated in 596 lung carcinomas of different types by Miettinen M and Sarlomo-Rikala M in 2003 [18]. MSLN reactivity was observed in 78 of 148 (53%) adenocarcinomas, 29 of 124 (23%) squamous cell carcinomas and 15 of 118 (13%) large cell carcinomas but was absent in small cell carcinomas. These results suggest that MSLN could act as an immunohistochemical biomarker for the determination of the subtype classification of mesotheliomas and lung cancer to a certain degree because of its specific expression pattern in these two cancers. MSLN is expressed in the majority of pancreatic cancers, and independent studies revealed that almost 100% of pancreatic cancers are positive for MSLN but that normal pancreatic tissues did not show evidence of MSLN [3, 19, 20]. Subsequent studies demonstrated the expression of MSLN in a broad spectrum of solid tumors with distinct frequency and distribution patterns, including extrahepatic biliary cancers (95%), triple negative breast cancer (66%), endometrial carcinomas (59%), colorectal carcinomas (30%), cervical carcinomas (25%) and esophageal (46%), endometrial (89%) and thymic cancer [3, 21–26]. A recent study reported that 25.6% of 117 patients with gastric carcinoma showed high levels of MSLN expression, which was associated with a poor prognosis [27]. We also detected MSLN expression to different degrees in 9 gastric cancer tissues but not in normal gastric tissue [28]. The elevated expression of MSLN was correlated with poorer prognoses in patients with ovarian cancer [29], cholangiocarcinoma [30, 31], lung adenocarcinoma [29, 32], triple negative breast cancer [4, 33] and resectable pancreatic adenocarcinoma [34–36].

In addition, MSLN is shed into the serum of patients with solid tumors, in which it is referred to as soluble MSLN-related protein (SMRP) [37]. The production of SMRP could be associated with abnormal splicing, which results in a secreted form or its cleavage from the membrane by the TNFα-converting enzyme ADAM17 [38]. SMRP was also identified as a promising cancer biomarker in the sera of patients with mesothelioma, in which elevated SMR levels in serum was correlated with advanced stage and increased disease burden [37, 39]. However, the sensitivity and specificity of SMRP as a tumor marker in ovarian cancer was limited [40]. The value of soluble MSLN in diagnosis and the prediction of cancer progression remains to be determined, and its combination with other tumor markers may be more precise for diagnosis.

**Targeted therapy**

Given that MSLN expression is rather limited in several normal tissues but highly elevated in the solid tumors mentioned above, MSLN is a potential target for antigen-specific therapy (Fig. 1).

**Antibody-based drugs**

Antibody-based drugs are used to target and kill tumor cells via neutralization by antibodies, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP) or antibodies conjugated with effector molecules (toxins or inhibitors), which mediate apoptosis or suppress cell proliferation.

The specific uptake of the indium111-labeled MSLN antibody K1 by tumor cells was observed by Hassen et al. [41]. The conjugation of a fragment of *Pseudomonas* exotoxin A (PE) to this antibody resulted in cytotoxicity in MSLN-expressing cell lines and tumor regression in tumor-bearing mice [42]. A new murine-derived antibody with higher affinity termed SS1 was produced via phage display and hotspot mutagenesis [43, 44]. The fusion of the PE38 portion to SS1 resulted in a recombinant immunotoxin (RIT) termed SS1P, which enters cells by receptor-mediated endocytosis and induces apoptosis by inactivating elongation factor 2 to impede protein synthesis [45]. Many drugs based on the MSLN antibody SS1 or other modified and humanized versions have been developed for targeted therapy (Table 1).
SS1P has been tested in several clinical trials that enrolled patients with advanced cancers. In an early phase I clinical trial (NCT00066651) [48], the dose-limiting toxicities (DLTs), maximum tolerated dose (MTD) and pharmacokinetics (PK) of SS1P were tested in 34 patients with mesothelioma (n = 20), ovarian cancer (n = 12) and pancreatic cancer (n = 2). With a limited sample size, this clinical trial demonstrated that the combination of SS1P with prednisone can reduce the risk of toxicity due to SS1P and allow the use of an increased drug dosage. No significant pericardial toxicity was observed in any of the patients, which suggested that the MSLN antibody SS1P presented less risk to pericardial mesothelial cells. Among the 33 evaluable patients, 4 had a partial response (PR), 19 had stable disease (SD), and 10 had progressive disease (PD). However, SS1P was proven to be immunogenic in a later clinical trial (NCT00006981) [49]. Twenty-four patients with chemo-resistant solid tumors received SS1P treatment at dosages of 4, 8, 12, 18, and 25 μg/kg/day (× 10). One patient had a PR, 12 had SD, and 11 had PD. It is noteworthy that high levels of neutralizing antibodies against SS1P were detected in 75% of patients, which could undermine the anti-tumor efficacy.

Given that the administration of SS1P alone showed a moderate effect, the combination therapy might be more effective. In the clinical trial NCT01362790 [47], 10 patients with chemotherapy-refractory mesothelioma received SS1P in combination with pentostatin and cyclophosphamide. Three patients had a PR (44% ~ 74%), 3 had SD and 4 had PD. Adverse events were evaluated for all patients. Grade 3 toxicities, including noncardiac chest pain, pleuritic pain, and back pain (9% each) were observed, but no grade 4 toxicities were observed in patients. Meanwhile, adverse events associated with pentostatin or cyclophosphamide, such as grade 4 lymphopenias, were observed in all patients. In contrast to the trials described above, the involvement of pentostatin and cyclophosphamide delayed the formation of neutralizing antibodies to SS1P, thereby allowing a prolonged period of therapy. SS1P combined with pemetrexed and cisplatin was further tested for treating chemotherapy-naive patients with...
advanced malignant pleural mesothelioma (MPM) (NCT01445392) [46]. Of the 20 evaluable subjects, 12 patients had a PR, 3 had SD, and 5 had PD. Notably, the changes in the relative serum levels of MSLN, MPF and CA125 were significantly correlated with responses (PR > SD > PD). These biomarker levels were generally decreased in 12 patients who received PR but were substantially increased in 5 patients who had PD.

