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Nanobody-enhanced T cell cancer immunotherapy: Advancing precision medicine

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1 **Mesothelin biology and the evolving landscape of** 2 **targeted immunotherapy**

3

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11 **ABSTRACT**

12 Due to its restricted expression in normal tissues, its frequent overexpression in various aggressive
13 malignancies, and its involvement in several pro-tumorigenic signaling pathways, mesothelin, a
14 glycosylphosphatidylinositol-anchored cell surface protein, has been identified as a promising tumor-
15 associated antigen. This review provides an up-to-date comprehensive overview regarding the role of
16 mesothelin in cancer. It encompasses the protein structural characteristics, expression profiles,
17 glycosylation patterns, shedding mechanisms, prognostic significance, and functional roles.
18 Subsequently, therapeutic strategies targeting mesothelin that have advanced to clinical trial stage are
19 discussed, including vaccine-based approaches, antibody-mediated immunotherapies, and cell therapies.
20 The remaining challenges of mesothelin targeted treatments are highlighted, along with the ongoing
21 options aimed at overcoming these limitations.

22 INTRODUCTION

23 Discovered in 1992 by Chang and Pastan, mesothelin (MSLN) is a glycosylphosphatidylinositol
24 (GPI)-anchored cell surface glycoprotein predominantly expressed on the mesothelial cells that line the
25 pleural, peritoneal, and pericardial cavities.^{1,2} A recent immunohistochemical study on 76 healthy organs
26 has revealed a broader expression of mesothelin, primarily observed in non-vital tissues in adults such
27 as the tongue, thymus, gallbladder, fallopian tubes, uterus, and placenta.³

28 The biological role of mesothelin in healthy tissues remains to be elucidated. Its function appears
29 non-essential, as MSLN gene invalidation experiments in mice did not result in any significant
30 abnormalities in development, fertility, or platelet count compared with wild-type mice.⁴

31 It has been hypothesized that MSLN, akin to several other GPI-anchored proteins, plays a role in
32 cell adhesion processes and signaling pathways. Sathyanarayana *et al.* proposed that MSLN contributes
33 to the modeling of the extracellular matrix through its interaction with matrix glycoproteins.⁵

34 The only described partner of mesothelin is the glycosylated transmembrane protein mucin 16
35 (MUC16) (~3-5 MDa), also known as CA125, expressed at the surface of various epithelial cells but
36 overexpressed in several cancer types.⁶⁻⁸ Notably, in healthy tissues, MSLN and MUC16 are expressed
37 at low levels and in distinct cell types, suggesting that no physiological interaction occurs under normal
38 conditions.^{3,8}

39 The mesothelin gene is located on chromosome 16 (16p13.3) and encodes the mesothelin precursor
40 protein (~70 kDa).² Three MSLN transcript variants have been described⁹. Variant 1 is regarded as the
41 canonical form because it is the predominant form expressed in both healthy and tumor tissues
42 **(Figure 1A)**.¹⁰

43 The MSLN precursor is transported and anchored to the cell membrane through a GPI anchor
44 **(Figure 1B)**. Subsequently, the MSLN precursor is cleaved by proteolysis, releasing the N-terminal
45 soluble fragment, designated as megakaryocyte potentiating factor (MPF) (~30 kDa). This process
46 leaves the mature form of MSLN (~40 kDa) anchored to the membrane.¹¹ A furin cleavage site
47 (RPRFRR) located between amino acids 286 and 295 has been identified as the primary proteolytic

48 processing site. However, several recent studies have shown that alternative proteolytic sites are also
49 present in this region.^{12,13}

50 The membrane-bound mature MSLN can be shed from cell surface under normal conditions,
51 contributing to serum MSLN levels (**Figure 1C**).^{14,15} However, the rate, the specific protease profile,
52 the regulatory pathways as well as its functional significance are not documented.

53 The three-dimensional structure of mature MSLN has been recently determined in complex with
54 therapeutic antibodies.^{16,17} In accordance with the predicted structure, MSLN exhibits a curved super-
55 helical structure comprising a series of ARM (3 helices) and HEAT (2 helices) repeats.⁵ MSLN is
56 organized in four domains: A (296-359), B (360-427), C (428-502), and D (503-598) (**Figure 1A**).¹⁶

57

58

59 **MESOTHELIN IN CANCER**

60 **Expression**

61 Mesothelin overexpression has been demonstrated in a multitude of tumor types, as evidenced by
62 an extensive immunohistochemical analysis of 15,050 tumors of 122 different types. The analysis
63 revealed that cancers with the highest prevalence and expression levels of MSLN include all types of
64 ovarian cancer, endometrial carcinoma, pancreatic adenocarcinoma, malignant mesothelioma of the
65 epithelioid type, and adenocarcinoma of the lung, stomach, esophagus and colorectum.³ Further
66 immunohistochemical analysis revealed a predominant membranous localization, with a less frequent
67 cytoplasmic localization, which is likely due to the detection of the MSLN precursor not yet anchored
68 to the membrane.^{18,19} Weidemann's study corroborates previous results obtained in a transcriptomic
69 study involving 19,746 samples covering 41 tumor types.²⁰ Recently, a transcriptomic study of
70 2051 pediatric and young adult patients with acute myeloid leukemia (AML) revealed that MSLN
71 overexpression is not exclusive to solid cancer.²¹ The authors demonstrated that MSLN is overexpressed
72 in approximately 36% of AML cases, despite its absence in normal bone marrow.

73 The mechanisms leading to MSLN overexpression in tumors have yet to be elucidated, but several
74 potential mechanisms have been postulated. As demonstrated in the literature, mesothelioma, lung,

75 ovarian, and pancreatic cancers with constitutive activation of the Wnt signaling pathway exhibit high
76 mesothelin expression.¹² The presence of an 18-base-pair sequence, CanScript, that drives high levels
77 of gene expression has been described in the MSLN promoter region in different MSLN-overexpressing
78 cancer lines.²² However, the co-factor involved in the regulation of MSLN expression remains to be
79 identified. The hypothesis of an epigenetic regulation has been postulated subsequent to the observation
80 of the hypomethylation of the MSLN promoter in pancreatic tumor samples.²³ However, this
81 phenomenon was not observed in other tumors overexpressing MSLN, such as ovarian cancer. In the
82 context of ovarian cancer, Park and Kim have demonstrated an upregulation of MSLN and MUC16
83 expression through the stimulation of TLR5 and TLR7 (Toll-Like Receptors). This upregulation is
84 mediated by the activation of WAVE3, a member of the Wiskott-Aldrich syndrome protein (WASP)
85 family. WAVE3 has been identified as a significant driver of the invasive and metastatic phenotypes in
86 multiple cancer types.²⁴

87

88 **Mesothelin binding partners**

89 The only well described partner of mesothelin is MUC16, overexpressed in several cancer types.⁶⁻
90 ⁸ MSLN/MUC16 interaction is a feature specific to malignant cells linked to the co-expression of
91 MUC16 and MSLN.

92 Mesothelin-MUC16-binding is mediated by protein-protein interaction between domain A (296-
93 359) of MSLN and MUC16 SEA (sea urchin sperm protein, enterokinase, and agrin) modules.^{7,25}
94 Involvement of the SEA modules suggests that MUC16 might engage MSLN molecules, thereby
95 generating an avidity phenomenon at the cell surface. However, enzymatic removal of MUC16 N-
96 glycans disrupts MUC16/MSLN interaction, indicating that glycans, by maintaining MUC16 stability,
97 are structurally critical for the binding.²⁶

98 Recently, it has been shown that, on portal fibroblasts, MSLN can also bind to Thy-1 (thymocyte
99 differentiation antigen 1, CD90) and form a MSLN/MUC16/Thy-1 complex in response to pro-fibrotic
100 signals, such as TGF- β 1. This complex by modulating TGF- β 1/TGF- β R1 signaling promotes fibroblast

101 activation and the transcription of fibrogenic and pro-tumorigenic genes via canonical TGF- β 1/Smad2/3
102 signaling, thereby contributing to desmoplasia and tumor progression.^{27–29}

103

104 **Glycosylation**

105 While glycosylation in general has been demonstrated to play critical roles in cancer progression,
106 including cellular adhesion, resistance to cell death, and metastatic potential, there is currently a lack of
107 knowledge regarding mesothelin glycosylation.^{30,31} To address this knowledge gap, Duran *et al.*
108 conducted a comprehensive analysis of the N-glycan structures of MSLN from pancreatic ductal
109 adenocarcinoma (PDAC) cell lines and tissues. Their findings revealed full occupancy of the three N-
110 glycosylation sites (N388, N488 and N515) and a structure predominantly corresponding to a complex
111 core fucosylated-N-glycan with terminal sialylation on MSLN from the cell-conditioned media.³² By
112 contrast, a decrease of core-fucosylated glycoforms of MSLN was observed in both serum and tissues
113 from PDAC patients. This observation was replicated by Zhan *et al.* who demonstrated that N-glycan
114 fucosylation was predominant on soluble MSLN (sMSLN) from a pancreatic cancer cell line, while in
115 ascites, sMSLN exhibited heavy sialylation and moderate fucosylation.¹⁶ These two studies indicate a
116 shift in the glycoforms of MSLN during the progression of pancreatic cancer, with a decrease in core-
117 fucosylated glycoforms and an increase in highly sialylated and moderately core-fucosylated
118 glycoforms.

119 Fujihira and colleagues have demonstrated that the glycosylation profile of MSLN in epithelial
120 mesothelioma differs from that of MSLN in normal mesothelial cells, thereby supporting a potential role
121 for glycosylation in the malignant transformation of cells.³³

122

123 **Shedding**

124 In cancer, MSLN shedding is mediated by upregulated and activated enzymes, such as members of
125 the ADAM, MMP, TACE and BACE families.¹⁵ These enzymes cleave MSLN at different sites in the
126 C-terminal region close to the cell surface (**Figure 1C**). The action of these proteases contributes to the
127 elevated shedding rate that appears to vary according to the cancer type and the tumor stage.³⁴ The

128 presence of sMSLN in the bloodstream has been demonstrated in patients diagnosed with mesothelioma
129 and ovarian cancer. Conversely, patients diagnosed with pancreatic adenocarcinoma exhibit minimal or
130 no increase in serum mesothelin concentration. A recent study has demonstrated, employing PDAC
131 models, that shed mesothelin is sequestered within the tumor microenvironment. The authors of the
132 study postulated that this phenomenon is associated with collagen density and elevated MUC16
133 expression.³⁴

134

135 **Prognostic value**

136 The sMSLN and the membrane-bound MSLN (mbMSLN) have been the focus of studies exploring
137 their potential as biomarkers for various cancers, particularly for mesothelioma, pancreatic cancer, and
138 ovarian cancer. However, as highlighted in an extensive review by Hagerty and Takabe, the
139 heterogeneity of study designs, outcomes, and protocols complicates the interpretation of their clinical
140 relevance.³⁵ Furthermore, the co-expression status of the ligand MUC16 is rarely addressed in studies
141 examining the prognostic value of MSLN. This information could offer valuable insights into
142 clinicopathological implications that could influence prognosis as MUC16 co-expression has been
143 linked to accelerated tumor growth, metastasis, and poor prognosis. Recently MSLN-MUC16 co-
144 expression has been associated to tumor aggressiveness and poor prognosis in patients with PDAC,³⁶⁻³⁸
145 endometrial serous carcinoma,^{39,40} perihilar bile duct carcinoma,⁴¹ and in luminal-type breast cancer.⁴²
146 Regarding the use of MSLN as a diagnostic biomarker, it has been demonstrated that neither elevated
147 levels of circulating mesothelin nor the expression of membrane-bound mesothelin, in isolation, are
148 adequate for establishing a reliable diagnosis of cancer. However, when considered in conjunction with
149 other markers, MSLN can serve as an indication of cancer pathology and provide a compelling rationale
150 for further investigations.⁴³

151 Several prognostic survival studies have been conducted on various MSLN-positive cancers with
152 the aim of determining the prognostic significance of soluble and/or membrane MSLN (**Figure 2**). A
153 significant number of these studies have validated the correlation between elevated MSLN expression

154 in immunohistochemistry and unfavorable prognosis in multiple cancers, including malignant pleural
155 mesothelioma (MPM),⁴⁴ ovarian,^{45,46} and triple-negative breast cancers (TNBC).⁴⁷

156 The cellular localization of MSLN has been demonstrated to possess potential prognostic value,
157 with luminal/membranous localization of MSLN being associated with a more unfavorable prognosis
158 in pancreatic cancers and extrahepatic bile duct cancers when compared with cytoplasmic localization.¹⁹
159 A recent observation by a Japanese research team has revealed a similar finding, demonstrating that
160 membrane MSLN positivity in luminal-type breast cancers appears to be associated with a poor
161 prognosis and an elevated risk of recurrence.^{42,48}

162 In the context of colorectal cancer, elevated MSLN expression has been associated with
163 chemoresistance, peritoneal metastases, and a lower 3-year overall survival rate.^{49,50}

164 The presence of sMSLN in the bloodstream has not yielded satisfactory prognostic results for
165 pancreatic cancers. This is due to the fact that serum levels of mesothelin do not differentiate between
166 stages of the disease nor reflect tumor burden.⁵¹

167 Conversely, a systematic review of 20 studies regarding MPM demonstrated that a decrease in
168 serum MSLN levels correlates with a positive response to treatment. This suggests that measuring serum
169 MSLN could enable the monitoring of disease progression, risk of recurrence after surgery, and response
170 to systemic therapy.⁵² Data from a clinical trial for a specific CAR-T cell -MSLN immunotherapy
171 approach in malignant pleural mesothelioma (NTC02414269) presented at the American Society of
172 Clinical Oncology (ASCO) and the American association for cancer research (AACR) in 2019 point in
173 the same direction. The observed decrease in serum sMSLN levels is associated with tumor regression
174 and persistence of CAR-T cells.⁵³

175 It is noteworthy that a recent publication reported the potential predictive value of MSLN regarding
176 therapies.⁵⁴ The study demonstrated a significant correlation between MSLN and both cetuximab and
177 gefitinib sensitivity in pancreatic cancer.

178

179 **Role in cancer**

180 Preclinical and clinical data suggest an active role of MSLN overexpression in the invasive and
181 aggressive phenotypes of MSLN-positive tumors. It is generally accepted that MSLN overexpression
182 promotes tumor cell survival, proliferation, migratory and invasive capacities, and contributes to
183 chemoresistance. However, the precise mechanisms by which MSLN exerts its effects and its
184 interactions with the tumor microenvironment (TME) are not yet fully understood. One hypothesis
185 suggests that MSLN may interact with various signaling transmembrane proteins within lipid rafts, a
186 phenomenon observed with GPI-anchored proteins.⁵⁵ It appears that MSLN can function in a MUC16-
187 dependent or MUC16-independent manner (**Figure 3**). However, distinguishing between these two
188 modes of action is challenging due to the lack of systematic assessment of MUC16 status in studies.
189 Additionally, the distinction between the soluble and membrane forms of mesothelin is not always clear
190 in existing literature.

191 MSLN overexpression has been demonstrated to regulate multiple signaling pathways identified as
192 contributing to cancer progression (**Figure 3**). This review does not aim to provide an exhaustive
193 overview of all these pathways. Instead, it will offer an overview of some of the pathophysiological
194 implications observed in different types of cancer.

195 In pancreatic cancer, MSLN-dependent NF- κ B pathway activation has been observed to result in
196 elevated levels of IL-6, which, in turn, have been found to be responsible for the activation of anti-
197 apoptotic factors, such as the Bcl-2 family.⁵⁶⁻⁵⁸ In mesothelioma and pancreatic cancer, MAPK pathway
198 activation through MSLN has been shown to promote cell migration and invasion by facilitating the
199 expression and activation of MMP-7 and MMP-9, which are metalloproteinases implicated in the
200 degradation of the extracellular matrix.^{37,59} In pancreatic cancer, independently of MUC16, MSLN
201 contributes to the development of peritoneal metastasis by enhancing angiogenesis, cell proliferation,
202 and invasion.⁶⁰ In breast cancer cell lines, ectopic expression of MSLN facilitates anchorage-
203 independent cell growth *in vitro* and induces resistance to anoikis.⁶¹

204 Recent studies have demonstrated that the inactivation of MSLN in various cancer cell lines
205 disrupts the expression of several genes associated with the epithelial-mesenchymal transition (EMT).
206 These results suggest that MSLN promotes the EMT and stem cell properties by enhancing the
207 expression of EMT regulators (snail, twist, slug, ABCG2) and reducing the expression of mesenchymal

208 markers (E-cadherin, caveolin-2, cytokeratins, claudins).^{62,63} *In vivo*, genetic knockdown of MSLN has
209 been demonstrated to decrease tumor growth and metastasis, while mesothelin overexpression in a non-
210 cancerous cell line has been shown to induce a malignant phenotype, arguing in favor of an oncogenic
211 role for mesothelin.⁶³

212 Bioinformatics approaches have demonstrated a positive correlation between high MSLN
213 expression and immune infiltration in ovarian cancers, including Th17, dendritic cells, and NK cells.
214 Additionally, a correlation has been observed between MSLN expression levels and chemoresistance.⁶⁴
215 A close association has been established between chemotherapy resistance and the level of MSLN
216 expression in malignant pleural mesothelioma and pancreatic cancers.^{45,62}

217 MSLN has been identified as a potential contributor to the progression of lung cancer, particularly
218 through its role in facilitating the disruption of the blood-brain barrier (BBB) in non-small cell lung
219 cancer (NSCLC).⁶⁵ Recent studies have demonstrated a correlation between high MSLN expression and
220 the remodeling of the tumor immune landscape. In microsatellite-stable colorectal tumors, MSLN
221 overexpression promotes immune checkpoint expression (PD-1, LAG-3, TIM-3), immune-related
222 marker expression (PD-L1), immune cell infiltration, and the activation of pro-tumoral pathways (TGF-
223 β and IL-6/JAK/Stat3).⁶⁶ In PDAC, sMSLN promotes the polarization of macrophages into an
224 immunosuppressive phenotype (CD106⁺), which supports tumor cell growth, neutrophil recruitment and
225 the spread of metastasis to the lungs.⁶⁷

226

227

228 **STRATEGIES IN CLINICAL TRIALS TARGETING**

229 **MESOTHELIN**

230 As described above, MSLN has emerged as a promising tumor-associated antigen (TAA). The
231 field of mesothelin-targeted therapies has undergone continuous evolution over the past 25 years,
232 encompassing the following three categories: vaccine, antibody-based immunotherapy, and cell therapy.
233 However, despite the extensive research conducted in this field, no MSLN-targeted treatment has yet

234 received the approval for clinical utilization from the food and drug administration (FDA). In the
235 following section, recent clinical trial data related to these approaches are compiled.

236

237 **Vaccines**

238 *Listeria monocytogenes*-based vaccines: CRS-207 and JNJ-757

239 CRS-207, developed by Aduro Biotech, is an attenuated strain of *Listeria monocytogenes*
240 engineered to express the human mesothelin within the cytosol of infected antigen-presenting cells
241 (APCs).^{12,68} This strain has been genetically modified through the deletion of two virulence genes,
242 significantly reducing its pathogenicity while preserving its capacity to elicit robust immune responses.⁶⁹

243 Since the first trial in 2007 with CRS-207, 11 clinical trials have been registered on
244 ClinicalTrials.gov: eight phase 2, one phase 1/2 and two phase 1 (**Table 1**).⁷⁰ CRS-207 has been
245 evaluated both as monotherapy and in combination with different therapies such immune checkpoint
246 inhibitors (ICIs) (ipilimumab, nivolumab, pembrolizumab), chemotherapies (epacadostat, an oral
247 inhibitor of the IDO1 enzyme, cisplatin, cyclophosphamide, pemetrexed), tadalafil or vaccine (GVAX
248 pancreas vaccine). The drug has been studied in patients with various types of cancer. In a clinical trial
249 for patients with advanced MPM (NCT01675765), the combination treatment of CRS-207 and
250 chemotherapy demonstrated a higher overall response rate compared to chemotherapy alone.⁷¹ By
251 contrast, the combination treatment of CRS-207 and pembrolizumab in patients with MPM exhibited no
252 clinical activity, resulting in the cessation of the clinical development of CRS-207 for this indication.⁷²
253 Despite this discontinuation, mesothelin-targeted CRS-207 in combination with immune checkpoint
254 inhibitors, dioxygenase (IDO) inhibitors, and CY/GVAX is currently being evaluated in other
255 indications, notably in patients with pancreatic cancers.⁷³

256 A similar vaccine (JNJ-757), developed by Janssen, a Johnson & Johnson subsidiary, has
257 undergone early phase trials, but the absence of clinical activity prompted its discontinuation.⁷⁴

258

259 *Neoantigen DNA and peptide vaccines*

260 The Washington University School of Medicine has developed personalized cancer DNA- and
261 peptide-based vaccines that incorporate prioritized neoantigens and mesothelin epitopes for patients
262 with pancreatic cancers.⁷⁵

263 Both approaches reached phase 1 clinical trials demonstrating an acceptable toxicity and a
264 neoantigen-specific immune response in all patients analyzed by ELISPOT (**Table 1**).⁷⁵ However,
265 despite the encouraging preliminary results, both trials were terminated due to insufficient funding.