Amatuximab
Amatuximab (MORAb-009) is a chimeric monoclonal antibody consisting of the SS1 scFv fused to the human IgG1 and κ constant regions. The binding of amatuximab to MSLN expressed on tumor cell surfaces leads to ADCC. Two clinical trials (NCT00570713 and NCT00738582) showed that no severe (grade 3 or 4) drug hypersensitivity adverse events (DHAEs) were observed in any of the subjects. Among the 20 of 24 patients evaluable for response, none had complete or partial responses, and only 11 patients had SD and 9 had PD [52]. MORAb-009 treatment resulted in a remarkable elevation in serum CA125 levels in all 8 patients under surveillance, possibly due to the interruption of binding between MSLN and CA125 by amatuximab, which could prevent the intraperitoneal/intrapleural metastasis of ovarian cancer and mesothelioma [53]. A clinical trial (NCT01018784) in Japanese patients with mesothelioma, pancreatic adenocarcinoma or other MSLN-positive solid tumors revealed that the weekly single administration of amatuximab in 4-week cycles at increasing doses ranging from 50 to 200 mg/m² led to limited treatment effects. Three of the 17 patients had SD, and 14 had PD [51].

The anti-tumor effect of amatuximab in combination with pemetrexed and cisplatin was elevated in 89 patients at 26 centers (NCT00738582) [54]. Amatuximab in combination with pemetrexed and cisplatin was administered according to the response (PR or SD) for up to 6 cycles. Thirty-three patients had a PR, and 42 had SD. The detection of the change in the MPF level in serum before and after treatment in 59 patients also showed that the decreased MPF level was correlated with good prognosis. The combination therapy led to severe adverse events, including hypersensitivity reactions, neutropenia, and atrial fibrillation. Dyspnea and fatigue were observed during the maintenance phase.

An 111Indium (111In) radiolabel was used to characterize the biodistribution and dosimetry of amatuximab in 6 patients (4 with malignant mesothelioma and 2 with pancreatic adenocarcinoma) [60]. SPECT/CT imaging showed 111In-amatuximab uptake in both primary tumors and metastatic sites and that uptake was increased in mesothelioma compared with that in pancreatic cancer. Notably, 111In-amatuximab uptake in the heart, liver, kidneys and spleen was also confirmed. Even so, amatuximab was generally well tolerated. Amatuximab PK was characterized in the clinical trial NCT02357147. It revealed that higher amatuximab exposure in combination with chemotherapy was associated with prolonged OS [50].

Anetumab ravtansine
Anetumab ravtansine, also referred to as BAY94–9343, is a human anti-MSLN antibody fused to DM4, which is a maytansinoid tubulin inhibitor that mainly affects proliferating cells. The specific binding of BAY94–9343 to MSLN with high affinity induces efficient antigen internalization. BAY94–9343 showed dose-dependent anti-tumor efficacy and bystander effects in xenogeneic tumor models [56]. The antitumor efficacy of anetumab ravtansine in combination with pegylated liposomal doxorubicin (PLD), carboplatin, copanlisib and bevacizumab was investigated for the treatment of ovarian cancer. The involvement of combination therapy showed enhanced anti-proliferative activity and increased apoptosis in vitro and improved in vivo efficacy in tumor-bearing mice [55]. The safety, tolerability, pharmacokinetics, and pharmacodynamics were then evaluated in clinical trials. Several phase 1/2 studies were carried out to explore the dosage and side effects of anetumab ravtansine when administered together with pemetrexed, cisplatin, PLD, irinotecan, gemcitabine, pembrolizumab, atezolizumab, gemcitabine hydrochloride, ipilimumab or nivolumab (Table 1). However, only one clinical trial data for anetumab ravtansine was submitted to ClinicalTrials.gov prior to the submission of this review.