266

267 **Antibody-based immunotherapies**

268 Currently, 17 antibody-based immunotherapies have advanced to the clinical research stage: two
269 immunotoxins (iTOXs), two antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-
270 dependent cell-mediated phagocytosis (ADCP) mediators, five antibody-drug conjugates (ADCs), two
271 immunostimulators, one targeted radionuclide therapy (TRT), and five T-cell engagers (TCEs)
272 (**Figure 4A**).

273 The development of anti-MSLN treatment was initiated in 1998 under the direction of Ira Pastan.
274 Ira Pastan's team used a mesothelin expression plasmid to immunize mice. This approach led to the
275 isolation of an anti-mesothelin single-chain variable fragment (scFv) known as SS scFv.⁷⁶ Mutations
276 were introduced in the complementarity determining region 3 (CDR3) of SS V_L to enhance its binding
277 affinity, resulting in the variant named SS1.⁷⁷ Notably, SS1 competes with MUC16 for MSLN binding.²⁵
278 SS1 was regularly used to develop anti-MSLN drugs including SS1P, LMB-100, amatuximab, and even
279 CAR-T cells (**Figure 4B**).^{77,78}

280

281 ***Immunotoxins: SS1P and LMB-100***

282 A recent review by Hagerty *et al.* provides a comprehensive summary of both pre-clinical and
283 clinical data regarding MSLN-targeted immunotoxin (iTOX).⁷⁹ The two iTOXs that advanced to the
284 clinical trial stage are derived from the scFv SS1 and conjugated to truncated forms of *Pseudomonas*
285 exotoxin A (PE) (**Figure 4A**). The mechanism of action of these iTOXs involves the impairment of
286 cellular functions, inducing cell death through apoptosis.⁸⁰ Following MSLN binding, the iTOX is

287 internalized through receptor-mediated endocytosis. During intracellular trafficking, the cleaved toxin
288 part induces the irreversible ADP ribosylation of elongation factor-2 (EF-2), which is crucial for protein
289 elongation during translation.

290 SS1P (SS1(dsFv)PE38 or CAT-5001), developed by the National Cancer Institute (NCI), is the
291 disulfide-stabilized SS1 fused to the truncated PE form known as PE38 (38 kDa).^{77,81} SS1P has been
292 evaluated in clinical trials both as monotherapy and in combination with bevacizumab, cisplatin,
293 carboplatin, cyclophosphamide, paclitaxel, pemetrexed and pentostatin, mainly in patients with MPM,
294 ovarian or pancreatic cancer (**Table 2**). Despite some evidence of anti-tumor effectiveness, reported
295 clinical activity has been modest and its development has faced critical challenges due to
296 immunogenicity with the development of neutralizing anti-drug antibodies (ADA) against PE and
297 toxicity.⁸²

298 To overcome the immunogenicity issues, the research shifted to a second generation of iTOX with
299 LMB-100. LMB-100 is a humanized anti-MSLN Fab fused to the deimmunized domain III of
300 *Pseudomonas* exotoxin A (24 kDa) to reduce immunogenicity.⁸¹ Nine clinical trials with LMB-100
301 alone or in combination with chemotherapy or ICIs have been reported, most of them in MPM (**Table 2**).
302 LMB-100 alone was found to be well tolerated with manageable side effects.^{83,84} However, despite a
303 lower immunogenicity than SS1P, it still faces the formation of ADA, limiting its long-term use in
304 patients.⁸⁵ According to the latest update from AdisInsight, its development has been discontinued for
305 solid tumors.

306 Interestingly, although most epithelioid MPMs express MUC16, the impact of its presence on the
307 therapeutic efficiency of iTOX that compete with MUC16 has not been thoroughly investigated.

308 As of March 2025, there is no evidence of ongoing clinical trials with SS1P or LMB-100
309 (**Figure 4B**).

310

311 ***ADCs: Anetumab ravtansine, DMOT4039A, BMS-986148, RC88, and SGN-MesoC2***

312 Antibody-drug conjugates (ADCs) combine the specificity of monoclonal antibodies with the
313 potency of cytotoxic payloads.⁸⁶

314 Anetumab ravtansine (BAY 94-9343 or AR) is an ADC developed by Bayer. It is a human anti-
315 mesothelin antibody that does not compete with MUC16. The antibody is conjugated to the
316 maytansinoid tubulin inhibitor DM4 via a non-cleavable disulfide-containing linker (**Figure 4A**).⁸⁷ To
317 date, 15 clinical trials have been conducted with AR, the first of which was initiated in 2011 (**Table 3**).⁸⁸
318 AR has been evaluated both as monotherapy and in combination with ICI (atezolizumab, bevacizumab,
319 ipilimumab, nivolumab, and pembrolizumab), chemotherapy (cisplatin, gemcitabine, pegylated
320 liposomal doxorubicin (PLD), pemetrexed), or fungicide (itraconazole). Regarding safety, clinical
321 studies have demonstrated that anetumab ravtansine exhibits a satisfactory level of tolerability and
322 favorable pharmacokinetics across multiple studies. Concerning its efficacy, there is evidence
323 supporting the clinical potential of AR in some MSLN-expressing tumors, with cases of complete
324 responses (CRs) and partial responses (PRs) reported.^{88,89} However, a direct comparison with other
325 treatments, such as vinorelbine, has not yet demonstrated the superiority of AR in terms of progression-
326 free survival (PFS) or overall survival (OS).^{88,89} While it has shown promise as part of combination
327 therapies in certain contexts, particularly in high mesothelin-expressing tumors, its clinical efficacy
328 remains inconsistent across indications.⁹⁰⁻⁹² Anetumab ravtansine is still in an active phase 2 clinical
329 trial, notably in combination with ICI.⁹⁰

330 DMOT4039A is an ADC developed by Genentech, a subsidiary of Roche. The structure of
331 DMOT4039A consists of a humanized anti-MSLN monoclonal antibody (h7D9.v3 or MMOT0530A),
332 which does not compete with MUC16 binding conjugated to the tubulin inhibitor monomethyl
333 auristatin E (MMAE) via a cathepsin-cleavable linker (**Figure 4A**).^{93,94} To date, two related phase 1
334 clinical trials have been conducted with DMOT4039A as monotherapy, focusing on pancreatic and
335 ovarian cancer (**Table 3**). The first study, initiated in 2011, demonstrated acceptable tolerability but
336 yielded disappointing results regarding clinical activity, particularly in patients with pancreatic cancer.⁹⁵
337 A confirmed partial response was observed in only 8% of patients, compared to 30% in ovarian cancer
338 patients.⁹⁵ The second one, a multicenter imaging study (NCT01832116) performed in parallel using
339 the same antibody conjugated to zirconium-89 showed a lower uptake in the pancreatic tumors than in
340 ovarian tumors, which may be due to the high level of fibrosis in the pancreatic microenvironment.⁹⁶
341 Since 2015, the development status of DMOT4039A remains unknown.

342 The ADC BMS-986148, a product of Bristol-Myers Squibb, is a fully human anti-MSLN IgG1
343 conjugated to a cytotoxic peptide, tubulysin, a tubulin polymerization inhibitor via a cleavable linker
344 (**Figure 4A**).⁹⁷ There is no information on the epitope targeted by BMS-986148. BMS-986148 has been
345 evaluated both in monotherapy and in combination with nivolumab in different indications (**Table 3**).
346 The phase 1 assay as monotherapy in advanced and/or metastatic solid tumors was completed in 2017
347 and the second trial was prematurely terminated. In both trials, BMS-986148 demonstrated a
348 manageable safety profile in patients with advanced tumors and a durable antitumor activity was
349 observed in some patients. Notably, treatment discontinuations were observed in both assays, primarily
350 due to treatment related adverse events (TRAEs).⁹⁷ The status of any ongoing research by Bristol-Myers
351 Squibb on this compound remains uncertain.

352 RC88, developed by RemeGen, consists of an anti-mesothelin antibody linked to MMAE via a
353 cleavable linker (**Figure 4A**).⁹⁸ No information regarding the targeted epitope on MSLN has been
354 disclosed. At present, five clinical trials are underway with RC88, either as monotherapy or in
355 combination with mAbs, including RC48 (anti-HER2), bevacizumab (anti-VEGF), and sintilimab (anti-
356 PD1) (**Table 3**). To date, there have been no published clinical trial data, apart from a poster presented
357 at the 2024 ASCO meeting that reported the tolerability and clinical efficacy of RC88 in patients with
358 mesothelin-expressing solid tumors.⁹⁹ Following the FDA's approval of the fast track designation for
359 RC88 in December 2023, a phase 2 clinical trial has been initiated to assess the efficacy of RC88 in
360 patients diagnosed with platinum-resistant recurrent epithelial ovarian, fallopian tube, and primary
361 peritoneal cancer.

362 SGN-MesoC2 (or PF-08052666) is an ADC initially developed by Harbour BioMed under the
363 name HBM9033 but now licensed by Pfizer. SGN-MesoC2, described as binding to membrane-bound
364 MSLN, comprises a camptothecin-based topoisomerase 1 inhibitor payload (SGD-12280) coupled to a
365 human IgG1 monoclonal antibody via a protease-cleavable linker (**Figure 4A**).¹⁰⁰ SGN-MesoC2 is the
366 only anti-MSLN ADC in clinical trials that does not use a microtubule inhibitor as payload.⁸⁶ The first
367 clinical trial started in 2024.

368

369 **TRT: BAY 2287411**

370 BAY 2287411 is a mesothelin-targeted thorium-227 conjugate developed by Bayer. The human
371 anti-mesothelin antibody (BAY 86-1903) is conjugated to the 3,2-HOPO chelator and radiolabeled with
372 the alpha emitter thorium-227 (**Figure 4A**).¹⁰¹ To date, only one clinical trial has been conducted with
373 BAY 2287411: a phase 1 monotherapy study that began in 2018 and ended in 2022 (**Table 4**). The
374 results of this study are not available. Preclinical studies have demonstrated that BAY 2287411 is well
375 tolerated in mice and in monkeys, induces a specific and significant antitumor activity, and activates
376 several immunostimulatory pathways while modulating the surface expression of various
377 immunomodulatory markers.¹⁰¹ Furthermore, the combination of BAY 2287411 with either ICIs or
378 DNA damage response inhibitors led to a significant potentiation of the antitumor activity of the
379 compound.^{101,102}

380

381 ***ADCC and ADCP mediators: Amatuximab and NI-1801***

382 Amatuximab (or Morab-009), developed by Morphotek, is a humanized chimeric IgG1 derived
383 from SS1, therefore competing with MUC16 (**Figure 4A**). Its mechanism of action includes inhibition
384 of the MSLN/MUC16 axis and ADCC/ADCP. Six clinical trials have been conducted with amatuximab
385 alone or in combination with perimetrexed and cisplatin (**Table 5**), in MPM and PDAC. However,
386 despite a good safety profile, clinical studies concluded that amatuximab cannot be regarded as an
387 effective treatment.^{103,104} Interestingly, based on the clinical trial NCT00738582, which did not achieve
388 its targeted PFS primary endpoint, Nicolaides *et al.* investigate the correlation between MUC16
389 expression levels and the efficacy of amatuximab-based treatments.^{105,106} The authors demonstrated that
390 amatuximab treatment induced a reversible increase in serum CA-125 levels due to the blockade of
391 MUC16–MSLN interactions. Furthermore, soluble CA-125 was shown to impair amatuximab-mediated
392 immune effector functions by binding to its (Fab')₂ domain, thereby disrupting its interactions with
393 CD16a (ADCC) and CD32a (ADCP) receptors.^{106,107} No major studies have been published since the
394 discontinued trial NCT02357147.

395 Amatuximab has also been investigated as an imaging agent. Two correlated phase 1 clinical trials
396 were conducted with ¹¹¹Indium-radiolabeled amatuximab in mesothelioma and PDAC patients.¹⁰⁸ The

397 efficacy of the agent in imaging tumor and metastatic sites was demonstrated, thus supporting its
398 potential application as a diagnostic tool.

399 NI-1801, developed by Light Chain Bioscience-NovImmune, is a bispecific human IgG1 $\kappa\lambda$ -body
400 targeting mesothelin and CD47 on tumors cells (**Figure 4A**). As described by the company, NI-1801 is
401 made of a common human IgG1 heavy chain and two light chains (one kappa and one lambda) with
402 distinct specificities. NI-1801 relies on ADCC and ADCP mechanisms, the ADCP process being
403 enhanced by the blocking of the CD47 ‘don’t eat me signal’. Based on the publication of Hatterer *et al.*,
404 it is likely that the MSLN arm targets a membrane-proximal epitope on MSLN, thus not competing with
405 MUC16.¹⁰⁹ A phase 1 study has started in 2022 with solid tumors.

406

407 ***Immunostimulators: ABBV-428 and AMG 994***

408 ABBV-428 and AMG 994, developed by AbbVie and Amgen, respectively, are bispecific
409 antibodies that exhibit bivalency toward mesothelin and CD40. ABBV-428 is a scFv-based antibody
410 composed of a silent human IgG1 Fc fused to anti-CD40 and anti-MSLN scFv at the N- and C-terminus,
411 respectively (**Figure 4A**).¹¹⁰ As described in the Amgen protocol 20190136, AMG 994, also known as
412 inezetamab, is an effector-silenced anti-CD40 IgG1 fused to an anti-MSLN scFv at the C-terminus.
413 There is no information about the targeted epitope on MSLN. The mechanism of action of both
414 molecules relies on the MSLN-dependent activation of CD40-expressing immune cells leading to an
415 enhanced priming and activation of cytotoxic T cells, activation of B lymphocytes, and reprogramming
416 of type 2 macrophages into type 1.^{110,111} Phase 1 clinical trials have been conducted to assess the efficacy
417 of both molecules in MSLN-positive solid tumors (**Table 6**). ABBV-428 evaluated as monotherapy
418 demonstrated acceptable tolerability, but only minimal clinical activity. No further development of this
419 molecule has been reported. Preliminary results suggest that AMG 994 demonstrates higher efficacy,
420 yet it concomitantly induces toxicity. However, the development of AMG 994 was discontinued
421 following a business decision.

422

423 ***T-Cell Engagers: HPN536, AMG 305, JNJ-79032421, ZW171, and CT-95***

424 HPN536, AMG 305, JNJ-79032421, ZW171, and CT-95 are all anti-MSLN / anti-CD3 TCEs able
425 to recruit and activate T cells against MSLN⁺ tumor cells (**Figure 4A**).

426 HPN536 is a trispecific T cell activating construct (TriTAC) developed by Harpoon Therapeutics,
427 a subsidiary of Merck.¹¹² It consists of a fusion of a humanized anti-MSLN llama single domain antibody
428 (sdAb), an anti-human serum albumin (HSA) sdAb for half-life extension through the FcRn loop, and a
429 humanized scFv anti-CD3 ϵ . The building blocks are connected by G₄SG₃S linkers.¹¹²

430 AMG 305, a trispecific antibody-like TCE directed against MSLN (monovalent), p-cadherin
431 (CDH3) (monovalent), and CD3 (bivalent), was developed by Amgen. This agent is a scFv-based
432 construct that requires binding to both CDH3 and MSLN for potent cytotoxic activity, CDH3 and MSLN
433 being co-expressed in multiple solid tumor types.¹¹³

434 JNJ-79032421 is a membrane restricted mesothelin targeting Fab fused to an anti-CD3 scFv
435 developed by Janssen, a Johnson & Johnson subsidiary.¹¹⁴ The precise structure of the compound
436 remains undisclosed.

437 ZW171 is a 2+1 scFv-Fab-based anti-MSLN (bivalent) and anti-CD3 (monovalent) TCE,
438 developed by Zymeworks.¹¹⁵

439 CT-95 is a 2+2 bispecific scFv-Fab-based TCE anti-MSLN (bivalent) and anti-CD3 (bivalent)
440 developed by Context Therapeutics. As described by the company, it has been engineered to target a
441 membrane proximal epitope on MSLN.

442 In cynomolgus monkeys, HPN536 demonstrated both good tolerability and MSLN-dependent
443 pharmacologic activity.¹¹² In cynomolgus monkeys, AMG 305 and ZW171 also demonstrated good
444 tolerability.¹¹³ In a CDH3⁺ MSLN⁺ xenograft tumor model, AMG 305 has been shown to demonstrate
445 *in vivo* antitumor activity that is dose dependent.¹¹³

446 To date, HPN536, AMG 305, JNJ-79032421, ZW171 and CT-95 are all involved in only one
447 monotherapy phase 1 study. The trials were initiated in 2019, 2023, 2024, 2024 and 2025, respectively.
448 The results of these studies are not yet available (**Table 7**).

449

450 **Adoptive Cell therapies targeting mesothelin**

451 Mesothelin-targeted cell therapies have made notable progress in recent years, with several
452 approaches progressing towards early-phase clinical trials. While the majority of adoptive cell therapies
453 targeting mesothelin are based on conventional $\alpha\beta$ T-cell engineering, in particular chimeric antigen
454 receptor (CAR)-T cells, the field is actively exploring other immune cell types (such as NK cells,
455 $\gamma\delta$ T cells, or macrophages) for CAR-based therapies, especially in the context of solid tumors.

456

457 *MSLN-targeting T cells*

458 The clinical research on anti-MSLN CAR-T cells was initiated in 2011 with the mRNA-transfected
459 SS1/CD3 ζ /4-1BB CAR-T cells developed by the University of Pennsylvania.¹¹⁶ Fifteen years later,
460 various strategies employing CAR-T cells have advanced to the clinical stage with currently 63 clinical
461 trials registered (**Table 8**).

462 Different strategies have been developed to improve MSLN CAR-T cells efficiency and to address
463 the immunosuppressive tumor microenvironment and CAR-T cell exhaustion, : i) CAR-T cells secreting
464 ICI, cytokine, or chemokine, ii) multispecific CAR-T cells, iii) logic gated CAR-T cells, iv) allogenic
465 CAR-T cells, and v) PD-1 KO CAR-T cells. Different routes of administration are also being evaluated
466 to improve tumor targeting and reduce systemic toxicity, notably regional delivery such as intrapleural
467 or intraperitoneal administration. In some cases, CAR-T cells have undergone modifications during their
468 development. For instance, M28z cells have been updated to M28z1XXPD1DNR, featuring a modified
469 CD3 ζ and a PD-1 dominant negative receptor. Similarly, α PD-1-mesoCAR-T cells, initially developed
470 to secrete anti-PD-1 antibody, have been re-engineered to secrete anti-PD-1 sdAb.¹¹⁷⁻¹¹⁹

471 Anti-mesothelin CAR-T trials are mainly in the early stages (phase I/II), to assess safety, tolerability
472 and early signs of efficacy. Data on the design and available results are limited. However, the results
473 published to date show acceptable tolerability and clinical responses in certain patients, notably in
474 malignant pleural mesothelioma using regional intrapleural delivery, particularly when combined with
475 pembrolizumab.¹¹⁷

476 Beyond the traditional CAR-T cell approach, alternative strategies for engineering T cells have also
477 been explored. These include: i) T cell receptor-engineered T cells (TCR-T cells), in which patient TCR
478 is genetically modified to recognize a mesothelin-specific peptide presented by a specific human

479 leukocyte antigen (HLA) molecule, typically HLA-A*02:01, as exemplified by the FH-TCR-T_{MSLN} cells
480 developed at the Fred Hutchinson Cancer Center, and ii) T cell receptor fusion construct (TRuC®)
481 T cells, which involve the fusion of an anti-mesothelin nanobody directly to the CD3ε subunit of the
482 native CD3/TCR complex.^{120,121} This platform has been tested both without (NCT03907852) and with
483 (NCT05451849) the co-expression of a chimeric switch receptor designed to convert inhibitory signals
484 from the tumor microenvironment, such as PD-L1, into stimulatory signals for the T cell.¹²¹

485 To date, a single clinical study has been conducted on CAR-γδT cells, and no data is yet available.

486

487

488 *Other immune cell types: CAR-PBMCs, CAR-NK cells, CAR-macrophages*

489 Since 2018, there has been an expansion of research interest on immune cell populations other than
490 T cells, which has led to the development of novel cell therapy approaches (**Table 8**).