DMOT4039A
DMOT4039A is a humanized anti-MSLN mAb (h7D9.v3) fused to the antimitotic agent monomethyl auristatin E (MMAE) [61]. It inhibited cell proliferation at an IC50 of 0.3 nmol/L and regressed tumor growth in a dose-dependent manner in a mouse model. In another clinical trial (NCT01469793), DMOT4039A was administered to 71 patients with pancreatic cancer (n = 40) or ovarian cancer (n = 31) [62]. Fifty-four patients received a DMOT4039A injection every 3 weeks (2.4–2.8 mg/kg; q3w), and 17 patients received an injection weekly (0.8–1.2 mg/ kg). Hyperglycemia (grade 3) and hypophosphatemia (grade 3) were observed in 2 patients treated with DMOT4039A every 3 weeks at a dosage of 2.8 mg/kg but no DLTs were observed in patients treated with other dosages. Related severe adverse events occurred in 5 patients at a dosage of 2.4–2.8 mg/kg every 3 weeks and one patient at a dosage of 1.2 mg/kg weekly. Cumulative peripheral neuropathy (grades 1–3) was observed in 14 patients due to microtubule inhibitors. Six patients
| Agent | NCT Number (Reference) | Title | Status (Results) | Interventions | Phases | Enrollment | Start Date | Locations |
|-------|------------------------|-------|------------------|---------------|--------|------------|------------|-----------|
| SS1P  | NCT01445392 [46]       | SS1(dsFv)PE38 Plus Pemetrexed and Cisplatin to Treat Malignant Pleural Mesothelioma | Terminated     | Biological: Multicycle SS1P Drug: Pemetrexed Drug: Cisplatin Biological: Single cycle SS1P Biological: Multicycle SS1P Drug: Pemetrexed Drug: Cisplatin Biological: Single cycle SS1P Biological: Multicycle SS1P Drug: Pemetrexed Drug: Cisplatin Biological: Single cycle SS1P | Phase 1 | 24         | 2007-11-14 | United States |
|       |                        |       |                  |               |        |            |            |           |
| NCT01362790 [47] | SS1P and Pentostatin Plus Cyclophosphamide for Mesothelioma | Unknown status (Results Submitted) | Drug: Pentostatin; Drug: Cyclophosphamide; Biological: SS1(dsFv)-PE38 - lot 0730809; Biological: SS1(dsFv)-PE38 - lot FIL12901 | Phase 1/2 | 55         | 2011-05-11 | United States |
|       |                        |       |                  |               |        |            |            |           |
| NCT01051934 | A Phase I Trial of SS1 (dsFv) PE38 With Paclitaxel, Carboplatin, and Bevacizumab in Subjects With Unresectable Non-Small Cell Lung Adenocarcinoma | Completed | Drug: SS1 (dsFv) PE38; Drug: Paclitaxel; Drug: Carboplatin; Drug: Bevacizumab | Phase 1 | 2          | 2009-12-29 | United States |
|       |                        |       |                  |               |        |            |            |           |
| NCT00066651 [48] | Immunotoxin Therapy in Treating Patients With Advanced Solid Tumors | Completed | Biological: SS1(dsFv)-PE38 immunotoxin | Phase 1 | 1          | 2003-07-01 | United States |
|       |                        |       |                  |               |        |            |            |           |
| NCT0006981 [49] | Immunotoxin Therapy in Treating Patients With Advanced Cancer | Completed | Biological: SS1(dsFv)-PE38 immunotoxin | Phase 1 | 1          | 2000-12-01 | United States |
| Amatuximab | NCT02357147 [50] | Study of the Safety and Efficacy of Amatuximab in Combination With Pemetrexed and Cisplatin in Subjects With Unresectable Malignant Pleural Mesothelioma (MPM) | Terminated | Drug: Placebo; Drug: Amatuximab; Drug: Pemetrexed; Drug: Cisplatin | Phase 2 | 108       | 2015-11-03 | Australia; France; Germany; Italy; United Kingdom; United States |
|       |                        |       |                  |               |        |            |            |           |
| NCT01521325 | A Single-Dose Pilot Study of Radiolabeled Amatuximab (MORAb-009) in Mesothelin Over Expressing Cancers | Completed | Drug: Amatuximab | Phase 1 | 6          | 2011-09-01 | United States |
|       |                        |       |                  |               |        |            |            |           |
| NCT01413451 | Amatuximab for High Mesothelin Cancers | Terminated | Drug: Amatuximab (MORab-009) | Early Phase 1 | 7          | 2011-07-12 | United States |
|       |                        |       |                  |               |        |            |            |           |
| NCT01018784 [51] | A Study of MORAb-009 in Patients With Solid Tumor | Completed | Drug: MORAb-009 | Phase 1 | 17         | 2009-11-01 | Japan |
|       |                        |       |                  |               |        |            |            |           |
| NCT00738582 [52–54] | An Efficacy Study of MORAb-009 (Amatuximab) in Subjects With Pleural Mesothe| Completed (Results Submitted) | Drug: MORAb-009(Amatuximab); Drug: Pemetrexed; Drug: Cisplatin | Phase 2 | 89         | 2008-12-01 | Canada; Germany; Netherlands; Spain; United States |
|       |                        |       |                  |               |        |            |            |           |
| NCT00570713 [52] | An Efficacy Study of MORAb-009 in Subjects With Pancreatic Cancer | Completed (Results Available) | Drug: MORAb-009; Drug: Placebo; Drug: Placebo; Drug: Gemcitabine | Phase 2 | 155        | 2007-12-01 | Belgium; Canada; Germany; Spain; United States |
| Agent                  | NCT Number   | Title                                                                 | Status        | Interventions                                                                 | Phases | Enrollment | Start Date | Locations                                                                 |
|------------------------|--------------|----------------------------------------------------------------------|---------------|-------------------------------------------------------------------------------|--------|------------|------------|---------------------------------------------------------------------------|
| A Study of MORAb-009   | NCT00325494  | in Subjects With Pancreatic Cancer, Mesothelioma, or Certain Types of Ovarian or Lung Cancer | Completed     | Drug: MORAb-009                                                                | 1      | 24         | 2006-05-01 | United States                                                             |
| Anetumab Ravtansine   | NCT03816358  | With Nivolumab, Ipilimumab, and Gemcitabine Hydrochloride in Treating Patients With Mesothelin Positive Advanced Pancreatic Cancer | Suspended     | Biological: Anetumab Ravtansine; Drug: Gemcitabine Hydrochloride; Biological: Ipilimumab; Biological: Nivolumab | 1      | 64         | 2019-07-01 | Canada                                                    |
| Anetumab Ravtansine   | NCT03455556  | and Atezolizumab in Treating Participants With Advanced Non-small Cell Lung Cancer | Recruiting    | Biological: Anetumab Ravtansine; Biological: Atezolizumab; Other: Laboratory Biomarker Analysis | 1/2    | 49         | 2018-08-10 | United States                                                             |
| Pembrolizumab With Or | NCT03126630  | Without Anetumab Ravtansine in Treating Patients With Mesothelin-Positive Pleural Mesothelioma | Recruiting    | Biological: Anetumab Ravtansine; Other: Laboratory Biomarker Analysis; Biological: Pembrolizumab; Other: Pharmacological Study | 1/2    | 134        | 2018-02-08 | United States; Canada                                                     |
| Anetumab Ravtansine   | NCT03102320  | and Atezolizumab in Mesothelin Expressing Advanced Solid Tumors       | Recruiting    | Drug: Cisplatin; Drug: Gemcitabine; Drug: Anetumab ravtansine (BAY94-9343)     | 1      | 348        | 2017-05-26 | United States; Australia; Belgium; France; Germany; Italy; Korea; Netherlands; Singapore; Spain; Switzerland; United Kingdom |
| Phase II Anetumab     | NCT03023722  | Ravtansine in Pre-treated Mesothelin-expressing Pancreatic Cancer     | Recruiting    | Drug: anetumab ravtansine                                                      | 2      | 30         | 2017-05-11 | United States                                                             |
| Phase Ib Study of     | NCT02824042  | Anetumab Ravtansine in Combination With Pemetrexed and Cisplatin in Mesothelin-expressing Solid Tumors | Active, not recruiting | Drug: Anetumab ravtansine (BAY94-9343); Drug: Itraconazole                     | 1      | 63         | 2016-09-12 | United States; Australia; Belgium; France; Netherlands; Spain             |
| Antimesothelin        | NCT02839681  | Antibody Drug Conjugate: Anetumab Ravtansine for Mesothelin Expressing Lung Adenocarcinoma | Terminated     | Drug: Anetumab Ravtansine; Device: Blood test                                | 2      | 2          | 2016-07-19 | United States                                                             |
| Phase Ib Study of     | NCT02751918  | Anetumab Ravtansine in Hepatic or Renal Impairment                  | Active, not recruiting | Drug: Anetumab ravtansine (BAY94-9343); Drug: Pegylated Liposomal Doxorubicin | 1      | 71         | 2016-06-08 | United States; Belgium; France; Japan; Moldova; Spain                     |
| Anetumab Ravtansine   | NCT02696642  | in Hepatic or Renal Impairment                                      | Active, not recruiting | Drug: Anetumab ravtansine (BAY94-9343)                                      | 1      | 54         | 2016-04-14 | France; Moldova                                                           |
| Anetumab Ravtansine   | NCT02639091  | in Combination With Pemetrexed and Cisplatin in Mesothelin-expressing Solid Tumors | Active, not recruiting | Drug: BAY 94-9343; Drug: Pemetrexed; Drug: Cisplatin                          | 1      | 36         | 2016-02-03 | United States; Italy                                                     |
| Agent | NCT Number (Reference) | Title | Status (Results) | Interventions | Phases | Enrollment | Start Date | Locations |
|-------|------------------------|-------|-----------------|---------------|--------|------------|------------|-----------|
| NCT02610140 | Phase II Anetumab Ravtansine as 2nd Line Treatment for Malignant Pleural Mesothelioma (MPM) | Active, not recruiting | Drug: Anetumab ravtansine (BAY 94–9343); Drug: Vinorelbine | Phase 2 | 248 | 2015-12-03 | United States; Australia; Belgium; Canada; Finland; France; Italy; Korea; Netherlands; Poland; Russian Federation; Spain; Turkey; United Kingdom |
| NCT02485119 | Phase I Dose Escalation Study of BAY94–9343 Given by Intravenous Infusion Every 3 Weeks in Japanese Subjects With Advanced Malignancies | Completed | Drug: BAY94–9343 | Phase 1 | 12 | 2015-08-14 | Japan |
| NCT01439152 | Phase I Study to Determine the Maximum Tolerable Dose of BAY94–9343 in Patients With Advanced Solid Tumors | Active, not recruiting | Drug: BAY94–9343; Drug: BAY94–9343 (Expansion); Drug: BAY94–9343 (1.8 mg/kg); Drug: BAY94–9343 (2.2 mg/kg) | Phase 1 | 147 | 2011-09-07 | United States |
| DMOT4039A | A Study of DMOT4039A in Participants With Unresectable Pancreatic or Platinum-Resistant Ovarian Cancer | Completed | Drug: DMOT4039A | Phase 1 | 71 | 2011-11-01 | United States |
| NCT01832116 | 89Zr-MMOT PET Imaging in Pancreatic and Ovarian Cancer Patients | Completed | Drug: 89Zr-MMOT0530A | Phase 1 | 11 | 2013-03-01 | Netherlands |
| BMS-986148 | Phase 1 Study of Mesothelin-ADC | Completed | Drug: BMS-986148 | Phase 1 | 8 | 2016-10-14 | Japan |
| NCT02810418 | A Study of BMS-986148 in Patients With Select Advanced Solid Tumors | Active, not recruiting | Drug: BMS-986148; Biological: Nivolumab | Phase 1/2 | 407 | 2015-06-17 | United States; Australia; Belgium; Canada; Italy; Netherlands; United Kingdom |
| LMB-100 | Anti-Mesothelin Immunotoxin LMB-100 Followed by Pembrolizumab in Malignant Mesothelioma | Recruiting | Drug: LMB-100; Biological: Pembrolizumab | Phase 2 | 38 | 2018-12-04 | United States |
| NCT03436732 | Mesothelin-Targeted Immunotoxin LMB-100 in Combination With SEL-110 in Subjects With Malignant Pleural or Peritoneal Mesothelioma | Suspended | Drug: LMB-100; Drug: SEL-110 | Phase 1 | 23 | 2018-02-28 | United States |
| NCT02798536 | Mesothelin-Targeted Immunotoxin LMB-100 in People With Malignant Mesothelioma | Active, not recruiting | Drug: LMB-100; Drug: nab-paclitaxel | Phase 1 | 21 | 2016-06-10 | United States |
| Agent             | NCT Number (Reference) | Title                                                                 | Status (Results) | Interventions                                                                 | Phases | Enrollment | Start Date | Locations                                    |
|-------------------|------------------------|----------------------------------------------------------------------|------------------|--------------------------------------------------------------------------------|--------|------------|------------|---------------------------------------------|
| BAY2287411        | NCT03507452            | First-in-human Study of BAY2287411 injection; Thorium-227 Labeled Antibody-chelator Conjugate, in Patients With Tumors Known to Express Mesothelin | Recruiting       | Drug: BAY2287411                                                              | Phase 1| 228        | 2018-06-13| United States; Finland; Netherlands; Sweden; United Kingdom |
| HPN536            | NCT03872206            | Study of HPN536 in Patients With Advanced Cancers Associated With Mesothelin Expression | Recruiting       | Biological: HPN536                                                             | Phase 1/2| 87         | 2019-04-16| United States                                |
| CRS-207           | NCT02004262            | Safety and Efficacy of Combination Listeria/GVAX Pancreas Vaccine in the Pancreatic Cancer Setting | Completed (Results Available) | Biological: GVAX Pancreas Vaccine; Biological: CRS-207; Drug: Chemotherapy; Drug: cyclophosphamide | Phase 2| 303        | 2014-02-05| Canada                                      |
| NCT01417000       | NCT00585845 [58]       | Study of Safety and Tolerability of Intravenous CRS-207 in Adults With Selected Advanced Solid Tumors Who Have Failed or Who Are Not Candidates for Standard Treatment | Terminated       | Biological: CRS-207, Live-attenuated Listeria monocytogenes expressing human Mesothelin | Phase 1| 17         | 2007-12-01| United States; Israel                         |
| JNJ-64041757      | NCT03371381            | An Efficacy and Safety Study of JNJ-64041757, a Live Attenuated Listeria Monocytogenes Immunotherapy, in Combination With Nivolumab Versus Nivolumab Monotherapy in Participants With Advanced Adenocarcinoma of the Lung | Terminated       | Biological: JNJ-64041757; Drug: Nivolumab                                     | Phase 1/2| 12         | 2018-01-02| United States; Belgium; Spain                 |
| NCT02592967       |                        | Safety & Immunogenicity of JNJ-64041757, Live-attenuated Double-deleted Listeria Immunotherapy, in Subjects With Non Small Cell Lung Cancer | Terminated       | Biological: JNJ-64041757(Cohort 1A and 1B); Biological: JNJ-64041757(Cohort 2A and 2B) | Phase 1| 18         | 2015-12-02| United States                                |
| Neoantigen DNA Vaccine | NCT03122106          | Neoantigen DNA Vaccine in Pancreatic Cancer Patients Following Surgical Resection and Adjuvant Chemotherapy | Recruiting       | Biological: Personalized neoantigen DNA vaccine; Device: TDS-IM Electrode Array System; Procedure: Peripheral blood draws | Phase 1| 15         | 2018-01-05| United States                                |
MSLN-positive malignancies. Clinical testing in combination therapy in patients with antitumor activity. LMB-100 is currently undergoing taxanes and panobinostat enhances its combination with actinomycin D, Nab-Paclitaxel and the damage response inhibitors ATRi and PARPi resulted in synergistic activity and increased anti-tumor efficacy. A clinical trial was carried out to evaluate the safety, tolerability, pharmacokinetics, immunogenicity, antitumor activity and pharmacodynamics of MSLN antibodies and toxins with reduced immunogenicity need to be developed. Many studies have attempted to do this. The insertion of a disulfide bond to protect the furin cleavage site of SS1-PE24 improves its serum half-life and decreases its toxicity. A study suggested that the involvement of albumin-binding domains could prolong the half-life and increase antitumor activity. In addition, the removal of B- and T-cell epitopes from RIT led to greatly reduced antigenicity. Fully human antibodies were also developed and verified in preclinical studies.

Vaccines
Cancer vaccines are designed to induce tumor-specific immune responses in the host. A large number of studies have tested multiple platforms, including peptides, proteins, antigen presenting cells, tumor cells, and viral vectors. The bacterium-based vaccine CRS-207, which uses a live-attenuated Listeria monocytogenes (Lm) strain ANZ-100 (Lm ΔactA/ΔinlB) engineered to express human MSLN, has been used to treat MSLN-positive cancers in clinical trials. CRS-207 was evaluated in 17 subjects (7 with pancreatic ductal carcinoma (PDA), 5 with mesothelioma, 3 with NSCLC, and 2 with ovarian cancer) in a dose-escalation study with up to 4 doses (NCT00585845). CRS-207 was well tolerated at the top dose (1 × 10⁹ cfu). Immune activation was confirmed by a multiplexed serum cytokine assay and phenotype analysis. Thirty-seven percent of subjects survived ≥15 months, but none of them had a PR. CRS-207 has also been used in combination with low-dose cyclophosphamide and another vaccine, GVAX pancreas, which is derived from an irradiated allogeneic GM-CSF secreting cell line, in patients with metastatic PDA.
Sixty-one patients who received CRS-207 and Cy/GVAX had longer overall survival (6.1 months) than 29 patients treated with Cy/GVAX alone (3.9 months). A follow-up study to test the immune responses and efficacy produced by the combination of CRS-207 and the GVAX pancreas vaccine (with cyclophosphamide) compared to those produced by chemotherapy or CRS-207 alone in adults with previously treated metastatic pancreatic adenocarcinoma was conducted. The overall survival was 3.8 months for the cohort treated with Cy/GVAX + CRS-207, 5.4 months for the cohort treated with CRS-207 alone, and 4.6 months for the cohort treated with chemotherapy (NCT02004262).