491 A single clinical study has been conducted on CAR-PBMCs targeting MSLN. However, this study
492 was prematurely stopped due to a change in the sponsor's strategic priorities. Despite an acceptable
493 tolerability, the available data fail to provide compelling evidence of efficacy.¹²²

494 Two exploratory clinical studies have been conducted with autologous CAR-NK cells for
495 evaluation of safety issues and preliminary efficacy mainly in gynecological cancers. No results have
496 yet been published.

497 In addition, two clinical studies have been conducted on CAR-macrophages, with results only
498 available for SY001 cells in combination with tislelizumab (anti-PD-1). The study's findings revealed
499 that 100% of patients experienced grade ≥ 3 adverse events (AEs), and no responses to the treatment
500 were observed.¹²³

501

502

503 **CONCLUSION**

504 Mesothelin has been established as a compelling target due to its high expression in several
505 aggressive tumors and limited expression in healthy tissues. Over recent years, a broad range of

506 mesothelin-targeted therapies have progressed into clinical trials, with satisfactory safety profiles and
507 encouraging early signs of efficacy, notably in mesothelioma, with consistent objective radiologic
508 responses following administration of anti-mesothelin CAR-T cells.¹²⁴

509 However, several aspects remain insufficiently investigated, although they could open new avenues
510 to improve the efficacy of mesothelin-targeted therapies and provide better interpretations of current
511 clinical outcomes. The presence of circulating sMSLN has been identified as a potential limiting factor,
512 as it may act as a decoy for antibody-based therapies, thereby sequestering therapeutic agents and
513 reducing their bioavailability at the tumor cell surface. This “antigen sink” phenomenon is frequently
514 cited among the reasons for the heterogeneous results reported in clinical trials. However, its impact
515 remains poorly documented due to the inconsistent measurement, reporting, and consideration of
516 circulating mesothelin levels in clinical studies.

517 To mitigate the decoy effect of sMSLN, strategies targeting membrane-proximal epitopes have
518 been developed, including the engineering of bispecific antibodies designed to enhance tumor cell
519 selectivity and preserve functional binding despite the presence of soluble antigen.¹²⁵

520 It should be noted that the occurrence of soluble forms of membrane receptors in patient serum is
521 not exclusive to mesothelin. Soluble form of many TAAs have been detected, some of them (EGFR,
522 CEA, and HER2) at concentrations comparable to those of mesothelin.^{126–128} Immune checkpoint
523 receptors, including PD-1, PD-L1, and CTLA-4, are also present as circulating molecules.^{129,130} These
524 soluble factors can either hinder or enhance antitumor therapy efficacy depending on their
525 concentrations, context, and interactions within the TME.

526 Another important but often insufficiently documented aspect in clinical trial reports is the MUC16
527 status of enrolled patients. Beyond its role in contributing to local immunosuppression and affecting
528 therapeutic efficacy, MUC16 may modulate the retention of circulating mesothelin in tumors by
529 facilitating mesothelin rebinding to the surface of MUC16-expressing tumor cells, resulting in localized
530 mesothelin enrichment.^{34,131} Both membrane-bound and soluble forms of MUC16/CA125 can also
531 interfere with the antibody recognition of mesothelin, particularly for antibodies targeting the same
532 epitope as MUC16.

533 A better understanding of the biological and clinical implications of mesothelin, combined with the
534 systematic integration of circulating mesothelin measurement and MUC16 status in clinical trials, could
535 enhance the interpretation of results and enable more appropriate patient selection.

536 Several emerging research directions are being pursued to develop novel mesothelin-targeting
537 therapies addressing current limitations and aiming to enhance efficacy, safety, and durability.

538 Innovative approaches such as conditional mesothelin binding via dual or multi-targeting and
539 affinity tuning are currently evaluated.^{132–135} Moreover, although amatuximab alone is not effective, the
540 “theranostic strategy”, combining ⁸⁹Zr- and ¹⁷⁷Lu-labeled amatuximab has shown promise with *in vivo*
541 specific tumor accumulation in PANC-1 MSLN⁺ models and therapeutic efficacy with minimal side
542 effects.¹³⁶

543 In summary, mesothelin remains an attractive therapeutic target, offering considerable hope for the
544 treatment of aggressive cancers. Nonetheless, it continues to hold many unresolved questions, making
545 it a particularly stimulating field for future research.

546

547

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551

552

553 **AUTHOR CONTRIBUTIONS**

554 RB: Conceptualization; Literature search and data curation; Formal analysis; Visualization;
555 Writing - original draft; Writing - review & editing. PC: Validation. BK: Conceptualization; Formal
556 analysis; Writing - review & editing; Supervision; Validation.

557 All authors have given approval to the final version of the manuscript.

558

559

560 **DECLARATION OF INTERESTS**

561 The authors have declared that no competing interest exists.

562

563

564 **KEYWORDS**

565 Mesothelin; Cancer; Clinical trials; Targeted therapy; Immunotherapy.

566

567

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1201

1202 **Table 1.** MSLN-targeted vaccines in clinical trials. Discontinued clinical trials are shown in dark grey. *AE: Adverse Event, CFU: Colony Forming Units, CR: Complete Response, CY/GVAX:*
1203 *Cyclophosphamide + GVAX pancreas vaccine, GEJ: Gastroesophageal Junction, id.: intradermal, iv.: intravenous, MPM: Malignant Pleural Mesothelioma, MTD: Maximum Tolerated Dose,*
1204 *NE: Not Evaluated, NSCLC: Non-Small-Cell Lung Cancer, OR: Overall Response, OS: Overall Survival, PD: Progressive Disease, PDAC: Pancreatic Ductal Adenocarcinoma, PFS: Progression-*
1205 *Free Survival, po.: per os, PR: Partial Response, Q2W: once every 2 weeks, Q3W: once every 3 weeks, Q4W: once every 4 weeks, Q6W: once every 6 weeks, RP2D: Recommended Phase 2 Dose,*
1206 *SAE: Serious Adverse Event, sc.: subcutaneous, SD: Stable Disease, TRAE: Treatment Related Adverse Event.*

Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CRS-207	NCT05014776	II	Est. 2025-04-03	17	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins	Evaluation of safety and efficacy of CRS-207 in combination with pembrolizumab, ipilimumab, and tadalafil <i>-Despite the use of CRS-207 this clinical trial is not focused on MSLN-</i>	PDAC	Posology: CRS-207 1×10^9 CFU iv. on day 2 of cycles 1-6 (3-week cycles) + pembrolizumab 200 mg iv. on day 1 of cycles 1-6 + ipilimumab 50 mg iv. on day 1 of cycles 1, 3, and 5 + tadalafil 20 mg po. on days 3-21 of cycles 1-6. Safety: N/A Efficacy: N/A	137
	NCT03006302	II	2024-08-05	41	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins	Evaluation of safety, tolerability and efficacy of CRS-207 in combination with epacadostat and pembrolizumab with or without CY/GVAX <i>-Despite the use of CRS-207 this clinical trial is not focused on MSLN-</i>	Pancreatic	Posology: CRS-207 1×10^9 CFU iv. on day 2 of cycles 3-6 (3-week cycles). (For epacadostat, pembrolizumab, cyclophosphamide and GVAX pancreas vaccine details: NCT03006302) Safety (n=40): 40% of patients experienced SAEs. Efficacy (n=26): OS: 46% of patients survived more than 6 months.	
	NCT03190265	II	2022-08-23	61	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins	Evaluation of safety and efficacy of nivolumab and ipilimumab in combination with either sequential administration of CY/GVAX followed by CRS-207 or with administration of CRS-207 alone <i>-Despite the use of CRS-207 this clinical trial is not focused on MSLN-</i>	Pancreatic	Posology: CRS-207 1×10^9 CFU iv. on day 2 of cycles 3-6 (3-week cycles). (For nivolumab, ipilimumab, cyclophosphamide and GVAX pancreas vaccine details: NCT03190265) Safety: Without CY/GVAX, 78% of patients experienced SAEs and 37% experienced grade ≥ 3 TRAEs. With CY/GVAX, 80% of patients experienced SAEs and 33% experienced grade ≥ 3 TRAEs. Efficacy nivolumab + ipilimumab + CRS-207 (n=30): OR: CR + PR 7%. Efficacy nivolumab + ipilimumab + CY/GVAX + CRS-207 (n=31): OR: CR + PR 0%.	
	NCT03122548	II	2018-01-31 (terminated due to low enrollment and lack of clinical activity in other CRS-207 studies)	5	Aduro Biotech	Evaluation of safety and efficacy of CRS-207 in combination with pembrolizumab	Gastric, GEJ and Esophageal	Posology: CRS-207 1×10^9 CFU iv. on day 2 of cycle 1 (3-week cycles), day 1 of cycles 2-4 and then on day 1 every 6 weeks + pembrolizumab 200 mg iv. on day 1 of each 3-week cycle. Safety (n=5): 80% of patients experienced SAEs. Efficacy (n=5): OS (n=5): 2.5 months. PFS (n=5): 1.2 months. OR (n=3): CR 0%, PR 0%, SD 0% and PD 100%.	138
	NCT03175172	II	2018-01-31 (terminated due to low enrollment and lack of clinical activity)	10	Aduro Biotech	Evaluation of safety and efficacy of CRS-207 in combination with pembrolizumab <i>-Despite the use of CRS-207 this clinical trial is not focused on MSLN-</i>	MPM	Posology: CRS-207 1×10^9 CFU iv. on day 2 (3-week cycles) + pembrolizumab 200 mg iv. on day 1. If tolerated, pembrolizumab and CRS-207 were administered on the same day for subsequent cycles. After 4 cycles, pembrolizumab was administered on day 1 every 3 weeks and CRS-207 once every 6 weeks. Safety (n=10): 40% of patients experienced SAEs. Efficacy (n=10): OS (n=10): 2.8 months. PFS (n=10): 1.4 months. OR (n=9): CR 0%, PR 10%, SD 11% and PD 89%.	72

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Drug name	NCT number	Phase	Completion date	Enrolment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CRS-207	NCT02243371	II	2017-07-21	93	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins	Evaluation of safety and efficacy of CY/GVAX followed by CRS-207 with or without nivolumab <i>-Despite the use of CRS-207 this clinical trial is not focused on MSLN-</i>	Pancreatic	Posology: CRS-207 1×10^9 CFU iv. on day 2 of cycles 3-6 (3-week cycles) + cyclophosphamide 200 mg/m ² iv. on day 1 of cycles 1 and 2 + GVAX 5×10^8 cells in 6 id. injections on day 2 of cycles 1 and 2, w/ or w/o nivolumab 3 mg/kg iv. on day 1 of cycles 1-6. Safety (n=93): 12% of patients treated with CY/GVAX + CRS-207 experienced grade ≥ 3 TRAEs (n=42). 35% of patients treated with CY/GVAX + CRS-207 + nivolumab experienced grade ≥ 3 TRAEs (n=51). Efficacy CY/GVAX + CRS-207 (n=42): OS: 6.1 months. PFS: 2.2 months. OR: CR 0%, PR 2%, SD 7% and PD 67%. Efficacy CY/GVAX + CRS-207 + nivolumab (n=51): OS: 5.9 months. PFS: 2.2 months. OR: CR 0%, PR 2%, SD 12% and PD 69%.	73
	NCT01417000	II	2017-02-10	93	Aduro Biotech	Evaluation of safety and efficacy of CY/GVAX in combination with CRS-207 and CY/GVAX alone	PDAC	Posology: In CY/GVAX alone condition, the treatment was on weeks 1, 4, 7, 10, 13, 16. In combination condition, CY 200 mg/m ² iv. on day 1 of weeks 1 and 4 + GVAX 1×10^8 cells id. on day 2 of weeks 1 and 4 + CRS-207 1×10^9 CFU iv. on day 1 of weeks 7, 10, 13, 16. Safety: In CY/GVAX alone condition, 4 grade ≥ 3 AEs occurred (n=29). In combination condition, 29 grade ≥ 3 AEs occurred (n=61). Efficacy CY/GVAX alone (n=29): OS: 3.9 months. OR: SD 24%. Efficacy CY/GVAX + CRS-207 (n=61): OS: 6.1 months. OR: SD 31%.	139
	NCT02004262	II	2016-08-23	303	Aduro Biotech	Evaluation of safety and efficacy of CRS-207 in combination with CY/GVAX vs. CRS-207 alone vs. single-agent chemotherapy <i>-Despite the use of CRS-207 this clinical trial is not focused on MSLN-</i>	Pancreatic	Posology: CRS-207 1×10^9 CFU iv. on day 1 of weeks 7, 10, 13 and 16. (For cyclophosphamide, GVAX pancreas vaccine, gemcitabine, capecitabine, fluorouracil, leucovorin, irinotecan and erlotinib details: NCT02004262) Safety (n=169): In the below order of treatment conditions, 47%, 37% and 28% of patients experienced SAEs. Most frequently reported AEs in all treatment groups were chills, pyrexia, fatigue, and nausea. No treatment-related deaths occurred. Efficacy single-agent chemotherapy (n=43, primary cohort): OS: 4.7 months. PFS: 2.1 months. Efficacy CRS-207 alone (n=58, primary cohort): OS: 5.2 months. PFS: 2.1 months. Efficacy CRS-207 + CY/GVAX (n=68, primary cohort): OS: 4.2 months. PFS: 2.3 months.	140

Drug name	NCT number	Phase	Completion date	Enrolment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CRS-207	NCT02575807	I / II	2018-05-08 (terminated due to low enrollment and lack of clinical activity)	35	Aduro Biotech	Evaluation of safety and efficacy of CRS-207 alone and in combination with epacadostat and/or pembolizumab <i>-Despite the use of CRS-207 this clinical trial is not focused on MSLN-</i>	Ovarian, Fallopian and Peritoneal	Posology: CRS-207 1×10^9 CFU iv. Q3W for 6 cycles, then Q6W (For epacadostat, and pembrolizumab details: NCT02575807) Safety (n=32): In combination with CRS-207, epacadostat RP2D was determined as 300 mg po. All patients included, 44% experienced SAEs. Efficacy epacadostat 300 mg (n=28): OS (n=28): 2.1 (CRS-207 + epacadostat + pembolizumab, n=1) to 11.3 months (CRS-207 alone, n=8). PFS (n=28): 1.2 (CRS-207 + epacadostat + pembolizumab, n=1) to 1.8 months (CRS-207 alone and CRS-207 + epacadostat, n=8 and n=16). OR (n=24): All patients combined the best response was SD (SD 31% with CRS-207 + epacadostat, n=13).	
	NCT01675765	I	2019-08-19	60	Aduro Biotech	Evaluation of safety and efficacy of CRS-207 in combination with pemetrexed and cisplatin w/ or w/o cyclophosphamide	MPM	Posology: CRS-207 1×10^9 CFU on weeks 1, 3, 23, 26 and every 8 weeks from week 34 + pemetrexed 500 mg/m ² and cisplatin 75 mg/m ² on weeks 5, 8, 11, 14, 17 and 20 (up to 6 3-week cycles), w/ or w/o cyclophosphamide 200 mg/m ² on weeks 1, 3, 23 and 26. Safety (n=60): 39% of patients treated with CRS-207 + pemetrexed + cisplatin experienced SAEs (n=38). 50% of patients treated with CRS-207 + pemetrexed + cisplatin + cyclophosphamide experienced SAEs (n=22). Efficacy CRS-207 + pemetrexed + cisplatin (n=21): OR: CR 3%, PR 53%, SD 14%, PD 3% and NE 3%. Efficacy CRS-207 + pemetrexed + cisplatin + cyclophosphamide (n=36): OR: CR 0%, PR 52%, SD 38% and PD 10%.	141
	NCT00585845	I	2009-02-01	17	Anza Therapeutics	Evaluation of safety, tolerability and pharmacokinetics of CRS-207	Solid	Posology: CRS-207 1×10^8 to 1×10^{10} CFU iv. up to 4 administrations every 3 weeks. Safety (n=17): MTD was determined to be 1×10^9 CFU. At the MTD, 100% of patients experienced grade 4 lymphopenia, 33% experienced grade 3 hypophosphatemia and 17% experienced grade 3 elevated gamma-glutamyl transferase (n=6). Efficacy CRS-207 1×10^9 CFU (n=6): OS: 33% of patients survived more than 15 months. OR: CR 0%, PR 0%, SD 33% and PD 67%.	70
JNJ-757 (JNJ-64041757)	NCT03371381	I / II	2018-10-09 (terminated due to lack of clinical benefit)	12	Janssen - Johnson & Johnson	Evaluation of safety, pharmacokinetics and efficacy of JNJ-757 in combination with nivolumab	NSCLC	Posology: JNJ-757 1×10^9 CFU iv. Q4W + nivolumab 240 mg iv. Q2W. Safety (n=12): 50% of patients experienced grade ≥ 3 AEs, including two cases of treatment-related fatal pneumonitis. Efficacy (n=11): OR: CR 0%, PR 0%, SD 36%, PD 45% and NE 18%.	74
	NCT02592967	I	2018-10-22 (terminated due to lack of efficacy)	18	Janssen - Johnson & Johnson	Evaluation of safety, pharmacokinetics and efficacy of JNJ-757	NSCLC	Posology: JNJ-757 1×10^8 or 1×10^9 CFU iv. Q3W. Safety (n=18): 67% of patients experienced grade ≥ 3 AEs. Efficacy (n=16): OR 1×10^8 CFU (n=5): CR 0%, PR 0%, SD 80% and PD 20%. OR 1×10^9 CFU (n=11): CR 0%, PR 0%, SD 36% and PD 64%.	74

Drug name	NCT number	Phase	Completion date	Enrolment	Sponsor	Study	Tumor type	Study outcomes	Ref.
Neoantigen DNA vaccine	NCT03122106	I	2022-08-13 (terminated due to loss of funding)	15	Washington University School of Medicine	Evaluation of safety and efficacy of neoantigen DNA vaccine	Pancreatic	<p>Posology: 2 injections (1 into each deltoid or lateralis) on days 1, 5, 9, 13, 17 and 21.</p> <p>Safety (n=11): No patients experienced SAE. No grade \geq 3 AEs were reported.</p> <p>Efficacy (n=9): 100% of patients analyzed by ELISPOT experienced a neoantigen-specific immune response.</p>	75
Neoantigen peptide vaccine	NCT03956056	I	2023-07-06 (terminated due to insufficient funding/staff)	12	Washington University School of Medicine	Evaluation of safety and efficacy of neoantigen peptide vaccine in combination with poly-ICLC	Pancreatic	<p>Posology: Neoantigen peptide vaccine + poly-ICLC on days 1, 4, 8, 15, 22, 50 and 78 sc.</p> <p>Safety (n=10): 10% of patients experienced SAEs.</p> <p>Efficacy (n=9): 100% of patients analyzed by ELISPOT experienced a neoantigen-specific immune response.</p>	75

1214 **Table 2.** MSLN-targeted antibody-based therapies in clinical trials. Discontinued clinical trials are shown in dark grey. *ADCC: ADCP: AE: Adverse Event, AR: Anetumab ravtansine, CHO:*
1215 *Cholangiocarcinoma, CR: Complete Response, DLT: Dose Limiting Toxicity, EOC: Epithelial Ovarian Cancer, FDA: Food and Drug Administration, HGOC: High Grade Ovarian Cancer, itu.:*
1216 *intratumoral, iv.: intravenous, MPM: Malignant Pleural Mesothelioma, MTD: Maximum Tolerated Dose, NCI: National Cancer Institute, NE: Not Evaluated, NSCLC: Non-Small-Cell Lung*
1217 *Cancer, OR: Overall Response, OS: Overall Survival, PD: Progressive Disease, PDAC: Pancreatic Ductal Adenocarcinoma, PFS: Progression-Free Survival, PLD: Pegylated Liposomal*
1218 *Doxorubicin, po.: per os, PR: Partial Response, QW: once weekly, Q2W: once every 2 weeks, Q3W: once every 3 weeks, Q6W: once every 6 weeks, QOD.: every other day, RP2D: Recommended*
1219 *Phase 2 Dose, SAE: Serious Adverse Event, SD: Stable Disease, TEAE: Treatment Emergent Adverse Event, TESAE: Treatment Emergent Serious Adverse Event, TNBC: Triple Negative Breast*
1220 *Cancer, TRAE: Treatment Related Adverse Event, TRSAE: Treatment Related Serious Adverse Event, TTP: Time To Progression.*

Drug Type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
Immunotoxin	SS1P (SS1(dsFv)PE38 or CAT-5001)	NCT01362790	I / II	2017-08-07	55	National Cancer Institute	Evaluation of safety, tolerability and efficacy of SS1P in combination with pentostatin and cyclophosphamide	Mesothelioma, Pancreatic and Lung adenocarcinoma	Posology: SS1P 35 µg/kg iv. on days 10, 12, and 14 for 1 cycle then on days 2, 4, and 6 for a maximum of 6 cycles + pentostatin + cyclophosphamide (regimen A). SS1P 35 µg/kg iv. on days 18, 20, and 22 for 1 cycle then on days 6, 8, and 10 for a maximum of 6 cycles + pentostatin + cyclophosphamide (regimen B). (For pentostatin and cyclophosphamide details: NCT01362790) Safety depending on the regimen (n=53): At 35 µg/kg, 25% to 100% patients experienced SAEs (n=45). No SS1P-related grade 4 AEs reported. MTD was determined to be 25 µg/kg (n=8). Efficacy depending on the regimen (n=45): OS: 4.2 - 29.3 months. PFS: 3.9 - 11.8 months. OR: CR 0%, PR 5%, SD 5%, PD 7% and NE 82%.	142
		NCT01445392	I	2016-10-03	24	National Cancer Institute	Evaluation of safety, tolerability, pharmacokinetics and efficacy of SS1P in combination with pemetrexed and cisplatin	MPM	Posology: SS1P 25 to 55 µg/kg iv. on days 1, 3, and 5 for 2 cycles (3-week cycle) + pemetrexed 500 mg/m ² iv. on day 1 for up to 6 cycles + cisplatin 75 mg/m ² iv. on day 1 for up to 6 cycles. Safety: MTD was determined to be 45 µg/kg. 100% of patients experienced AEs. No SS1P-related grade 4 AEs. Efficacy (n=24): OS: 13.6 months. PFS: 6.0 months. OR: CR 0%, PR 50%, SD 13%, PD 21% and NE 17%. Efficacy SS1P 45 µg/mg (n=13): OR: CR 0%, PR 77%, SD 8% and PD 15%.	143
		NCT01051934	I	2011-09-28	2	National Cancer Institute	Evaluation of safety, tolerability, and pharmacokinetics of SS1P in combination with paclitaxel, carboplatin and bevacizumab	NSCLC	Posology: N/A Safety: N/A Efficacy: N/A	
		NCT00006981	I	N/A (publication 2009)	24	National Cancer Institute	Evaluation of safety, tolerability, pharmacokinetics and efficacy of SS1P	Mesothelioma, Ovarian and Pancreatic	Posology: 10 injections of SS1P 4 to 25 µg/kg/day iv. Safety (n=24): MTD was determined to be 25 µg/kg/day. All patients experienced at least 1 AE. 13% of patients experienced grade ≥ 3 AEs. Efficacy (n=24): OR: CR 0%, PR 4%, SD 50% and PD 46%.	82
		NCT00066651	I	N/A (publication 2007)	34	National Cancer Institute	Evaluation of safety, tolerability, pharmacokinetics and efficacy of SS1P	Mesothelioma, Ovarian and Pancreatic	Posology: With 6 injections QOD of SS1P: 8 to 25 µg/kg iv. With 3 injections QOD of SS1P: 25 to 60 µg/kg iv. Safety (n=34): MTD with 6 injections was determined to be 18 µg/kg/dose (n=17) and with 3 injections to be 45 µg/kg/dose (n=17). SS1P is well-tolerated with pleuritis as the DLT at the highest dose level. Efficacy (n=34): OR: CR 0%, PR 12%, SD 56%, PD 29% and NE 3%.	144

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Drug Type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
Immunotoxin	SS1P (SS1(dsFv)PE38 or CAT-5001)	NCT00024674	I	2001-02-01 (withdrawn, continued by NCI NCT00006981)	0	INSYS Therapeutics	Evaluation of safety and efficacy of SS1P	N/A	Posology: 10-day continuous iv. Safety: N/A Efficacy: N/A	
		NCT00024687	I	2000-11-01 (withdrawn, continued by NCI NCT00006981)	0	INSYS Therapeutics	Evaluation of safety, tolerability, pharmacokinetics and efficacy of SS1P	N/A	Posology: 6 injections iv. QOD. Safety: N/A Efficacy: N/A	
	LMB-100 (RG7787 or RO6927005)	NCT04027946	II	2023-12-22 (terminated due to slow accrual)	6	National Cancer Institute	Evaluation of efficacy of LMB-100 followed by pembrolizumab	NSCLC	Posology: LMB-100 140 µg/kg on days 1, 3 and 5 of 3-week cycles (up to 2) + 200 mg of pembrolizumab on day 1 of each subsequent 3-week cycles. Safety (n=3): 0% of patients experienced grade ≥ 3 TRAEs. 100% of patients analyzed experienced SAEs. Efficacy (n=3): OS: 22.9 months. PFS: 3 months. OR: CR 0%, PR 0%, SD 67% and PD 33%.	83
		NCT03644550	II	2020-11-02 (terminated due to principal investigator decision after FDA's approval of nivolumab + ipilimumab as first line treatment for mesothelioma)	18	National Cancer Institute	Evaluation of safety and efficacy of LMB-100 followed by pembrolizumab	Mesothelioma	Posology: LMB-100 140 µg/kg iv. on days 1, 3 and 5 of two 3-week cycles + pembrolizumab 200 mg iv. Q3W from cycle 3. Safety (n=18): 78% of patients experienced SAEs. Efficacy (n=18): OS (n=18): 17.1 months. PFS (n=18): 7.1 months. OR (n=17): PR 18%.	83
		NCT02810418	I/II	2021-06-22	40	National Cancer Institute	Evaluation of safety, tolerability and efficacy of LMB-100 alone and in combination with nab-paclitaxel	PDAC	Posology: LMB-100 65 or 100 µg/kg iv. on days 1, 3, and 5 of 3-week cycles for 1–3 cycles w/ or w/o nab-paclitaxel 125 mg/m ² iv. on days 1 and 8. Safety (n=20): With nab-paclitaxel, LMB-100 MTD was determined to be 65 µg/kg. Most common AEs were hypoalbuminemia (100%), edema (65%), fatigue (50%), hyponatremia (50%), and myalgia (45%). Efficacy LMB-100 65 µg/kg + nab-paclitaxel 125mg/m² (n=14): OS: 5.3 months. OR: PR 7%. Although clinical activity was observed, the combination was not well tolerated.	84
		NCT05375825	I	2024-01-31 (withdrawn)	0	National Cancer Institute	Evaluation of safety, tolerability and pharmacokinetics of LMB-100	MPM	Posology: LMB-100 1 mg/mL intrathoracic perfusion using a closed circuit and roller pump with a heat exchanger following maximal cytoreductive surgery. Safety: N/A Efficacy: N/A	
		NCT02798536	I	2022-04-21	21	National Cancer Institute	Evaluation of safety, tolerability, pharmacokinetics and efficacy of LMB-100 alone and in combination with nab-paclitaxel	Mesothelioma	Posology: LMB-100 100 to 170 µg iv. on days 1, 3 and 5 of each 3-week cycle (up to 2-4), w/ or w/o nab-paclitaxel 125 mg/m ² iv. on days 1 and 8 of each 3-week cycle (up to 6). Safety (n=21): LMB-100 alone RP2D was determined to be 140 µg (n=10). In combination with nab-paclitaxel, LMB-100 RP2D was determined to be 100 µg (n=11). 0% of patients treated with LMB-100 at 140 µg experienced SAEs (n=7). 33% of patients treated with LMB-100 at 170 µg experienced SAEs (n=3). 36% of patients treated with LMB-100 at 100µg + nab-paclitaxel experienced SAEs (n=11). Efficacy LMB-100 140 µg (n=7): OS: 16.9 months. PFS: 2.7 months. OR: CR 0% and PR 0%. Efficacy LMB-100 170 µg (n=3): OS: 33.5 months. PFS: 2.7 months. Efficacy LMB-100 100 µg + nab-paclitaxel (n=11): OS: 10.1 months. PFS: 2.7 months. OR: CR 0% and PR 0%.	84

Drug Type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
Immunotoxin	LMB-100 (RG7787 or RO6927005)	NCT04840615	I	2022-01-12 (terminated due to slow accrual)	2	National Cancer Institute	Evaluation of feasibility and safety of LMB-100 intratumoral injections in combination with ipilimumab	Mesothelioma	Posology: LMB-100 400 µg itu. + ipilimumab itu. on days 1 and 4 each 3-week cycle (up to 4). Safety (n=2): 100% of patients experienced SAEs. Efficacy (n=2): OS: 3.4 months. PFS: 1.4 months. OR: CR 0% and PR 0%.	
		NCT04034238	I	2021-11-19	19	National Cancer Institute	Evaluation of safety, tolerability, pharmacokinetics and efficacy of LMB-100 in combination with tofacitinib	Mesothelioma, CHO and PDAC	Posology: LMB-100 65 to 140 µg/kg iv. on days 4, 6 and 8 each 3-week cycles + tofacitinib 10 mg po. twice daily on days 1-10. Safety (n=10): With tofacitinib, LMB-100 MTD was determined to be 100 µg/kg on days 4, 6 and 8. At the MTD, 14% of patients experienced SAEs that may be related to LMB-100 (n=7). Efficacy: N/A	145
	LMB-100 (RG7787 or RO6927005)	NCT03436732	I	2019-04-30 (terminated due to fatal occurrence attributed to one of the study drugs)	5	National Cancer Institute	Evaluation of safety and tolerability of LMB-100 in combination with SEL-110	Mesothelioma	Posology: LMB-100 100 or 140 µg/kg on days 1, 3 and 5 each 3-week cycles (up to 4) + SEL-110 on day 1 of each cycle at escalating doses. Safety (n=5): The study was closed due to a fatal occurrence of pneumonitis attributed to one of the drugs. Efficacy (n=5): OR: CR 0% and PR 0%.	
		NCT02317419	I	2015-08-01 (terminated due to the emerging benefit:risk ratio that did not justify continuing dosing patients)	15	Roche	Evaluation of safety, tolerability, pharmacokinetics and efficacy of LMB-100 alone and in combination with gemcitabine and nab-paclitaxel	Solid	Posology: LMB-100 iv. on days 1, 3, and 5 of each 3-4-week cycles, w/ or w/o gemcitabine + nab-paclitaxel on days 1, 8, and 15 of each 4-week cycles. Safety: N/A Efficacy: N/A	84
Antibody-drug conjugate	Anatumab ravtansine (BAY 94-9343)	NCT03587311	II	Estimated 2025-10-21	96	National Cancer Institute	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AR in combination with bevacizumab vs. paclitaxel in combination bevacizumab	HGOC	Data cut-off date: 2024-11-27 Posology: AR 2.2 mg/kg iv. QW + bevacizumab 10 mg/kg iv. Q2W. Paclitaxel 80 mg/m ² iv. QW + bevacizumab 10 mg/kg iv. Q2W. Safety (n=57): With bevacizumab, AR RP2D was determined to be 2.2 mg/kg. 26 grade ≥ 3 TRAEs were reported with paclitaxel + bevacizumab (n=29). 29 grade ≥ 3 TRAEs were reported with AR + bevacizumab (n=28) Efficacy paclitaxel + bevacizumab (n=29): PFS: 12.7 months. OR: CR 0%, PR 66%, SD 20% and PD 14%. Efficacy AR + bevacizumab (n=28): PFS: 5.3 months. OR: CR 4%, PR 18%, SD 50% and PD 28%.	90
		NCT03926143	II	2022-05-18 (terminated due to strategic company decisions)	9	Bayer	Evaluation of long-term safety and efficacy of AR alone and in combination with gemcitabine	Solid	Posology: N/A Safety (n=9): 100% of patients experienced TEAEs. 89% of patients experienced TRAEs. 22% of patients experienced TESAEs. 0% of patients experienced TRSAEs Efficacy (n=9): OS: 34.1 months.	

Drug Type	Drug name	NCT number	Phase	Completion date	Enrolment	Sponsor	Study	Tumor type	Study outcomes	Ref.
Antibody-drug conjugate	Anetumab ravtansine (BAY 94-9343)	NCT03587311	II	Estimated 2025-10-21	96	National Cancer Institute	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AR in combination with bevacizumab vs. paclitaxel in combination bevacizumab	HGOC	<p>Data cut-off date: 2024-11-27</p> <p>Posology: AR 2.2 mg/kg iv. QW + bevacizumab 10 mg/kg iv. Q2W. Paclitaxel 80 mg/m² iv. QW + bevacizumab 10 mg/kg iv. Q2W.</p> <p>Safety (n=57): With bevacizumab, AR RP2D was determined to be 2.2 mg/kg. 26 grade ≥ 3 TRAEs were reported with paclitaxel + bevacizumab (n=29). 29 grade ≥ 3 TRAEs were reported with AR + bevacizumab (n=28)</p> <p>Efficacy paclitaxel + bevacizumab (n=29): PFS: 12.7 months. OR: CR 0%, PR 66%, SD 20% and PD 14%.</p> <p>Efficacy AR + bevacizumab (n=28): PFS: 5.3 months. OR: CR 4%, PR 18%, SD 50% and PD 28%.</p>	90
		NCT03926143	II	2022-05-18 (terminated due to strategic company decisions)	9	Bayer	Evaluation of long-term safety and efficacy of AR alone and in combination with gemcitabine	Solid	<p>Posology: N/A</p> <p>Safety (n=9): 100% of patients experienced TEAEs. 89% of patients experienced TRAEs. 22% of patients experienced TESAEs. 0% of patients experienced TRSAEs</p> <p>Efficacy (n=9): OS: 34.1 months.</p>	
		NCT03023722	II	2019-12-11	18	Yale University	Evaluation of safety and efficacy of AR	Pancreatic	<p>Posology: AR 6.5 mg/kg iv. Q3W.</p> <p>Safety (n=18): 61% of patients experienced SAEs.</p> <p>Efficacy (n=14): TTP: 2.1 months. OR: CR 0%, PR 0%, SD 14% and PD 86%.</p>	146
		NCT02610140	II	2019-09-06	248	Bayer	Evaluation of safety, pharmacokinetics and efficacy of AR vs. vinorelbine	MPM	<p>Posology: AR 6.5 mg/kg Q3W. Vinorelbine 30 mg/m² Q3W.</p> <p>Safety (n=235): 74% of patients treated with vinorelbine experienced grade ≥ 3 AEs and 15% experienced TESAEs (n=72). 48% of patients treated with AR experienced grade ≥ 3 AEs and 7% experienced TESAEs (n=163).</p> <p>Efficacy vinorelbine (n=82): OS (n=82): 11.6 months. PFS (n=82): 4.5 months. OR (n=65): CR 0%, PR 9%, SD 77% and PD 14%.</p> <p>Efficacy AR (n=166): OS (n=166): 9.5 months. PFS (n=166): 4.3 months. OR (n=146): CR 0%, PR 11%, SD 73% and PD 16%.</p>	89
		NCT02839681	II	2018-07-25 (terminated due to slow / insufficient accrual)	2	National Cancer Institute	Evaluation of safety, tolerability and efficacy of AR	Lung	<p>Posology: AR iv. Q3W.</p> <p>Safety (n=2): 50% of patients experienced a SAE (respiratory failure).</p> <p>Efficacy (n=2): OS: 1.7 months. PFS: 1.3 months. OR: CR 0% and PR 0%.</p>	
		NCT03126630	I / II	Estimated 2025-09-19	49	National Cancer Institute	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AR in combination with pembrolizumab	MPM	<p>Data cut-off date: 2024-08-08</p> <p>Posology: Pembrolizumab 200 mg iv. w/ or w/o AR 6.5 mg/kg iv. Q3W. (up to 1-2 years).</p> <p>Safety (n=35): 53% of patients treated with pembrolizumab alone experienced grade ≥ 3 AEs (n=17) and 61% of patients treated with AR + pembrolizumab experienced grade ≥ 3 AEs (n=18).</p> <p>Efficacy pembrolizumab alone (n=17): PFS: 3.9 months. OR: CR 0%, PR 6%, SD 29%, PD 53% and NE 12%.</p> <p>Efficacy AR + pembrolizumab (n=18): PFS: 12.2 months. OR: CR 0%, PR 17%, SD 50%, PD 20% and NE 13%.</p>	91

Drug Type	Drug name	NCT number	Phase	Completion date	Enrolment	Sponsor	Study	Tumor type	Study outcomes	Ref.
Antibody-drug conjugate	Anetumab ravtansil	NCT03455556	I / II	2020-01-07 (terminated due to slow accrual)	1	Mayo Clinic	Evaluation of safety, tolerability and efficacy of AR in combination with atezolizumab	NSCLC	Posology: AR iv. + atezolizumab iv. Q3W. Safety: N/A Efficacy: N/A	88
	Anetumab ravtansil (BAY 94-9343)	NCT03816358	I	Estimated 2025-01-31	74	National Cancer Institute	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AR in combination with nivolumab, w/ or w/o ipilimumab or gemcitabine	PDAC	Data cut-off date: 2022-01-22 Posology: AR 5.5 or 6.5 mg/kg iv. on day 1 (3 to 4-week cycles) + nivolumab iv. on day 8 of cycle 1 and on day 1 of subsequent cycles (up to 17), w/ or w/o ipilimumab iv. on day 8 of cycle 1 and on day 1 of cycles 2-4 or gemcitabine on days 1 and 8. Safety (n=28): 2 DLTs were reported for AR 6.5 mg/kg + nivolumab + ipilimumab. RP2D was determined to be 6.5 mg/kg. At 6.5 mg/kg, 5, 17 and 20% of patients experienced TRAEs, for AR + nivolumab, AR + nivolumab + ipilimumab, AR + nivolumab + gemcitabine, respectively. Efficacy (n=33): Following data correspond to AR + nivolumab (n=11), AR + nivolumab + ipilimumab (n=13), AR + nivolumab + gemcitabine (n=9). OR: CR 0%, PR 0%, SD 18, 15, 89%, PD 64, 46, 0% and NE 18, 38, 11%.	147
		NCT03102320	I	2021-07-26	173	Bayer	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AR alone and in combination with cisplatin or gemcitabine	Gastric, NSCLC, TNBC, Thymic and PDAC	Posology: AR 6.5 mg/kg iv. on day 1 (3-week cycle), w/ or w/o cisplatin 25 mg/m ² iv. or gemcitabine 1000 mg/m ² on day 1 and 8 (up to 6 cycles). Safety: N/A Efficacy: N/A	148
		NCT02751918	I	2019-10-31	65	Bayer	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AR in combination with PLD	Ovarian, Fallopian and Peritoneal	Posology: AR 5.5 or 6.5 mg/kg iv. + PLD 30 mg/m ² iv. Q3W. Safety (n=65): With PLD, AR MTD was determined to be 6.5 mg/kg (n=9). No DLTs reported. More than 10% of patients experienced grade ≥ 3 AEs, fatigue (12%), neutrophil count decrease (11%) and neutropenia (11%) (n=65). Efficacy (n=65): PFS: 5.0 months. OR: CR 2%, PR 26%, SD 43%, PD 17% and NE 12%. High MSLN expression increases treatment efficacy.	92
		NCT02639091	I	2019-10-17	36	Bayer	Evaluation of safety, tolerability and pharmacokinetics of AR in combination with pemetrexed and cisplatin	Mesothelioma and NSCLC	Data cut-off date: 2017-06-12 Posology: AR 5.5 or 6.5 mg/kg iv. + pemetrexed 500 mg/m ² iv. + cisplatin 75 mg/m ² iv. Q3W. Safety (n=25): With pemetrexed + cisplatin, AR MTD was determined to be 6.5 mg/kg. 2 grade 3 AEs occurred (corneal microcystic and hypertension). Efficacy (n=17): OR (n=17): PR 47% and NE 6%. OR with AR 6.5 mg/kg (n=13): PR 46%.	149
		NCT02696642	I	2019-08-19	54	Bayer	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AR	Solid	Posology: AR 6.5 mg/kg iv. Q3W. Safety: N/A Efficacy: N/A	
		NCT02824042	I	2019-08-05	63	Bayer	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AR alone and in combination with itraconazole	Solid	Posology: AR iv. + atezolizumab iv. on day 1 (3-week cycles), w/ or w/o itraconazole 200 mg po. twice daily on day 18, once daily on days 19-21, and once daily on days 22-30. Safety: N/A Efficacy: N/A	