JNJ-64041757 (previously referred to as ADU-214) is a live-attenuated, double-deleted (LADD) Listeria monocytogenes strain used as a potential treatment for NSCLC that was engineered by Aduro Biotech, Inc. in 2014. However, two clinical trials that attempted to evaluate its efficacy alone or in combination with nivolumab were both terminated due to a lack of clinical benefit (NCT02592967 and NCT03371381). A neoantigen DNA vaccine strategy is currently being evaluated in pancreatic cancer patients following surgical resection and adjuvant chemotherapy in an ongoing phase 1 clinical trial (NCT03122106). Neoantigen DNA vaccines incorporate prioritized neoantigens, and personalized MSLN epitopes will be administered intramuscularly using the TDS-IM system. The estimated completion date of this study is March 2022.

Despite the fact that there are few clinical trials of MSLN-targeted vaccines and the results of these trials have been disappointing, many preclinical studies are still ongoing. One study showed that a cell-based vaccine, Meso-VAX, in combination with the adenosassociated virus (AAV)-IL-12 increased the number of MSLN-specific T cells and the levels of anti-MSLN Abs and enhanced tumor clearance activity in mice [80]. The anti-tumor effects of the chimeric DNA vaccine CTGF/MSLN (containing an antigen-specific connective tissue growth factor linked to MSLN) in combination with an anti-CD40 Ab and the TLR 3 ligand poly(I:C), which are essential adjuvants for DC maturation, the immuno-modulator EGCG and Meso-VAX in combination with (AAV)-IL-12 were proven [81]. Recently, a MSLN-derived epitope peptide restricted to HLA-A*2402 was shown to be effective in inducing peptide-specific CTLs. The MSLN-10-5 peptide-specific CTL clones showed specific cytotoxic activity against HLA-A*2402-positive MSLN-expressing pancreatic cancer cells, indicating that the peptide-based vaccine is a promising candidate for therapy [82].

**CAR-T therapy**

**The development of MSLN-targeting CAR-T cells**

Chimeric antigen receptor T (CAR-T) cells are designed to target cell surface antigens without MHC restriction. Therefore, the CAR-T cells could be broadly applicable in HLA-diverse allogeneic recipients. The CARs are recombinant receptors commonly consisting of an extracellular antigen recognition domain, which is generally derived from the single chain variable fragment (scFv) of antibodies, transmembrane domains that function as anchors in the cytoplasmic membrane, and an intracellular domain that transmits T cell activation signals. The first-generation CARs consisted of only one intracellular signaling domain, which was usually a CD3z chain, and this was sufficient to initiate T cell activation but produced only short-term proliferative activity and a low level of cytotoxicity. The second-generation CARs had greatly improved potency through the incorporation of another costimulatory molecule (CD28, 4-1BB, or OX40) [83–85]. Furthermore, our team and other groups demonstrated that the third-generation MSLN-targeting CARs containing two costimulatory domains (CD28, 4-1BB, TLR2, or DAP10) and a hinge domain were superior in terms of cell proliferation, cytotoxicity, persistence and tumor suppression efficacy [86–89]. The latest iteration, the fourth-generation CARs, can additionally secrete cytokines or other effector molecules, such as IL-12, IL-15, IL-7, CCL19, or oPD-1, to regulate the immune microenvironment [90–95].

Because MSLN is a highly specific antigen in several cancers, CAR-T therapy has been proven to be a promising strategy for the treatment of these cancers. TNBC is intractable due to the lack of an effective targeted therapy. The presence of MSLN in 67% of TNBCs provides a candidate target for CAR-T therapy of TNBC [23]. MSLN-directed CAR-T cells were demonstrated to induce cytotoxicity in MSLN-expressing pancreatic cancer cells in vivo depending on the MSLN expression level to delay tumor growth and eliminate lung metastases in vivo [96, 97]. Our team previously demonstrated that MSLN was also a promising target for treating lung cancer and gastric cancer [28, 87]. We proved that third-generation CAR-T could effectively delay tumor growth or even completely eradicate subcutaneous tumors, eliminate pulmonary and intraperitoneal metastases of gastric cancer cells in mice and prolong survival. Similarly, the effectiveness of this targeted strategy has also been proven in bile duct carcinoma [98] and ovarian cancer [99].

CAR-T cells are generally produced via lentivirus transduction. The CAR genes are cloned into lentiviral vectors and subsequently integrated into the host T cell genome, allowing for the stable and permanent expression of the CAR. This method has been widely adopted.
because it is simple and reliable. Another method used for the stable integration of the CAR gene into the T cell genome is the piggyBac transposon system. The piggyBac transposon system is an efficient nonviral method for the genomic engineering of mammalian cells, including pluripotent stem cells and human T lymphocytes, and its advantages include a large cargo capacity, nonrandom integration and the elimination of virus-associated issues [100]. MSLN-targeting CAR-T cells engineered by the piggyBac transposon system have been proven to be cytotoxic to pancreatic cancer cells [97] and bile duct carcinoma cells [98]. To avoid the risks associated with genomic integration, several studies have proposed that CAR-T cells targeting MSLN could be generated by RNA electroporation [99, 101]. The expression of the CAR was shown to be detectable 7 days after electroporation. Multiple injections of RNA-electroporated CAR T cells reduced tumor volumes in mice. However, the CAR is transiently expressed and will be completely eliminated over time as a result of the degradation of the CAR mRNA [99, 101].

CAR-T cells are generally administered by systemic delivery, such as intravenous injection. However, systemically delivered T cells need to pass through the barriers created by multiple tissues before infiltrating into tumors. Therefore, inefficient T cell infiltration and short persistence are common obstacles for solid tumor therapy by CAR-T. A recent preclinical study revealed that regional intrapleural administration of CAR T cells resulted in more robust proliferation and increased antitumor efficacy with a long persistence of 200 days in an orthotopic MPM model compared with that induced by systemically infused T cells [102]. Similarly, we found that the regional peritumoral delivery of CAR-T cells produced enhanced tumor clearance in a subcutaneous GC model [28]. The subcutaneous tumors in some mice in the peritumoral delivery group were completely eliminated, whereas a moderate effect was observed in the group treated with intravenously injected CAR-T cells. In addition, we found improved T cell infiltration in tumors in the peritumoral delivery group. Overall, regional delivery might enhance the therapeutic effects, but this requires verification in clinical trials. To enhance T cell infiltration, the MSLN-targeting CAR-T cells were also engineered to express CCR2b, a chemokine receptor that is minimally expressed on T cells, while the CCR2b ligand and CCL2 is highly secreted by MPM [103]. The overexpression of CCR2b enhanced CAR-T cell cytotoxicity in tumor cells and chemotaxis in response to CCL2 in vitro. A 12.5-fold increase in T cell infiltration into tumors and significantly enhanced tumor clearance were observed in mice [103].

The tumor immune microenvironment is crucial in regulating T cell immunosurveillance. The upregulation of PD-L1 in tumor cells and the expression of inhibitory receptors, including PD1, CTLA-4, TIM3, LAG3, and 2B4, on T cells always reduces the infiltration of T cells into tumors and induces T cell exhaustion [95]. Recent preclinical studies showed that PD-1/PD-L1 blockade or CRISPR/Cas9-mediated PD-1 disruption could rescue MSLN-targeted CAR-T cell responses in vivo [104, 105]. Based on this, CAR-T cells engineered to express immune checkpoint antibodies (CTLA-4 and PD-1) or to knock out PD-1 are being evaluated in clinical trials [95] (NCT03030001, NCT03182803, NCT03615313, NCT03545815, and NCT03747965). In addition to being restricted by immune checkpoint molecules, the function of T cells is regulated by a variety of cytokines. The depletion of IL-10 with a blocking antibody or via the elevation of TNF-α and IL-2 levels by an oncolytic adenovirus enhanced and prolonged the functioning of MSLN-redirected CAR-T cells [106, 107].

MSLN-redirected CAR-T cells are also associated with the “on target, off tumor” issue. Despite the fact that no extensive or severe on-target toxicity against normal tissues has been observed, a great deal of effort has been made to avoid this problem. A promising strategy for this involves the achievement of accurate tumor recognition by combinatorial antigen-sensing circuits, while bispecific antibodies have proven more specific and potent [108]. Another potential approach is to physically separate the CD3ζ module from the costimulation module by using two distinct CARs specific for different antigens [109–111]. This structural design allows for comparable anticancer activity and persistence with the second-generation CAR-T cells only encounter both antigens. Another strategy is to engineer T cells with a synthetic Notch receptor that contains the core regulatory domain derived from the signaling receptor Notch [112]. An extracellular antigen recognition domain and a synthetic intracellular transcriptional domain were designed to replace the native Notch domain. Upon binding to the first antigen, the synthetic Notch receptor is cleaved and releases the intracellular transcriptional domain to activate the expression of the CAR, which recognizes the second antigen.