Drug Type	Drug name	NCT number	Phase	Completion date	Enrol lment	Sponsor	Study	Tumor type	Study outcomes	Ref.
Antibody-drug conjugate	Anetumab ravtansine (BAY 94-9343)	NCT01439152	I	2019-07-30	148	Bayer	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AR	Solid	Posology: AR 0.15 to 7.5 mg/kg Q3W. or AR 1.8 or 2.2 mg/kg QW. Safety (n=148): Q3W MTD was determined to be 6.5 mg/kg (n=45), 39% of patients experienced grade ≥ 3 TESAEs (n=38). QW MTD was determined to be 2.2 mg/kg (n=71), 42% of patients experienced grade ≥ 3 TESAEs (n=36). Efficacy (n=148): PFS (n=103): 2.8 months. PFS AR 6.5 mg/kg Q3W (mesothelioma-ovarian, n=12-20): 14.1-4.1 months. PFS AR 2.2 mg/kg QW (mesothelioma-ovarian, n=15-21): 2.6-2.2 months. OR (n=148): CR 1%, PR 7%, SD 45%, PD 41% and NE 7%.	88
	Anetumab ravtansine (BAY 94-9343)	NCT02485119	I	2017-07-04	12	Bayer	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AR	Solid	Posology: AR 4.5 or 6.5 mg/kg iv. Q3W. Safety: N/A Efficacy: N/A	
	DMOT4039A	NCT01469793	I	2015-12-01	71	Genentech - Roche	Evaluation of safety, tolerability, pharmacokinetics and efficacy of DMOT4039A	Pancreatic and Ovarian	Posology: DMOT4039A 0.2 to 2.8 mg/kg iv. Q3W, or 0.8 to 1.2 mg/kg iv. QW. Safety: Most frequent TRAEs occurred in 10% of patients on Q3W and QW schedules. RP2D was determined to be 2.4 mg/kg for Q3W regimen and 1 mg/kg for QW regimen. Efficacy pancreatic cancer DMOT4039A 2.4 mg/kg Q3W. (n=26): PFS: 1.7 months. OR: CR 0%, PR 8%, SD 35% and PD 57%. Efficacy ovarian cancer DMOT4039A 2.4 mg/kg Q3W. (n=10): PFS: 4.9 months. OR: CR 0% and PR 30%.	95
		NCT01832116	I	2014-05-01	11	University Medical Center Groningen	Evaluation of pharmacokinetics of 89Zr-MMOT0530A before the clinical trial NCT01469793	Pancreatic and Ovarian	Posology: N/A Safety: N/A Efficacy: N/A	150
	BMS-986148	NCT02341625	I / II	2020-05-07 (terminated due to business reasons)	126	Bristol-Myers Squibb	Evaluation of safety, tolerability, pharmacokinetics and efficacy of BMS-986148 alone and in combination with nivolumab	Solid	Posology: Monotherapy, BMS-986148 0.1 to 1.6 mg/kg iv. Q3W, or BMS-986148 0.4 to 0.6 mg/kg iv. QW. In combination, BMS-986148 0.8 mg/kg iv. Q3W + nivolumab 360 mg iv. Q3W. Safety (n=126): BMS-986148 MTD and RP2D was determined to be 1.2 mg/kg Q3W (n=84). The dose level of 0.6 mg/kg QW was considered above the MTD (n=12). 49% of patients treated with BMS-986148 monotherapy Q3W experienced grade ≥ 3 TRAEs (n=84). 25% of patients treated with BMS-986148 monotherapy QW experienced grade ≥ 3 TRAEs (n=12). 33% of patients treated with BMS-986148 + nivolumab Q3W experienced grade ≥ 3 TRAEs (n=30). Efficacy BMS-986148 alone 1.2 mg/kg Q3W. (n=51): PFS (n=51): 2.8 months. PFS (mesothelioma, n=25): 3.8 months. OR (n=44): CR 0%, PR 7%, SD 57% and PD 36%. Efficacy BMS-986148 0.8 mg/kg + nivolumab 360 mg Q3W. (n=30): PFS (n=30): N/A. PFS (mesothelioma, n=13): 6.5 months. OR (n=26): CR 0%, PR 23%, SD 54% and PD 23%.	97
		NCT02884726	I	2017-09-06	8	Bristol-Myers Squibb	Evaluation of safety and tolerability of BMS-986148	N/A	Posology: BMS-986148 0.8 mg/kg iv. Q3W. Safety (n=7): 29% of patients experienced grade 3 AEs. No grade 4 AEs or SAEs were reported. One DLT was reported. Efficacy: N/A	97

Drug Type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
Antibody-drug conjugate	RC88	NCT06173037	II	Estimated 2026-12-31	88	RemeGen	Evaluation of safety, pharmacokinetics and efficacy of RC88	EOC, Fallopian and Peritoneal	Posology: RC88 2 mg/kg iv. Q3W. Safety: N/A Efficacy: N/A	
		NCT06016062	I / II	Estimated 2025-12-31	221	RemeGen	Evaluation of safety, tolerability, pharmacokinetics and efficacy of RC148 alone and in combination with docetaxel, RC48, RC88 or RC88 with bevacizumab	Solid	Posology: RC88 2 mg/kg iv. Q3W + RC148 20 mg/kg iv. Q3W, w/ or w/o bevacizumab 15 mg/kg iv. Q3W. (For docetaxel and RC48 details: NCT06016062) Safety: N/A Efficacy: N/A	
		NCT05804526	I / II	Estimated 2025-12-01	82	RemeGen	Evaluation of safety, pharmacokinetics and efficacy of RC88 in combination with sintilimab	Solid	Posology: RC88 1.5 to 2.5 mg/kg iv. Q3W + sintilimab 200 mg iv. Q3W. Safety: N/A Efficacy: N/A	
	RC88	NCT04175847	I / II	Estimated 2025-12-01	200	RemeGen	Evaluation of safety, tolerability, pharmacokinetics and efficacy of RC88	Solid	Data cut-off date: 2023-12-19 Posology: RC88 0.1 to 2.5 mg/kg iv. Q3W. Safety (n=164): 40% of patients experienced grade \geq 3 TEAEs. 14% of patients experienced TRSAEs. RP2D was determined to be 2 mg/kg Q3W. Efficacy (n=6-43): OR: CR 0-7% and PR 22-46%.	99
		NCT05508334	I	Estimated 2025-12-30	81	RemeGen	Evaluation of safety, tolerability and efficacy of RC88	Solid	Posology: RC88 dose escalation iv. Q2W. Safety: N/A Efficacy: N/A	98
	SGN-MesoC2 (PF-08052666 or HBM9033)	NCT06466187	I	Estimated 2029-02-16	365	Seagen - Pfizer	Evaluation of safety, tolerability, pharmacokinetics and efficacy of SGN-MesoC2	Solid	Posology: SGN-MesoC2 dose escalation iv. Safety: N/A Efficacy: N/A	100
Radiionuclide therapy	BAY 2287411	NCT03507452	I	2022-03-29	36	Bayer	Evaluation of safety, tolerability, pharmacokinetics and efficacy of BAY2287411	Mesothelioma and Ovarian	Posology: Thorium-227 start at 1.5 MBq and increase in steps of 1.0 to 1.5 MBq, with antibody doses of 10 to 400 mg iv. Q6W. Safety: N/A Efficacy: N/A	151

Drug Type	Drug name	NCT number	Phase	Completion date	Enrolment	Sponsor	Study	Tumor type	Study outcomes	Ref.
ADCC and ADCP mediator	Amatuximab (MORAb-009)	NCT02357147	II	2018-11-30 (terminated due to business reason)	124	Morphotek	Evaluation of safety and efficacy of amatuximab in combination with pemetrexed and cisplatin	MPM	Posology: Amatuximab 5 mg/kg iv. + pemetrexed 500 mg/m ² iv. + cisplatin 75 mg/m ² iv. Q3W for 6 cycles. Safety (n=106): 20% of patients treated with placebo + pemetrexed + cisplatin experienced SAEs (n=54). 29% of patients treated with amatuximab + pemetrexed + cisplatin experienced SAEs (n=52). Efficacy: N/A	152
		NCT00738582	II	2014-01-10	89	Morphotek	Evaluation of safety, tolerability and efficacy of amatuximab in combination with pemetrexed and cisplatin	MPM	Posology: Amatuximab 5 mg/kg iv. on days 1 and 8 + pemetrexed 500 mg/m ² iv. on day 1 + cisplatin 75 mg/m ² iv. on day 1 of each 3-week cycle for 5 cycles. Safety (n=89): 100% of patients experienced TEAEs. 43% of patients experienced SAEs. Efficacy (n=89): OS (n=89): 14.8 months. PFS (n=84): 6.1 months. OR (n=83): CR 0%, PR 40%, SD 51% and PD 10%.	105
		NCT00570713	II	2009-12-01	155	Morphotek	Evaluation of safety and efficacy of amatuximab in combination with gemcitabine	Pancreatic	Posology: Amatuximab 5 mg/kg iv. + gemcitabine 200 mg/m ² iv. QW. Safety: 72% of patients treated with placebo + gemcitabine experienced SAEs (n=75). 67% of patients treated with amatuximab + gemcitabine experienced SAEs (n=73). Efficacy placebo + gemcitabine (n=77): OS: 6.9 months. PFS: 3.5 months. OR: CR 0%, PR 8%, SD 56%, PD 23% and NE 13%. Efficacy amatuximab + gemcitabine (n=78): OS: 6.5 months. PFS: 3.4 months. OR: CR 0%, PR 6%, SD 47%, PD 19% and NE 27%.	104
		NCT01413451	I	2013-11-15	7	National Cancer Institute	Evaluation of safety, tolerability and pharmacokinetics of ¹¹¹ Indium-radiolabeled amatuximab	Mesothelioma and PDAC	Posology: Single dose injection. Safety (n=6): Radiotracer dose of 5 mCi was well-tolerated. Efficacy (n=6): Tumor and metastatic sites imaged. Higher uptake in mesothelioma than pancreatic cancer.	108
		NCT01521325	I	2013-03-01	6	Morphotek	Evaluation of safety, tolerability and pharmacokinetics of ¹¹¹ Indium-radiolabeled amatuximab	Mesothelioma and PDAC	Posology: Single dose injection. Safety (n=6): Radiotracer dose of 5 mCi was well-tolerated. Efficacy (n=6): Tumor and metastatic sites imaged. Higher uptake in mesothelioma than pancreatic cancer.	108
		NCT01018784	I	2013-02-01	17	Eisai	Evaluation of safety and tolerability of amatuximab	Solid	Posology: Amatuximab 50 to 200 mg/m ² iv. QW. Safety (n=17): MTD was determined to be 200 mg/m ² . 77% of patients experienced AEs. Efficacy (n=17): OR: CR 0%, PR 0%, SD 18% and PD 82%.	103
		NCT00325494	I	2008-09-01	24	Morphotek	Evaluation of safety, tolerability, and pharmacokinetics of amatuximab	Mesothelioma, Pancreatic and Ovarian	Posology: Amatuximab 12.5 to 400 mg/m ² iv. on days 1, 8, 15 and 22. Safety (n=24): MTD was determined to be 200 mg/m ² . 41% patients experienced SAEs, among them 2 patients treated at 400 mg/m ² experienced DLT. Efficacy (n=24): OS: 6.5 months. PFS: 3.4 months. OR: CR 0%, PR 0%, SD 46%, PD 38% and NE 17%.	104
	NI-1801	NCT05403554	I	Estimated 2026-09-30	70	Light Chain Bioscience - NovImmune	Evaluation of safety, tolerability, pharmacokinetics and efficacy of NI-1801 alone and in combination with pembrolizumab or paclitaxel	Ovarian, TNBC, NSCLC and PDAC	Posology: NI-1801 Q2W or Q3W, w/ or w/o pembrolizumab 400 mg Q6W (up to 4 cycles) or paclitaxel 80 mg/m ² QW (up to 4 cycles). Safety: N/A Efficacy: N/A	

Drug Type	Drug name	NCT number	Phase	Completion date	Enrolment	Sponsor	Study	Tumor type	Study outcomes	Ref.
Immunostimulator	ABBV-428	NCT02955251	I	2019-10-29	61	AbbVie	Evaluation of safety, tolerability, pharmacokinetics and efficacy of ABBV-428	Solid	Posology: ABBV-428 0.01 to 3.6 mg/kg iv. Q2W. Safety (n=59): 3 grade \geq 3 AEs were reported. RP2D was determined to be 3.6 mg/kg. Efficacy at 3.6 mg/kg (n=25): OR: CR 0%, PR 0%, SD 36% and PD 64%.	111
	AMG 994 (Inezetamab)	NCT04727554	I	2023-06-05	11	Amgen	Evaluation of safety, tolerability, pharmacokinetics and efficacy of AMG 994 alone and in combination with AMG 404 (anti-PD-1 IgG1)	Solid	Posology: AMG 994 (low or high dose) iv. QW + AMG 404 iv. Q4W. for 12 cycles (4-week cycles) Safety (n=11): With low dose regimen, 0% of patients experienced DLTs and 100% experienced SAEs (n=3). With high dose regimen, 38% of patients experienced DLTs and 75% experienced SAEs (n=8). 100% of patients experienced TEAEs (n=11). Efficacy low dose (n=3): OS: 6.0 months. PFS: 2.6 months. OR: CR 0% and PR 0%. Efficacy high dose (n=8): OS: 12.5 months. PFS: 1.6 months. OR: CR 0% and PR 25%.	
T-cell engager	HPN536	NCT03872206	I	2023-01-04	95	Harpoon Therapeutics - Merck	Evaluation of safety, tolerability, pharmacokinetics and efficacy of HPN536	N/A	Posology: HPN536 multiple injection doses of 6 to 14400 ng/kg iv. Safety: N/A Efficacy: N/A	112
	AMG 305	NCT05800964	I	Estimated 2028-08-12	220	Amgen	Evaluation of safety and tolerability of AMG 305	Solid	Posology: iv. Safety: N/A Efficacy: N/A	113
	JNJ-79032421	NCT06255665	I	Estimated 2028-03-07	120	Janssen - Johnson & Johnson	Evaluation of safety and tolerability of JNJ-79032421	Solid	Posology: N/A Safety: N/A Efficacy: N/A	114
	ZW171	NCT06523803	I	Estimated 2027-12-01	160	Zymeworks	Evaluation of safety, tolerability and efficacy of ZW171	N/A	Posology: N/A Safety: N/A Efficacy: N/A	
	CT-95	NCT06756035	I	Estimated 2028-12-01	50	Context Therapeutics	Evaluation of safety, tolerability and efficacy of CT-95	Solid	Posology: CT-95 dose escalation iv. QW. Safety: N/A Efficacy: N/A	

1238 **Table 3.** MSLN-targeted cell therapies in clinical trials. Discontinued clinical trials are shown in dark grey. Clinical trials with an unknown status are shown in light grey. *AE: Adverse Event,*
1239 *CHO: Cholangiocarcinoma, CR: Complete Response, CRS: Cytokine Release Syndrome, DLT: Dose Limiting Toxicity, EOC: Epithelial Ovarian Cancer, ip.: Intrapleural, itu.: intratumoral, iv.:*
1240 *intravenous, LD: Lymphodepletion, MPM: Malignant Pleural Mesothelioma, NE: Not Evaluated, NSCLC: Non-Small-Cell Lung Cancer, OR: Overall Response, OS: Overall Survival, PD:*
1241 *Progressive Disease, PDAC: Pancreatic Ductal Adenocarcinoma, PFS: Progression-Free Survival, PR: Partial Response, Q3W: once every 3 weeks, QW: once weekly, RP2D:*
1242 *Recommended Phase 2 Dose, SAE: Serious Adverse Event, SD: Stable Disease, TNBC: Triple Negative Breast Cancer, TRAE: Treatment Related Adverse Event.*

Cell type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CAR-T cells	anti-MSLN CAR-T cells (mRNA transfected SS1/CD3 ζ /4-1BB CAR-T cells)	NCT01897415	I	2017-03-01	16	University of Pennsylvania	Evaluation of feasibility, safety and efficacy of anti-MSLN CAR-T cells	PDAC	Posology: No LD. 1 to 3 \times 10 ⁸ cells/m ² iv. 3 times weekly for 3 weeks. Safety (n=6): No DLT reported. No CRS reported. 3 grade 3 AEs were reported. 2 grade 4 TRAEs were reported. Efficacy (n=6): PFS: 4.6 months. OR: CR 0%, PR 0%, SD 33% and PD 67%.	153
	anti-MSLN CAR-T cells (retroviral transduced CAR-T cells)	NCT01355965	I	2015-10-01	18	University of Pennsylvania	Evaluation of feasibility, safety, pharmacokinetics and efficacy of anti-MSLN CAR-T cells	MPM and PDAC	Data cut-off date: 2014-02-01 Efficacy: No LD. Different protocols were used with multiple injections at 1 \times 10 ⁸ cells iv., 1 \times 10 ⁹ cells iv., 3 \times 10 ⁸ cells/m ² iv. and/or 2 \times 10 ⁸ cells itu. Safety (n=2): Treatment not tolerated, the first patient died because of the treatment and the second experienced 2 grade \geq 3 AEs. Efficacy (n=2): Results suggest a role of anti-MSLN CAR-T cells in inducing an antitumor effect.	116
	anti-MSLN CAR-T cells (retroviral transduced CAR-T cells)	NCT01583686	I / II	2018-12-17 (terminated due to slow / insufficient accrual)	15	National Cancer Institute	Evaluation of safety, tolerability and efficacy of anti-MSLN CAR-T cells in combination with aldesleukin	Solid	Posology: LD. Single dose injection of 1 \times 10 ⁶ to 1 \times 10 ⁸ cells iv. + aldesleukin 72,000 IU/kg iv. (up to 15 dose injections). Safety (n=15): 45% of patients experienced SAEs. The only group without SAEs is the one with 1 \times 10 ⁷ cells. Efficacy (n=15): OR: CR 0%, PR 0%, SD 7% and PD 93%. The only patient with a SD was injected with 3 \times 10 ⁶ CAR-PBL.	
	huCART-meso cells (lentiviral transduced SS1/4-1BB/CD3 ζ CAR-T cells)	NCT05057715	I	Estimated 2038-09-01	13	University of Pennsylvania	Evaluation of feasibility and safety of huCART-meso cells in combination with VCN-01	Pancreatic and Ovarian	Posology: No LD. VCN-01 3.3 \times 10 ¹² vp iv. on day 0 + huCART-meso 5 \times 10 ⁷ cells iv. on day 14 (regimen A). VCN-01 1 \times 10 ¹³ vp iv. on day 0 + huCART-meso 5 \times 10 ⁷ cells iv. on day 14 (regimen B). huCART-meso cells iv. on day 0 + VCN-01 3.3 \times 10 ¹² vp iv. on day 14 (regimen C). Safety: N/A Efficacy: N/A	
	huCART-meso cells (lentiviral transduced SS1/4-1BB/CD3 ζ CAR-T cells)	NCT05623488	I	Estimated 2038-02-01	12	University of Pennsylvania	Evaluation of feasibility and safety of intratumoral huCART-meso cells injection	TNBC	Posology: No LD. 3 \times 10 ⁶ to 3 \times 10 ⁷ cells itu. Safety: N/A Efficacy: N/A	
	huCART-meso cells (lentiviral transduced SS1/4-1BB/CD3 ζ CAR-T cells)	NCT03323944	I	Estimated 2025-09-01	18	University of Pennsylvania	Evaluation of feasibility and safety of iv. injection and local delivery of huCART-meso cells	Pancreatic	Safety: No LD. Single dose injection of 1 \times 10 ⁶ to 3 \times 10 ⁷ cells/m ² iv., ip. or intrahepatic. Safety: N/A Efficacy: N/A	78
	huCART-meso cells (lentiviral transduced SS1/4-1BB/CD3 ζ CAR-T cells)	NCT03054298	I	2024-07-30	54	University of Pennsylvania	Evaluation of feasibility and safety of iv injection and local delivery of huCART-meso cells	Mesothelioma, Ovarian and Pancreatic	Data cut-off date: 2019-07-17 Posology: w/ or w/o LD. Single dose injection of 1 to 3 \times 10 ⁷ cells/m ² iv. or 1 to 3 \times 10 ⁸ cells/m ² iv. Safety (n=15): 7 grade \geq 3 AEs were reported. Efficacy (n=15): PFS: 2.1 months. OR: CR 0%, PR 0%, SD 73% and PD 27%.	78

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Cell type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CCAR-T cells	huCART-meso cells (lentiviral transduced SS1/4-1BB/CD3ζ CAR-T cells)	NCT02465983	I	2017-11-01 (terminated due to lack of efficacy and funding)	4	University of Pennsylvania	Evaluation of feasibility, safety, pharmacokinetics and efficacy of huCART-meso cells in combination with anti-CD19 CAR-T cells	PDAC	Posology: LD. huCART-meso cells 3×10 ⁷ cells/m ² iv. + anti-CD19 CAR-T cells 3×10 ⁷ cells/m ² iv. (two separate injections). Safety (n=3): No DLT reported. Efficacy (n=3): OR: CR 0%, PR 0%, SD 33%, PD 67%. CART-19 cells deplete normal B cells but at the dose tested no improvement of huCART-meso cells persistence.	154
		NCT02159716	I	2015-11-01	19	University of Pennsylvania	Evaluation of feasibility, safety, pharmacokinetics and efficacy of huCART-meso cells	PDAC, EOC and MPM	Posology: w/ or w/o LD. Single dose injection of 1 to 3×10 ⁷ cells/m ² iv. or 1 to 3×10 ⁸ cells/m ² iv. Safety (n=15): Without LD, at 1 to 3×10 ⁷ cells/m ² a DLT occurred. Efficacy (n=15): PFS: 2.1 months. OR: CR 0%, PR 0%, SD 73% and PD 27%.	78
		NCT02388828	N/A	2019-07-01	10	University of Pennsylvania	Evaluation of long-term safety of huCART-meso cells	N/A	Posology: No LD. Safety: N/A Efficacy: N/A	
	M28z cells (retroviral transduced anti-MSLN M912 scFv/CD28/CD3ζ CAR-T cells)	NCT02414269	I / II	Estimated 2025-04-30	113	Memorial Sloan Kettering Cancer Center	Evaluation of safety and efficacy of M28z cells alone and in combination with pembrolizumab	Solid	Data cut-off date: 2019-04-01 Posology: w/ or w/o LD. Single dose injection of 1.3×10 ⁵ to 8.6×10 ⁷ cells/kg ip. (at PD stage 15% of patients received a second injection) + pembrolizumab 200 mg iv. 3 times and 4 weeks after completing CAR-T cells injection. Safety (n=27): No DLTs reported. 22% of patients experienced grade 3 AEs. Grade 4 AEs were reversible. Efficacy M28z + pembrolizumab (n=16): OS: 23.9 months. OR: CR 0%, PR 13%, SD 56% and PD 31%.	117
	anti-MSLN CAR-T cells (retroviral transduced anti-MSLN/4-1BB/CD3ζ CAR-T cells)	NCT02580747	I	Estimated 2018-11-01*	20	Chinese PLA General Hospital	Evaluation of feasibility, safety and efficacy of anti-MSLN CAR-T cells	Solid	Posology: No LD. Injection on days 0, 1 and 2. Safety: N/A Efficacy: N/A	
	TAI-meso-CART cells	NCT02706782	I	Estimated 2018-09-01*	30	Shanghai GeneChem	Evaluation of safety and efficacy of TAI-meso-CART cells	Pancreatic	Posology: LD. Single dose injection of 1×10 ⁶ to 10×10 ⁶ cells/kg iv. Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-T cells (transduced anti-MSLN CAR-T cells)	NCT02792114	I	Estimated 2025-06-01	186	Memorial Sloan Kettering Cancer Center	Evaluation of safety and tolerability of anti-MSLN CAR-T cells	Breast	Posology: LD. Single dose injection iv. Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-T cells (lentiviral transduced CAR-T cells)	NCT02959151	I / II	Estimated 2018-07-01*	20	Shanghai GeneChem	Evaluation of safety and efficacy of anti-MSLN CAR-T cells in combination with interventional therapy	Pancreatic	Posology: No LD. Single dose injection of 1.25 to 4×10 ⁷ cells/cm ³ of tumor bulk iv. or itu. Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-T cells	NCT02930993	I	Estimated 2019-08-01*	20	China Meitan General Hospital	Evaluation of safety and efficacy of anti-MSLN CAR-T cells	Solid	Posology: LD. 3-day split-dose of 5×10 ⁴ to 1×10 ⁷ cells/kg iv. (day0, 10%; day1, 30%; day2, 60%). Safety: N/A Efficacy: N/A	
	αPD-1-mesoCAR-T cells (DNA transfected anti-MSLN CAR-T cells secreting PD-1 antibody)	NCT03615313	I / II	Estimated 2020-12-03*	50	Shanghai Cell Therapy Research Institute	Evaluation of safety and efficacy of αPD-1-mesoCAR-T cells	Solid	Data cut-off date: 2020-05-25 Posology: LD. 5×10 ⁶ cells/kg, 5×10 ⁷ cells/kg or 1×10 ⁸ cells/kg iv. from day 1 to day 3. Safety (n=10): Most common AEs were mild fatigue and fever. Efficacy (n=10): PFS: 3.2 months. OR: CR 0%, PR 20%, SD 40% and PD 40%.	119
NCT03030001		I / II	Estimated 2019-02-01*	40	Ningbo Cancer Hospital	Evaluation of safety and efficacy of anti-MSLN CAR-T cells expressing PD-1 antibody	Solid	Posology: No LD. Single dose injection iv. Safety: N/A Efficacy: N/A		

1245 * unknown status

Cell type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CAR-T cells	anti-MSLN CAR-T cells secreting CTLA-4/PD-1 antibodies	NCT03182803	I / II	Estimated 2019-04-20 (unknown status)	40	Shanghai Cell Therapy Research Institute	Evaluation of safety, pharmacokinetics and efficacy of anti-MSLN CAR-T cells secreting CTLA-4/PD-1 antibodies	Solid	Posology: LD. 2 to 5×10 ⁷ cells/kg iv. from day 18 to day 19 (±2 days), 2 cycles. Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-T cells	NCT03267173	I	Estimated 2019-06-01 (unknown status)	10	First Affiliated Hospital of Harbin Medical University	Evaluation of safety and efficacy of anti-MSLN CAR-T cells	Pancreatic	Posology: No LD. Single dose injection. Safety: N/A Efficacy: N/A	
		NCT04203459	N/A	Estimated 2022-12-31 (unknown status)	80	First Affiliated Hospital of Harbin Medical University	Evaluation of the effects and mechanism of gut microbiota on anti-MSLN CAR-T cells	Pancreatic	Posology: N/A Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-T cells (anti-MSLN CD8 ⁺ CAR-T cells + anti-TGF-β CD4 ⁺ CAR-T cells secreting IL-7/CCL19 and/or anti-PD-1/CTLA-4/TIGIT scFvs)	NCT03198052	I	Estimated 2036-08-01	30	Second Affiliated Hospital of Guangzhou Medical University	Evaluation of safety, tolerability and efficacy of anti-MSLN CD8 ⁺ CAR-T cells + anti-TGF-β CD4 ⁺ CAR-T cells secreting IL-7/CCL19 and/or anti-PD-1/CTLA-4/TIGIT scFvs	Lung	Posology: No LD. 1 to 10×10 ⁶ cells/kg iv. or regional injections for 3 or more cycles. Safety: N/A Efficacy: N/A	
	anti-MSLN-7 × 19 CAR-T cells (lentiviral transduced anti-MSLN scFv/TLR2/CD3ζ CAR-T cells secreting human IL-7 and CCL19)	NCT03198546	I	Estimated 2036-08-01	30	Second Affiliated Hospital of Guangzhou Medical University	Evaluation of safety and efficacy of anti-MSLN-7 × 19 CAR-T cells	Pancreatic and Ovarian	Data cut-off date: 2021-07-17 Posology: No LD. Injections every 1 to 2 months iv. or local. Safety (n=6): Injection every 1 to 2 months. No grade ≥ 2 AEs reported. Efficacy (n=2): OR: CR 50%, PR 0%, SD 0% and PD 50%.	155
	anti-MSLN CAR-T cells (lentiviral transduced anti-MSLN scFv/4-1BB/CD3ζ CAR-T cells)	NCT03497819	I	Estimated 2020-10-31 (unknown status)	10	First Affiliated Hospital of Wenzhou Medical University	Evaluation of safety and efficacy of anti-MSLN CAR-T cells in combination with anti-CD19 CAR-T cells	Pancreatic	Posology: LD. iv. or artery injection. Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-T cells (GIMI-IRB cells)	NCT03356808	I / II	Estimated 2020-12-31 (unknown status)	20	Shenzhen Geno-Immune Medical Institute	Evaluation of feasibility, safety, pharmacokinetics and efficacy of anti-MSLN CAR-T cells	Lung (SCLC and NSCLC)	Posology: Single dose injection of 1×10 ⁶ to 1×10 ⁷ cells/kg iv. Safety: N/A Efficacy: N/A	

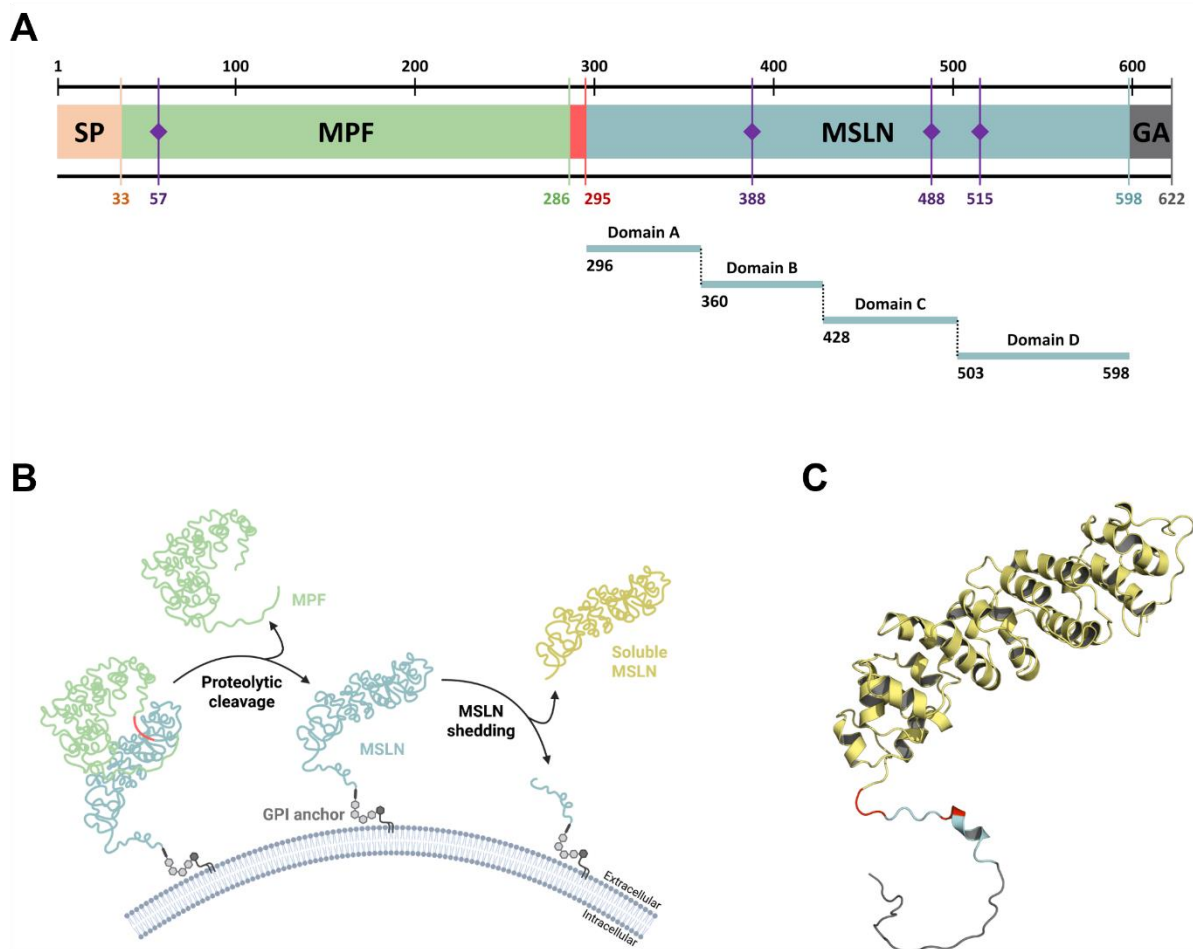
Cell type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CAR-T cells	anti-MSLN CAR-T cells (GIMI-IRB cells)	NCT03356795	I / II	Estimated 2020-12-01 (unknown status)	20	Shenzhen Geno-Immune Medical Institute	Evaluation of feasibility, safety, pharmacokinetics and efficacy of anti-MSLN CAR-T cells	Cervical	Posology: No LD. Single dose injection of 1×10^6 to 1×10^7 cells/kg iv. Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-T cells	NCT03638206	I / II	Estimated 2023-03-01 (unknown status)	73	Shenzhen BinDeBio	Evaluation of safety and efficacy of anti-MSLN CAR-T cells	Gastric, Pancreatic and Mesothelioma	Posology: LD. Single dose injection. Safety: N/A Efficacy: N/A	
		NCT03941626	I / II	Estimated 2021-12-01 (unknown status)	50	Shenzhen BinDeBio	Evaluation of safety and efficacy of anti-MSLN CAR-T cells	Gastric	Posology: No LD. Safety: N/A Efficacy: N/A	
	MPTK-CAR-T cells (PD-1 and TCR deficient lentiviral transduced P4 scFv/CD28/CD3 ζ CAR-T cells)	NCT03545815	I	Estimated 2020-12-30 (unknown status)	15	Chinese PLA General Hospital	Evaluation of feasibility, safety, pharmacokinetics and efficacy of MPTK-CAR-T cells	Solid	Posology: No LD. Single dose injection of 0.1 to 9×10^6 cells/kg iv. If undetectable circulating MPTK-CAR-T cells, possibility of a second or a third infusion (8/15). Safety (n=15): 5 grade ≥ 3 AEs were reported. Efficacy (n=15): PFS: 1.6 months. OR: CR 0%, PR 0%, SD 13% and PD 87%.	156
	GC008t cells ((PD-1 deficient lentiviral transduced P4 scFv/CD28/CD3 ζ CAR-T cells))	NCT03747965	I	Estimated 2020-05-01 (unknown status)	10	Chinese PLA General Hospital	Evaluate the feasibility, safety and efficacy of GC008t cells	Pancreatic, Ovarian and Colorectal	Data cut-off date: 2020-01-20 Posology: w/ or w/o LD. 1×10^8 to 5×10^7 cells/kg. 4/9 patients received repeat injections of GC008t. Safety (n=9): CRS noticed only at the highest dose. Efficacy (n=9): PFS (PR condition, n=2): 3.9 months. OR (n=9): CR 0%, PR 22%, SD 44%, PD 11% and NE 22%.	157
	anti-MSLN CAR-T cells (lentiviral transduced SS1/TCR ζ /4-1BB CAR-T cells)	NCT03638193	N/A	Estimated 2022-02-01 (unknown status)	10	Shenzhen BinDeBio	Evaluation of safety and efficacy of CART-meso cells	Pancreatic	Posology: LD. Single dose injection of 1×10^7 to 3×10^8 cells/m ² iv. Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-T cells (retroviral transduced CAR-T cells)	NCT03799913	I	Estimated 2022-04-01 (unknown status)	20	Zhejiang University	Evaluation of feasibility, safety, pharmacokinetics and efficacy of anti-MSLN CAR-T cells	Ovarian	Posology: LD. Safety: N/A Efficacy: N/A	
	gavo-cel (gavocabtagene autoleucel or TC-210 or lentiviral transduced anti-MSLN sdAb (MH1)/CD3 ζ CAR-T cells)	NCT03907852	I / II	Estimated 2028-11-02	36	TCR2 Therapeutics	Evaluation of safety and efficacy of gavo-cel	MPM, Ovarian and CHO	Data cut-off date: 2023-01-17 Posology: w/ or w/o LD. Single dose injection of 5×10^7 to 5×10^8 cells/m ² iv. Safety (n=32): 97% of patients experienced AEs. 78% of patients experienced CRS. The RP2D was determined to be 1×10^8 cells/m ² , after LD. At the RP2D, no cases of pneumonitis were reported and severe CRS events (2/13, 15%) were rapidly reversible. Efficacy in MPM (n=23): OS: 11.1 months. PFS: 5.6 months. Efficacy in ovarian cancer (n=8): OS: 9.4 months. PFS: 5.8 months.	124
	anti-MSLN CAR-T cells (retroviral transduced CAR-T cells)	NCT03916679	I / II	Estimated 2023-04-20 (unknown status)	20	Second Affiliated Hospital, School of Medicine, Zhejiang University	Evaluation of feasibility, safety, pharmacokinetics and efficacy of anti-MSLN CAR-T cells	EOC	Posology: LD. Safety: N/A Efficacy: N/A	