We have noted that the immunogenicity of murine-derived antibodies would limit their therapeutic effects in humans. Similarly, the use of a CAR of murine origin also limited the persistence of CAR-T cells in recipients. The development of a CAR with a human-derived scFv is needed to address this issue. A fully human MSLN-targeting CAR (P4) was constructed and shown to be enhanced in terms of cytokine secretion and cytotoxicity in vitro and anti-tumor activity in vivo [113]. P4 CAR-T cells were shown to be able to lyse MSLN-positive tumor cells in vitro and in vivo, even in the presence of soluble MSLN protein.
| NCT Number      | Title                                                                 | Status               | Interventions                                                                 | Phases   | Enrollment | Start Date | Locations |
|-----------------|------------------------------------------------------------------------|----------------------|-------------------------------------------------------------------------------|----------|------------|------------|-----------|
| NCT03814447     | The Fourth Generation CART-cell Therapy for Refractory-Relapsed Ovarian Cancer | Not yet recruiting  | Drug: anti-MESO CART-T cells; Drug: Fludarabine; Drug: Cyclophosphamide         | Early Phase 1 | 10         | 2019-04-01 | China     |
| NCT03749765     | Study of PD-1 Gene-knocked Out Mesothelin-directed CART-T Cells With the Conditioning of PC in Mesothelin Positive Multiple Solid Tumors | Recruiting           | Biological: Mesothelin-directed CART-T cells                                 | Phase 1  | 10         | 2018-11-01 | China     |
| NCT03608618     | Intrapitoneal MCY-M11 (Mesothelin-targeting CAR) for Treatment of Advanced Ovarian Cancer and Peritoneal Mesothelioma | Recruiting           | Biological: MCY-M11                                                          | Phase 1  | 15         | 2018-08-27 | United States |
| NCT03615313     | PD-1 Antibody Expressing mesoCAR-T Cells for Mesothelin Positive Advanced Solid Tumor | Recruiting           | Biological: PD-1 antibody expressing mesoCAR-T cells                           | Phase 1/2 | 50         | 2018-08-06 | China     |
| NCT03638193     | Study of Autologous T-cells in Patients With Metastatic Pancreatic Cancer | Recruiting           | Biological: CART-meso cells                                                   | Not Applicable | 10     | 2018-07-11 | China     |
| NCT03545815     | Study of CRISPR-Cas9 Mediated PD-1 and TCR Gene-knocked Out Mesothelin-directed CART-T Cells in Patients With Mesothelin Positive Multiple Solid Tumors. | Recruiting           | Biological: anti-mesothelin CART-T cells                                      | Phase 1  | 10         | 2018-03-01 | China     |
| NCT03356808     | Antigen-specific T Cells Against Lung Cancer                            | Recruiting           | Biological: Lung cancer-specific T cells                                       | Phase 1/2 | 20         | 2017-12-15 | China     |
| NCT03356795     | Intervention of CAR-T Against Cervical Cancer                           | Recruiting           | Biological: Cervical cancer-specific CAR-T cells                               | Phase 1/2 | 20         | 2017-11-15 | China     |
| NCT03497819     | Autologous CARTmeso/19 Against Pancreatic Cancer                        | Active, not recruiting | Biological: CARTmeso CART19                                                    | Early Phase 1 | 10     | 2017-10-01 | China     |
| NCT03323944     | CAR T Cell Immunotherapy for Pancreatic Cancer                          | Recruiting           | Biological: huCART-meso cells                                                  | Phase 1  | 18         | 2017-09-15 | United States |
| NCT03198052     | HER2/Mesothelin/Lewis-Y/PSCA/MUC1/PD-L1/CD80/86-CAR-T Cells Immunotherapy Against Cancers | Recruiting           | Biological: CAR-T cells targeting HER2, Mesothelin, PSCA, MUC1, Lewis-Y, or CD80/86 | Phase 1  | 30         | 2017-07-01 | China     |
| NCT03267173     | Evaluate the Safety and Efficacy of CAR-T in the Treatment of Pancreatic Cancer. | Recruiting           | Drug: Chimeric antigen receptor T cell                                          | Early Phase 1 | 10     | 2017-06-15 | China     |
| NCT03182803     | CTLA-4 and PD-1 Antibodies Expressing Mesothelin-CAR-T Cells for Mesothelin Positive Advanced Solid Tumor | Recruiting           | Biological: CTLA-4/PD-1 antibodies expressing mesoCAR-T                      | Phase 1/2 | 40         | 2017-06-07 | China     |
| NCT03054298     | CAR T Cells in Mesothelin Expressing Cancers                            | Recruiting           | Biological: huCART-meso cells                                                  | Phase 1  | 30         | 2017-03-01 | United States |
| NCT03030001     | PD-1 Antibody Expressing CAR T Cells for Mesothelin Positive Advanced Malignancies | Unknown status       | Biological: PD-1 antibody expressing mesothelin specific CAR-T cells            | Phase 1/2 | 40         | 2017-02-15 | China     |
| NCT02930993     | Anti-mesothelin CAR T Cells for Patients With Recurrent or Metastatic Malignant Tumors | Recruiting           | Biological: anti-mesothelin CART T cells                                      | Phase 1  | 20         | 2016-08-01 | China     |
| NCT02921151     | A Study of Chimeric Antigen Receptor T Cells Combined With Interventional Therapy in Advanced Liver Malignancy | Unknown status       | Drug: CART-T cell                                                              | Phase 1/2 | 20         | 2016-07-01 | China     |
| NCT02792114     | T-Cell Therapy for Advanced Breast Cancer                                | Recruiting           | Drug: Cyclophosphamide; Biological: Mesothelin-targeted T cells; Drug: AP1903 | Phase 1  | 36         | 2016-06-01 | United States |
| NCT Number   | Title                                                                 | Status               | Interventions                                                                 | Phases  | Enrollment | Start Date  | Locations      |
|-------------|-----------------------------------------------------------------------|----------------------|-------------------------------------------------------------------------------|---------|------------|-------------|----------------|
| NCT02706782 | A Study of Mesothelin Redirected Autologous T Cells for Advanced Pancreatic Carcinoma | Unknown status       | Drug: TAI-meso-CART                                                           | Phase 1 | 30         | 2016-03-01  | China          |
| NCT02580747 | Treatment of Relapsed and/or Chemotherapy Refractory Advanced Malignancies by CART-meso | Unknown status       | Biological: anti-meso-CAR vector transduced T cells                          | Phase 1 | 20         | 2015-10-01  | China          |
| NCT02414269 | Malignant Pleural Disease Treated With Autologous T Cells Genetically Engineered to Target the Cancer-Cell Surface Antigen Mesothelin | Recruiting           | Genetic: iCasp9M28z T cell infusions; Drug: cyclophosphamide                 | Phase 1 | 48         | 2015-05-01  | United States  |
| NCT02465983 | Pilot Study of Autologous T-cells in Patients With Metastatic Pancreatic Cancer | Completed            | Biological: CART-meso-19 T cells; Drug: Cyclophosphamide                     | Phase 1 | 4          | 2015-05-01  | United States  |
| NCT02388828 | CART-meso Long-term Follow-up                                         | Active, not recruiting | Biological: lentiviral-based CART mesothelin therapy                         |         | 10         | 2015-03-01  | United States  |
| NCT02159716 | CART-meso in Mesothelin Expressing Cancers                             | Completed            | Biological: CART-meso                                                         | Phase 1 | 19         | 2014-06-01  | United States  |
| NCT01897415 | Autologous Redirected RNA Meso CAR T Cells for Pancreatic Cancer       | Completed            | Biological: Autologous T cells transfected with chimeric anti-mesothelin immunoreceptor SS1 | Phase 1 | 16         | 2013-07-01  | United States  |
| NCT01583686 | CART T Cell Receptor Immunotherapy Targeting Mesothelin for Patients With Metastatic Cancer | Terminated           | Drug: Fludarabine; Biological: Anti-mesothelin CAR transduced PBL; Drug: Cyclophosphamide; Drug: Aldesleukin | Phase 1/2 | 15         | 2012-05-04  | United States  |
| NCT01355965 | Autologous Redirected RNA Meso-CIR T Cells                             | Completed            | Biological: Autologous T cells                                               | Phase 1 | 18         | 2011-03-01  | United States  |
Clinical trials of MSLN-targeting CAR-T cells