Cell type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CAR-T cells	anti-MSLN CAR-T cells (transduced anti-MSLN CAR-T cells)	NCT03814447	I	Estimated 2023-01-01 (unknown status)	10	Shanghai 6th People's Hospital	Evaluation of feasibility, safety, pharmacokinetics and efficacy of anti-MSLN CAR-T cells	Ovarian	Posology: LD. Single dose injection of 5×10^6 cells/kg. Safety: N/A Efficacy: N/A	
	α PD1-MSLN-CAR T cells (anti-MSLN CAR-T cells secreting anti-PD-1 sdAb)	NCT05089266	I	Estimated 2025-01-30	30	Shanghai Cell Therapy Group	Evaluation of safety and efficacy of α PD1-MSLN-CAR T cells	Colorectal	Posology: No LD. Safety: N/A Efficacy: N/A	
		NCT05373147	I	Estimated 2023-08-01 (unknown status)	21	Shanghai Mengchao Cancer Hospital	Evaluation of safety, tolerability, pharmacokinetics and efficacy of α PD1-MSLN-CAR T cells	Solid	Posology: No LD. Single dose injection of 1×10^5 to 3×10^6 cells/kg. Safety: N/A Efficacy: N/A	
		NCT04489862	I	Estimated 2022-12-01 (unknown status)	10	Wuhan Union Hospital, China	Evaluation of safety, tolerability, pharmacokinetics and efficacy of α PD1-MSLN-CAR T cells	NSCLC and Mesothelioma	Posology: LD. Single dose injection of 1×10^5 to 3×10^6 cells/kg iv.. Safety: N/A Efficacy: N/A	
		NCT04503980	I	Estimated 2022-06-01 (unknown status)	10	Shanghai Cell Therapy Group	Evaluation of safety, tolerability, pharmacokinetics and efficacy of α PD1-MSLN-CAR T cells	Colorectal and Ovarian	Safety: LD. 1×10^5 to 3×10^6 cells/kg iv. Safety: N/A Efficacy: N/A	
	M28z1XXPD1DNR cells (M28z equipped with a modified CD3 ζ (1XX), and a PD-1 dominant negative receptor (PD1DNR))	NCT06623396	I	Estimated 2028-09-30	18	Memorial Sloan Kettering Cancer Center	Evaluation of safety and tolerability of M28z1XXPD1DNR cells	Esophagogastric	Posology: No LD. Single dose injection ip. Safety: N/A Efficacy: N/A	
		NCT04577326	I	Estimated 2025-09-30	14	Memorial Sloan Kettering Cancer Center	Evaluation of safety and pharmacokinetics of M28z1XXPD1DNR cells	MPM	Data cut-off date: 2021-12-01 Posology: LD. Single dose injection of 1 to 3×10^6 cells/kg ip. Safety (n=4): No grade ≥ 3 TRAEs reported. Efficacy: N/A	118
	LCAR-M23 cells	NCT04562298	I	2022-06-07 (terminated due to sponsors and collaborator decision)	15	Shanghai East Hospital	Evaluation of safety, tolerability, pharmacokinetics and efficacy of LCAR-M23 cells	EOC	Posology: LD. Single dose injection. Safety: N/A Efficacy: N/A	
	PD-1 KO anti-MSLN CAR-TILs secreting anti-PD-1/CTLA-4 scFvs	NCT04842812	I	Estimated 2035-01-01	40	Second Affiliated Hospital of Guangzhou Medical University	Evaluation of safety, tolerability and efficacy of PD-1 KO anti-MSLN CAR-TILs secreting anti-PD-1/CTLA-4 scFvs	Solid	Posology: No LD. 1 to 10×10^7 cells/kg for each treatment, 3 or more cycles. Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-T cells	NCT05531708	I	2021-04-02 (withdrawn)	0	Shanghai Pudong Hospital	Evaluation of safety and efficacy of anti-MSLN CAR-T cells	Solid	Posology: LD. Single dose injection iv. Safety: N/A Efficacy: N/A	
anti-MSLN CAR-T cells (mRNA transfected CAR-T cells)	NCT04981691	I	Estimated 2022-07-09 (unknown status)	12	Ruijin Hospital	Evaluation of safety, pharmacokinetics and efficacy of anti-MSLN CAR-T cells	Solid	Posology: LD. First week, 1 to 3×10^9 cells iv. divided into 3 injections. Second week, 1 to 3×10^9 cells iv. for each injection (3 injections). Safety: N/A Efficacy: N/A		

Cell type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CAR-T cells	FH-TCR-TMSLN cells	NCT04809766	I	Estimated 2025-09-04	9	Fred Hutchinson Cancer Center	Evaluation of safety, pharmacokinetics and efficacy of FH-TCR-TMSLN	PDAC	Data cut-off date: 2023-07-01 Posology: LD. 3 injections of 1×10 ⁹ or 3.3×10 ⁹ cells Q3W. Safety (n=6): No DLTs occurred. 17% of patients experienced SAEs. Efficacy: N/A	120
	RD133 cells	NCT05166070	I	Estimated 2037-01-01	24	The First Affiliated Hospital with Nanjing Medical University	Evaluation of safety, tolerability and efficacy of RD133 cells	Solid	Posology: LD. Single dose injection of 1 to 6×10 ⁶ cells/kg iv. Safety: N/A Efficacy: N/A	
	LD013 cells	NCT05372692	N/A	2023-02-01	3	Weijia Fang, MD	Evaluation of safety of LD013 cells	Ovarian	Posology: No LD. Safety: N/A Efficacy: N/A	
	TC-510 cells (lentiviral transduced anti-MSLN sdAb (MH1)/CD3ζ TCR-T cells with PD-1:CD28 switch receptor)	NCT05451849	I / II	Estimated 2028-10-30	6	TCR2 Therapeutics	Evaluation of safety and efficacy of TC-510	Solid	Posology: LD. Safety: N/A Efficacy: N/A	121
	BZE2209 cells (anti-MSLN CAR-T cells secreting anti-PD-1/CTLA-4 sdAbs)	NCT06248697	I	Estimated 2026-12-31	16	Shanghai Cell Therapy Group	Evaluation of safety and tolerability of BZE2209 cells	Solid	Posology: No LD. iv. Safety: N/A Efficacy: N/A	
	UCLM802 cells	NCT05783089	I	Estimated 2027-04-01	87	Cancer Institute and Hospital, Chinese Academy of Medical Sciences	Evaluation of safety, tolerability and efficacy of anti-MSLN CAR-T cells	Solid	Posology: LD. Single dose injection of 0.1 to 2×10 ⁶ cells/kg iv. or local injection, w/ or w/o checkpoint inhibitors. Safety: N/A Efficacy: N/A	
			I	Estimated 2025-07-01	87	Zhejiang University	Evaluation of safety, tolerability and efficacy of UCLM802	Solid	Posology: LD. Single dose injection of 0.1 to 2×10 ⁶ cells/kg iv. Safety: N/A Efficacy: N/A	
I			Estimated 2025-01-01	87	Peking University	Evaluation of safety, tolerability and efficacy of UCLM802 cells	Solid	Posology: LD. Safety: N/A Efficacy: N/A		
anti-MSLN CAR-T cells secreting IL-21 and anti-PD-1 scFv	NCT05779917	I	Estimated 2036-03-10	30	Second Affiliated Hospital of Guangzhou Medical University	Evaluation of safety, tolerability and efficacy of anti-MSLN CAR-T cells secreting IL-21 and anti-PD-1 scFv	Pancreatic	Posology: No LD. Safety: N/A Efficacy: N/A		

Cell type	Drug name	NCT number	Phase	Completion date	Enrollment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CAR-T cells	SynKIR-110 cells (anti-MSLN KIR-CAR-T cells)	NCT05568680	I	Estimated 2027-12-01	42	Verismo Therapeutics	Evaluation of feasibility, safety, tolerability and efficacy of SynKIR-110 cells	Ovarian, CHO and Mesothelioma	Posology: No LD. Single dose injection iv. Safety: N/A Efficacy: N/A	
		NCT06701201	N/A	Estimated 2042-02-01	60	Verismo Therapeutics	Evaluation of long-term safety and efficacy of SynKIR-110 cells	N/A	Posology: N/A Safety: N/A Efficacy: N/A	
		NCT06377202	N/A	2023-11-15 (withdrawn and replaced by NCT06701201)	0	Verismo Therapeutics	Evaluation of long-term safety of SynKIR-110 cells	Solid	Posology: No LD. Single dose injection. Safety: N/A Efficacy: N/A	
	BZT2312 cells (anti-MSLN CAR-T cells secreting anti-PD-1 sdAb)	NCT06249256	I	Estimated 2026-12-31	12	Shanghai Cell Therapy Group	Evaluation of safety, tolerability and efficacy of BZT2312 cells	Solid	Posology: LD. Single dose injection of 5×10^5 to 5×10^6 cells/kg iv. Safety: N/A Efficacy: N/A	
	anti-MSLN TCR-like CAR-T cells (retroviral transduced CAR-T cells)	NCT05963100	I / II	Estimated 2026-05-16	10	Zhongda Hospital	Evaluation of safety and efficacy of anti-MSLN TCR-like CAR-T cells	Ovarian	Safety: No LD. 1×10^6 to 2×10^7 cells/kg. Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-T cells	NCT06054308	N/A	2024-02-02 (withdrawn)	0	Chinese University of Hong Kong	Evaluation of feasibility of endoscopic ultrasound guided injection of anti-MSLN CAR-T cells	PDAC	Posology: No LD. Endoscopic ultrasound guided injection. Safety: N/A Efficacy: N/A	
	UCMYM802 cells (mRNA transfected anti-MSLN CAR-T cells)	NCT06256055	I	Estimated 2025-04-01	24	UTC Therapeutics	Evaluation of safety, tolerability, pharmacokinetics and efficacy of UCMYM802 cells	Solid	Posology: 1×10^8 to 2×10^9 cells iv. QW (4 injections). Safety: N/A Efficacy: N/A	
	BZE2203 cells (anti-MSLN CAR-T cells secreting anti-PD-1 sdAb)	NCT06327997	I	Estimated 2027-12-31	20	Shanghai Cell Therapy Group	Evaluation of safety, tolerability, pharmacokinetics and efficacy of BZE2203 cells	Solid	Posology: No LD. Single dose injection of 5×10^5 to 5×10^6 cells/kg iv. Safety: N/A Efficacy: N/A	
	A2B694 cells (logic-gated Tmod™ CAR-T cells with anti-MSLN activating receptor and HLA-A*02 blocking receptor)	NCT06051695	I / II	Estimated 2029-06-01	230	A2 Biotherapeutics	Evaluation of safety, tolerability and efficacy of A2B694 cells	Solid	Posology: LD. Single dose injection iv. Safety: N/A Efficacy: N/A	158
CHT102 cells (anti-MSLN UCAR-T cells)	NCT06760364	I	Estimated 2039-12-24	21	Tianjin Medical University Cancer Institute and Hospital	Evaluation of safety, pharmacokinetics and efficacy of CHT102 cells	Pancreatic	Posology: No LD. Arterial injection. Safety: N/A Efficacy: N/A		
	NCT06717022	N/A	Estimated 2039-05-01	24	Tianjin Medical University Cancer Institute and Hospital	Evaluation of safety and efficacy of CHT102 cells	N/A	Posology: No LD. 2.5×10^6 to 3×10^7 cells/kg iv. on days 1, 5 and 9. Safety: N/A Efficacy: N/A		

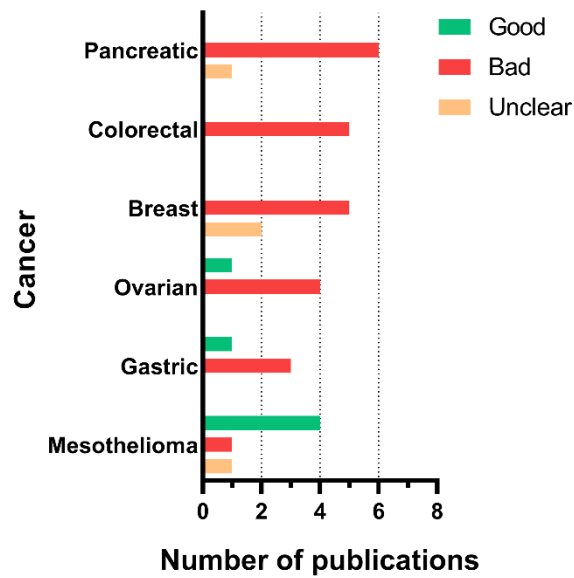
Cell type	Drug name	NCT number	Phase	Completion date	Enrolment	Sponsor	Study	Tumor type	Study outcomes	Ref.
CAR-T cells	MSLN-CAR- $\gamma\delta$ T cells	NCT06196294	I	Estimated 2036-12-30	30	Second Affiliated Hospital of Guangzhou Medical University	Evaluation of safety, tolerability and efficacy of MSLN-CAR- $\gamma\delta$ T cells	Pancreatic, Lung, Liver and Mesothelioma	Posology: No LD. Safety: N/A Efficacy: N/A	
CAR-PBMCs	MCY-M11 cells (mRNA transfected CAR-PBMCs)	NCT03608618	I	2021-08-24 (terminated due to shift of sponsor's focus)	14	MaxCyte	Evaluation of feasibility, safety and tolerability of MCY-M11 cells administered intraperitoneally	Solid	Data cut-off date: 2020-01-01 Posology: No LD. Injections of 1×10^7 to 5×10^8 cells ip. QW for 3 weeks. Safety (n=11): No TRAEs reported. No DLTs reported. Efficacy at 5×10^7 (n=3): PFS: 4 months. OR: CR 0%, PR 0%, SD 100% and PD 0%.	122
CAR-NK cells	anti-MSLN CAR-NK cells	NCT03692637	I	Estimated 2021-11-01 (unknown status)	30	Allife Medical Science and Technology	Evaluation of safety, tolerability and efficacy of anti-MSLN CAR-NK cells	EOC	Posology: No LD. Single dose injection of 5×10^5 to 3×10^6 cells/kg iv. Safety: N/A Efficacy: N/A	
	anti-MSLN CAR-NK cells; anti-MSLN CAR-NK cells secreting IL-7/CCL19; anti-MSLN CAR-NK secreting anti-PD-1/PD-L1/CTLA-4 scFvs	NCT05410717	I	Estimated 2036-05-31	200	Second Affiliated Hospital of Guangzhou Medical University	Evaluation of safety and efficacy of anti-MSLN CAR-NK cells; anti-MSLN CAR-NK cells secreting IL-7/CCL19; anti-MSLN CAR-NK secreting anti-PD-1/PD-L1/CTLA-4 scFvs alone and in combination with cannabidiol or nicotinamide adenine dinucleotide	Ovarian, Testis and Endometrial	Posology: No LD. Safety: N/A Efficacy: N/A	159
CAR-macrophages	TAK-103 cells	NCT05164666	I	Estimated 2027-10-31	2	Takeda	Evaluation of safety, pharmacokinetics and efficacy of TAK-103 cells	Solid	Posology: No LD. Single dose injection of 1×10^6 to 5×10^8 cells iv. Safety: N/A Efficacy: N/A	
	SY001 cells (adenoviral transduced anti-MSLN scFv/4-1BB/CD3 ζ CAR-macrophages)	NCT06562647	N/A	Estimated 2025-04-01	2	CellOrigin Biotech	Evaluation of safety, tolerability, pharmacokinetics and efficacy of SY001 cells in combination with tislelizumab	Ovarian	Posology: No LD. SY001 cells injections iv. on days 1, 3 and/or 5 + tislelizumab iv. on day 1. Safety (n=2): 100% of patients experienced transient cytokine fluctuations and reductions in lymphocytes or neutrophils post-treatment but no other grade ≥ 3 AEs or CRS were observed. Efficacy (n=2): OR: SD 100% (28-day follow-up period).	123



1258

1259 **Figure 1.** Mesothelin structure and processing. (A) Mesothelin precursor is a 622 amino acids protein with 4 glycosylation
 1260 sites (purple) and 5 regions: signal peptide (SP, orange); megakaryocyte potentiating factor (MPF, green); proteolytic cleavage
 1261 site (red); mature mesothelin (MSLN, blue); and the GPI anchor (GA, gray). (B) Schematic representation of the mesothelin
 1262 processing from precursor to soluble mesothelin. (C) Three-dimensional AlphaFold ribbon representation of mature MSLN
 1263 with major cleavage sites (MCS, red) locations leading to soluble mesothelin (yellow).

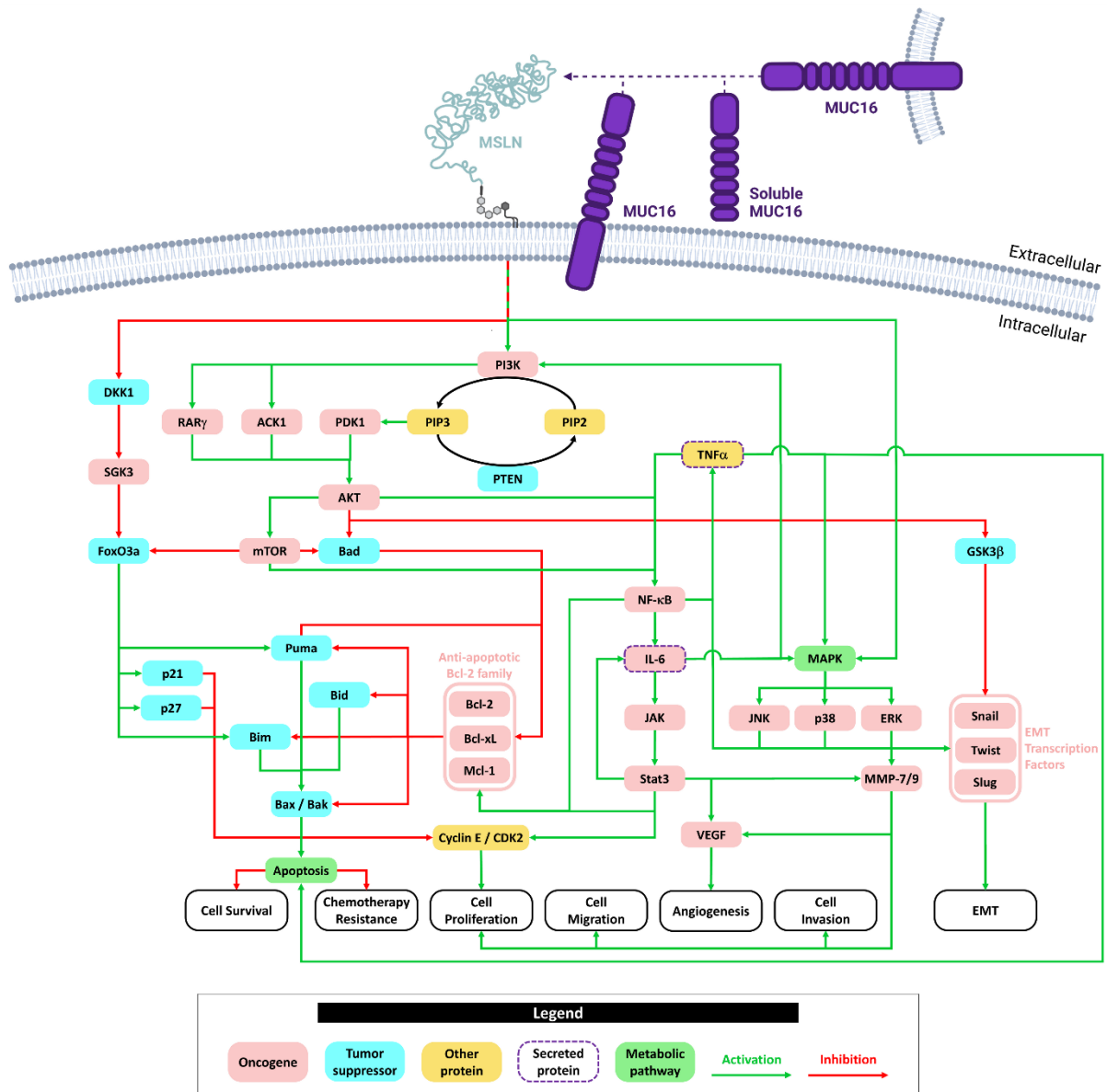
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1266 **Figure 2.** Prognostic value regarding MSLN overexpression in cancers. The cancers presented are those for which at least 3
 1267 studies have demonstrated the prognostic value of MSLN. Cancers are listed in descending order of publication demonstrating
 1268 a correlation between MSLN level expression and bad prognosis: Pancreatic;^{36-38,160-163} Colorectal;^{49,50,164-166} Breast;^{42,47,48,167-}
 1269 ¹⁷⁰ Ovarian;^{45,46,64,171,172} Gastric;¹⁷³⁻¹⁷⁶ and Mesothelioma.^{18,44,177-180}