The majority of newly registered clinical trials targeting MSLN in the past 3 years are related to CAR-T therapy. CAR-T therapy has been a potent strategy for treating MSLN-expressing tumors [86, 114]. CAR design has been greatly optimized to enhance its performance [85]. The safety, effects and the maximum tolerated dose of MSLN-targeting CAR-T cell therapy are currently being evaluated in multiple phase 1/2 clinical trials (Table 2).

In a preclinical study, MSLN-targeting CAR-T cells generated by the transfection of mRNA showed robust antitumor activity and the transient expression of the CAR. mRNA-based CAR-T cells (SS1-4-1BB CAR) were proven to be well tolerated after multiple intravenous or intratumoral infusions (NCT01355965) [115, 116]. A confirmed partial response was observed in patients with MPM or PDA. The serum levels of inflammatory cytokines, including MIP-1β, granulocyte colony-stimulating factor (G-CSF), IL-6, and IL-17, were transiently elevated after each infusion of CAR-T cells [115]. CAR-T cells were detected in tumors with reduced CAR transcripts several days after administration. Notably, MSLN-targeting CAR-T cells were able to lyse primary tumor cells and elicit a systemic antitumor immune response by inducing epitope spreading [116].

In another recent phase 1 clinical trial, 6 patients with chemotherapy-refractory metastatic PDAC were intravenously administered autologous MSLN-targeting CAR-T cells 3 times weekly for 3 weeks [117]. Two patients had stable disease with PFS of 3.8 and 5.4 months. A decrease in MSLN expression by 69.2% in one patient was confirmed by biopsy. None of the 6 patients experienced cytokine release syndrome or neurological symptoms. Noteworthy, no evident on-target/off-tumor toxicity against normal tissues was observed in these patients [116, 117]. However, in addition to the short life span of the CAR, another issue that might limit its potency is the production of human anti-CAR antibodies [115–117]. An anaphylactic response reported in one patient was attributed to the high production of IgE antibodies specific to the CAR [115]. This suggests that a fully human anti-MSLN scFv is urgently needed for clinical use. Interestingly, a clinical trial that aims to impede the production of antibodies via the depletion of B cells by CD19-targeting CAR-T cells has been initiated (NCT03497819). This clinical trial is active but is not recruiting yet.

Regional delivery was proven to enhance T cell proliferation, persistence and function in mice. Because of this, regional delivery was applied to the clinical treatment of patients. CAR-T cells were administered intrapleurally, intratumorally, or by vascular interventional mediated injection (NCT02414269, NCT02706782, NCT02959151, NCT03267173, and NCT03198052). We still await the publication of the clinical outcomes to determine the importance of regional delivery in the clinic.

CAR-T therapy is always accompanied by cytokine release syndrome (CRS) and neurotoxicity due to the excessive immune activation of CAR-T or non-CAR-T cells, and the severity of this is associated with disease burden, the CAR-T cell dose, high-intensity lymphodepletion and preexisting endothelial activation [118]. To decrease the CAR-T-induced side effects, debulking chemotherapy is recommended to reduce tumor burden and the subsequent CAR-T dose, and tocilizumab could be used to prevent severe CRS in the clinic [118]. To enhance the safety of CAR-T therapy and controllably eliminate CAR-T cells when SAEs occur or tumors are eliminated, inducible suicide genes, including iCaspase-9, HSV-TK or EGFRA, could co-transduced with the CAR genes [25]. Exposure to a synthetic dimerizing drug would induce the dimerization of iCaspase-9 and lead to cell apoptosis. This inducible T-cell safety switch involving iCaspase-9 has been proven to eliminate over 90% of modified T cells within 30 min [119]. A MSLN-targeting CAR-T therapy trial involving the use of iCaspase-9 is currently recruiting (NCT03747965).

Conclusions

The expression pattern of MSLN provides an exciting opportunity for its use in targeted therapy in various types of malignant tumors, including pancreatic cancer, ovarian cancer, lung cancer, TNBC and gastric cancer. To date, antibody-based drugs have been effective in inhibiting cancer progression and show acceptable “on target, off tumor” toxicity, while vaccines have showed moderate effects. The great improvements in CAR-T design allows them to be a promising therapeutic strategy to treat MSLN-expressing tumors. The immunogenicity of drugs and CAR-T cells, the low level of T cell infiltration into tumors and the high level of immunosuppression in the tumor microenvironment are obstacles that need to be overcome. The combined use with checkpoint inhibitors as well as additional strategies to reduce drug resistance and optimize delivery regimens might show promise in the future.

Abbreviations

ADCC: Antibody-dependent cell-mediated cytotoxicity; ADCP: Antibody-dependent cell-mediated phagocytosis; cfu: Colony forming units; CRS: Cytokine release syndrome; DHAEs: Drug hypersensitivity adverse events; DLTs: Dose-limiting toxicities; G-CSF: Granulocyte colony-stimulating factor; GPI: Glycosylphosphatidylinositol; HSV: Herpes Simplex Virus; Lm: Listeria monocytogenes; MAAE: Monomethyl auristatin E; MPF: Megakaryocyte potentiating factor; MPA: Malignant pleural mesothelioma; MSLN: Mesothelin; MTD: Maximum tolerated dose; NSCLC: Non-small cell lung cancer; PD: Progressive disease; PDA: Pancreatic ductal carcinoma; PE: Pseudomonas exotoxin; PK: Pharmacokinetics; PLD: Pegylated liposomal doxorubicin; PR: Partial responses; RIT: Recombination immunotoxin; scFv: Single chain variable fragment; SD: Stable disease; SMRP: Soluble MSLN-related protein; TNBC: Triple negative breast cancer.
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