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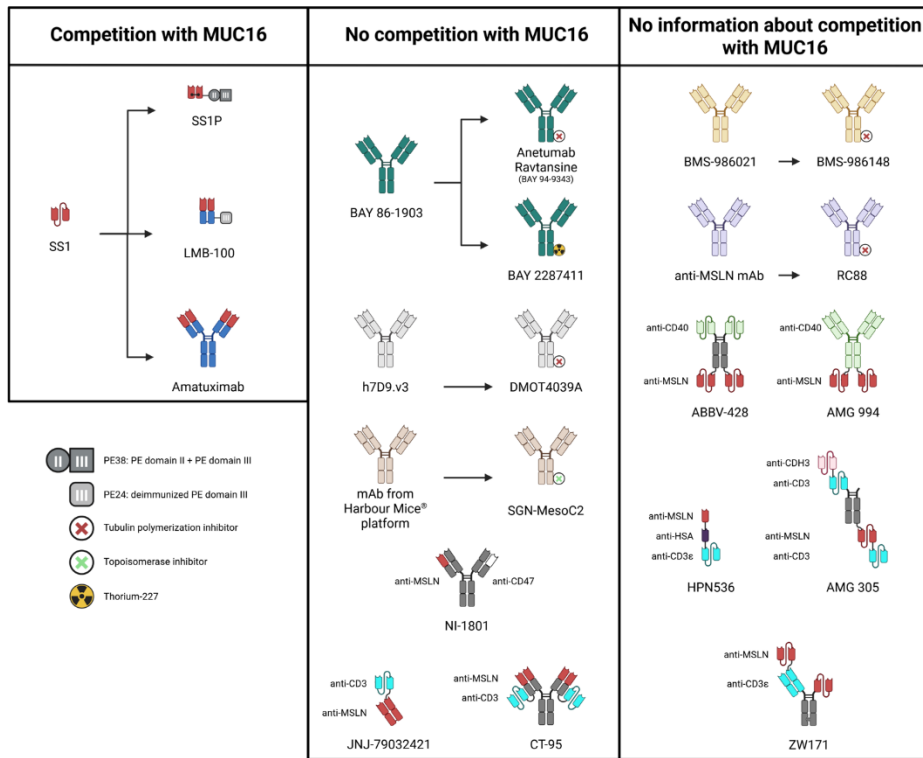
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1272 **Figure 3.** Schematic representation of MSLN-related metabolic pathways in cancer. Regarding apoptosis pathways, under
 1273 PI3K activation mesothelin induced AKT phosphorylation and regulates RARG and TNK2 (encoding RAR γ and ACK1),
 1274 known to phosphorylate and activate AKT, promoting cell survival and disease progression in pancreatic and hepatocellular
 1275 carcinoma.^{181,182} AKT activation, through RAR γ , ACK1, and PDK1 leads to the inhibition of the transcription factor Forkhead
 1276 box O3a (FoxO3a) through mTOR.¹⁸³ This inhibition is emphasized by MUC16-MSLN interaction leading to SGK3/FOXO3
 1277 pathway activation as DDK1 is inhibited stopping SGK3 kinase inhibition, a FoxO3a inhibitor.¹⁸⁴ Phosphorylated-FoxO3a is
 1278 translocated to the cytoplasm, ubiquitinated and degraded.¹⁸⁵ Thus, two BH3-only sensitizers are inhibited: Bad, by AKT and
 1279 mTOR, and Puma, no longer activated by FoxO3a.¹⁸⁶ In healthy condition, Bad and Puma inhibit anti-apoptotic multidomain
 1280 Bcl-2 proteins (eg. Bcl-2, Bcl-xL, Mcl-1). In MSLN-positive cancers, these proteins are no longer inhibited. Furthermore, anti-
 1281 apoptotic Bcl-2 proteins are activated by NF- κ B and Stat3 both overactivated in MSLN⁺ cancers. In normal condition, the
 1282 BH3-only activator family, made up of Bim, Bid and Puma, promote Bax/Bak heterodimerization leading to death cell.¹⁸⁶ In

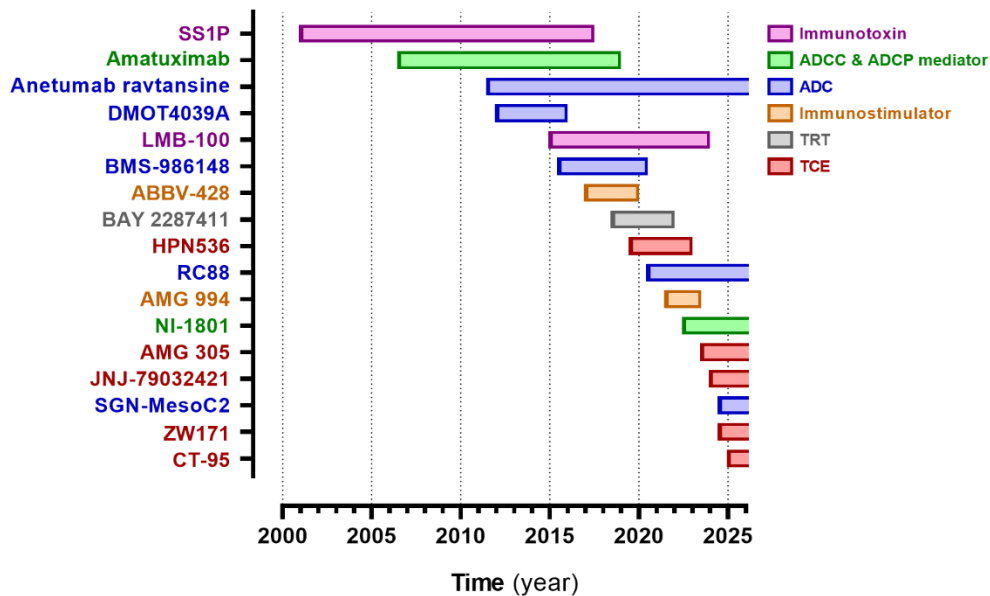
1283 MSLN-positive cancers, Bim is partly activated by FoxO3a, which is inhibited by MSLN, and the BH3-only activator family
1284 is inhibited by the now activated anti-apoptotic Bcl-2 proteins.¹⁸⁶ Thus, MSLN in cancer decreases apoptosis, thereby
1285 promoting cell survival. Furthermore, the expression level of MSLN has been demonstrated to be correlated with chemotherapy
1286 resistance.^{45,49,62,64} This phenomenon can be explained by the inhibition of the apoptosis pathway. Regarding cell proliferation
1287 pathways, AKT and mTOR activate NF- κ B pathway, leading to the production of IL-6 known to activate AKT through PI3K,
1288 generating previously discussed events.^{187,188} In pancreatic cancers, mesothelin expression level correlates with IL-6
1289 expression.⁵⁶ NF- κ B also generates a self-activation loop through TNF- α and promote anti-apoptotic Bcl-2 proteins.¹⁸⁹ Despite
1290 TNF- α overexpression, a protein considered as inducing death cells, tumor cells might survive using various mechanisms and
1291 may acquire resistance.⁵⁷ Autocrine IL-6 binds its receptor, inducing JAK phosphorylation, leading to Stat3 activation through
1292 homodimerization mediated by subunit phosphorylation.^{187,190} The IL-6/Stat3 pathway is renown in cancer for promoting cell
1293 survival, proliferation and migration.^{191,192} Stat3 acts as a transcription factor for oncogenes like IL-6, anti-apoptotic Bcl-2
1294 proteins and cyclin E, which, through the cyclin E/CDK2 complex, induces cell proliferation.⁵⁸ Under normal condition, cyclin
1295 E/CDK2 complex is closely regulated by p21 and p27, both activated by FoxO3a, inhibited in MSLN-positive cancers.¹⁹³ Cell
1296 proliferation, cell migration and cell invasion pathways appear closely related to MMP-7 and MMP-9.^{37,59} Mesothelin activates
1297 directly MAPK cascades, and indirectly through IL-6 and TNF- α , leading to ERK, JNK and p38 activation.^{189,194,195} These
1298 three proteins, along with Stat3, promote MMP-7 leading to cell migration and invasion.^{37,196} Additionally, cases of increased
1299 angiogenesis through MSLN have been reported, notably in PDAC.⁶⁰ IL-6 seems to play a role in angiogenesis mediated by
1300 MSLN as a correlation between IL-6 and VEGF expression in cancers is well established.¹⁹⁷ While no study has clearly proven
1301 a pathway involving MSLN, IL-6, Stat3, HIF-1 α , and VEGF, the Stat3, HIF-1 α , and VEGF correlation is well established in
1302 many cancers, suggesting MSLN could regulate angiogenesis through this same pathway.¹⁹⁸ Moreover, MMP-7 is also
1303 increasing angiogenesis, at least in part, by stimulating the proliferation of VEGF.¹⁹⁶ Finally, MSLN has been demonstrated to
1304 induce EMT by activating EMT transcription factors.^{62,63} This mechanism is known in cancer to be activated through AKT,
1305 which inhibits GSK3 β and activates NF- κ B, and through ERK, JNK, and p38.¹⁹⁹ *ACK1: Activated CDC42-associated Kinase*
1306 *1, Bad: Bcl-2 Associated agonist of cell Death, Bak: Bcl-2 Antagonist/Killer, Bax: Bcl-2 Associated X-protein, Bcl-2: B Cell*
1307 *Lymphoma-2, Bcl-xL: B Cell Lymphoma-extra Large, Bid: BH3 Interacting Domain death agonist, Bim: Bcl-2 Interacting*
1308 *Mediator of cell death, CDK2: Cyclin-Dependent Kinase 2, DKK1: Dickkopf 1, EMT: Epithelial-Mesenchymal Transition,*
1309 *ERK: Extracellular signal-Regulated Kinase, FoxO3a: Forkhead box class O 3a, GSK3 β : Glycogen Synthase Kinase-3 β , IL-*
1310 *6: Interleukin 6, JAK: Janus Kinase, JNK: c-Jun N-terminal Kinase, MAPK: Mitogen-Activated Protein Kinase, Mcl-1:*
1311 *Myeloid Cell Leukemia-1, MMP-7/9: Matrix Metalloproteinase-7 / Matrix Metalloproteinase-9, mTOR: Mammalian Target*
1312 *Of Rapamycin, NF- κ B: Nuclear Factor Kappa-light-chain-enhancer of activated B cells, PDK1: Phosphoinositide-Dependent*
1313 *protein Kinase-1, PI3K: Phosphoinositide 3-Kinase, PIP2: Phosphatidylinositol 4,5-bisphosphate, PIP3:*
1314 *Phosphatidylinositol-3,4,5-trisphosphate, PTEN: Phosphatase and Tensin homolog, Puma: p53 Upregulated Modulator of*

- 1315 *Apoptosis, RAR γ : Retinoic Acid Receptor γ , SGK3: Serum and Glucocorticoid inducible protein Kinase 3, Stat3: Signal*
- 1316 *Transducer and Activator of Transcription 3, TNF α : Tumor Necrosis Factor α .*
- 1317

A



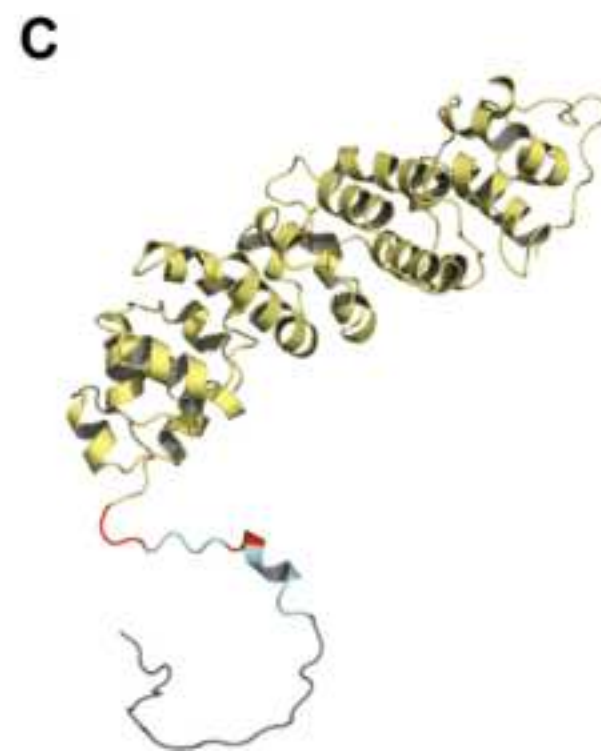
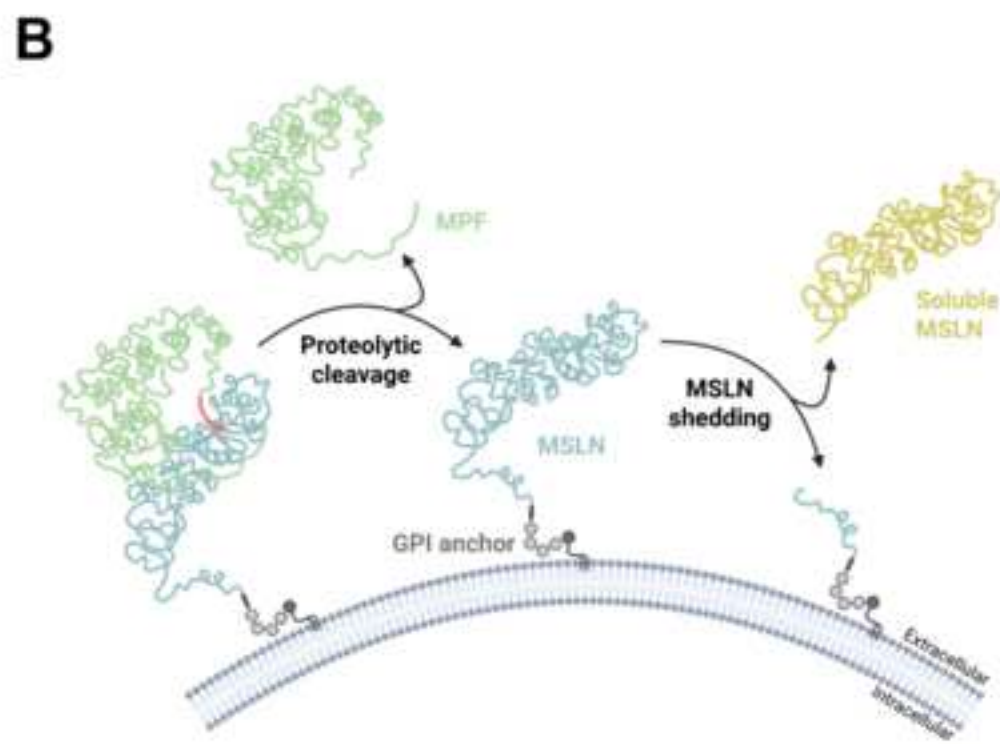
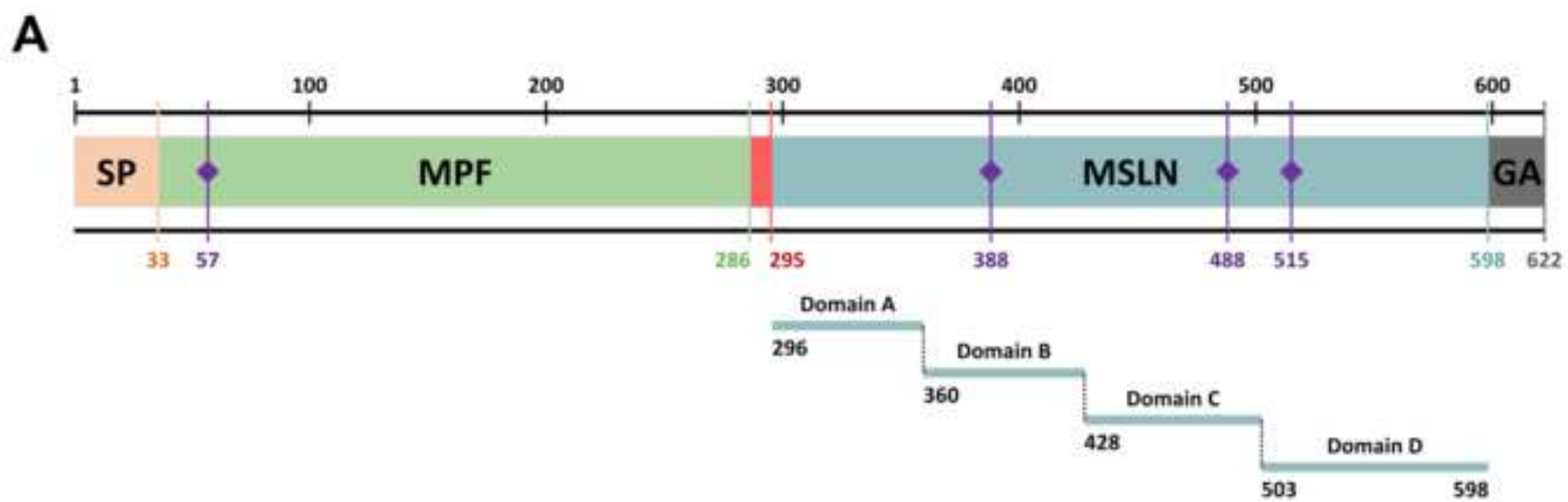
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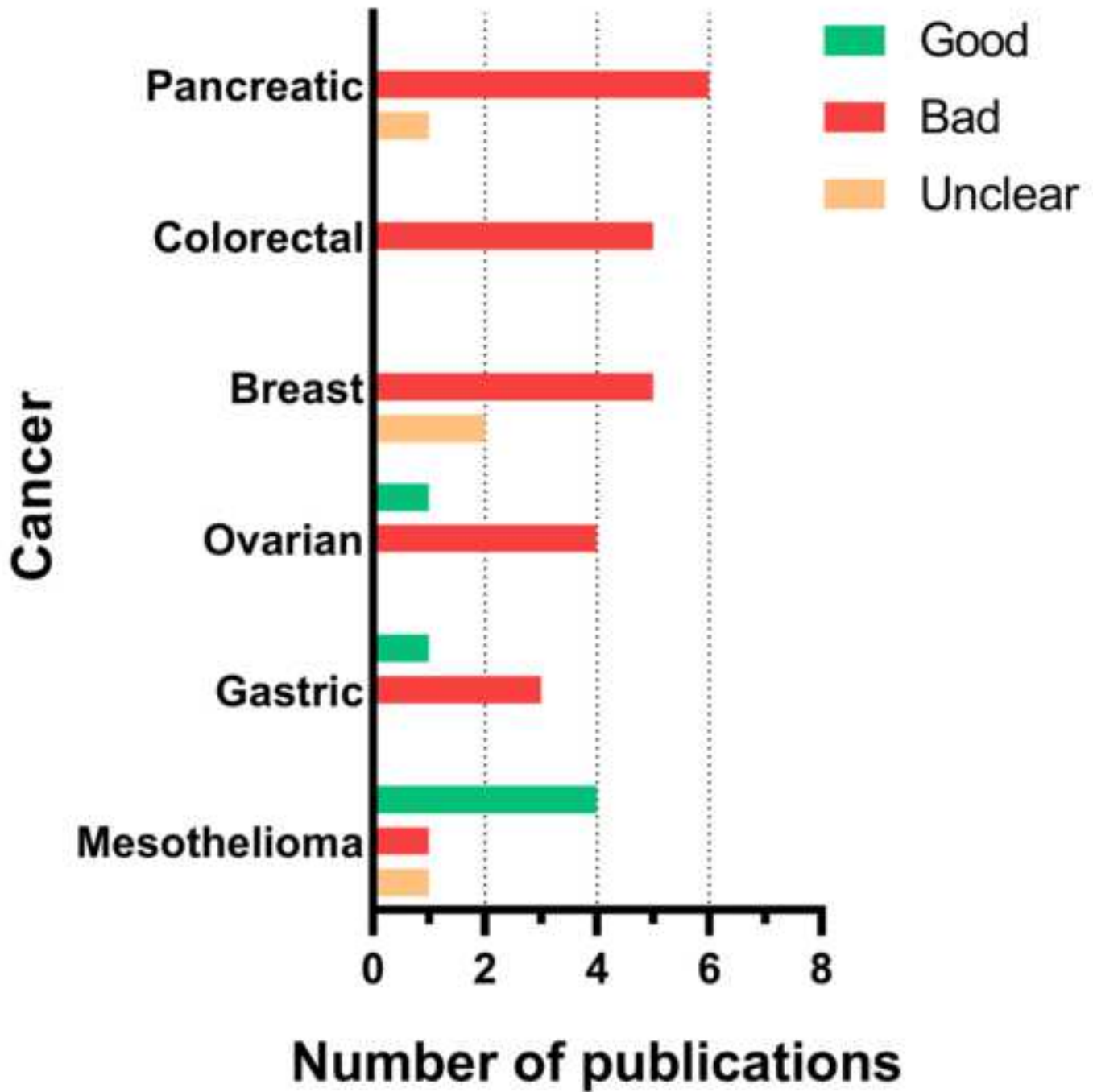


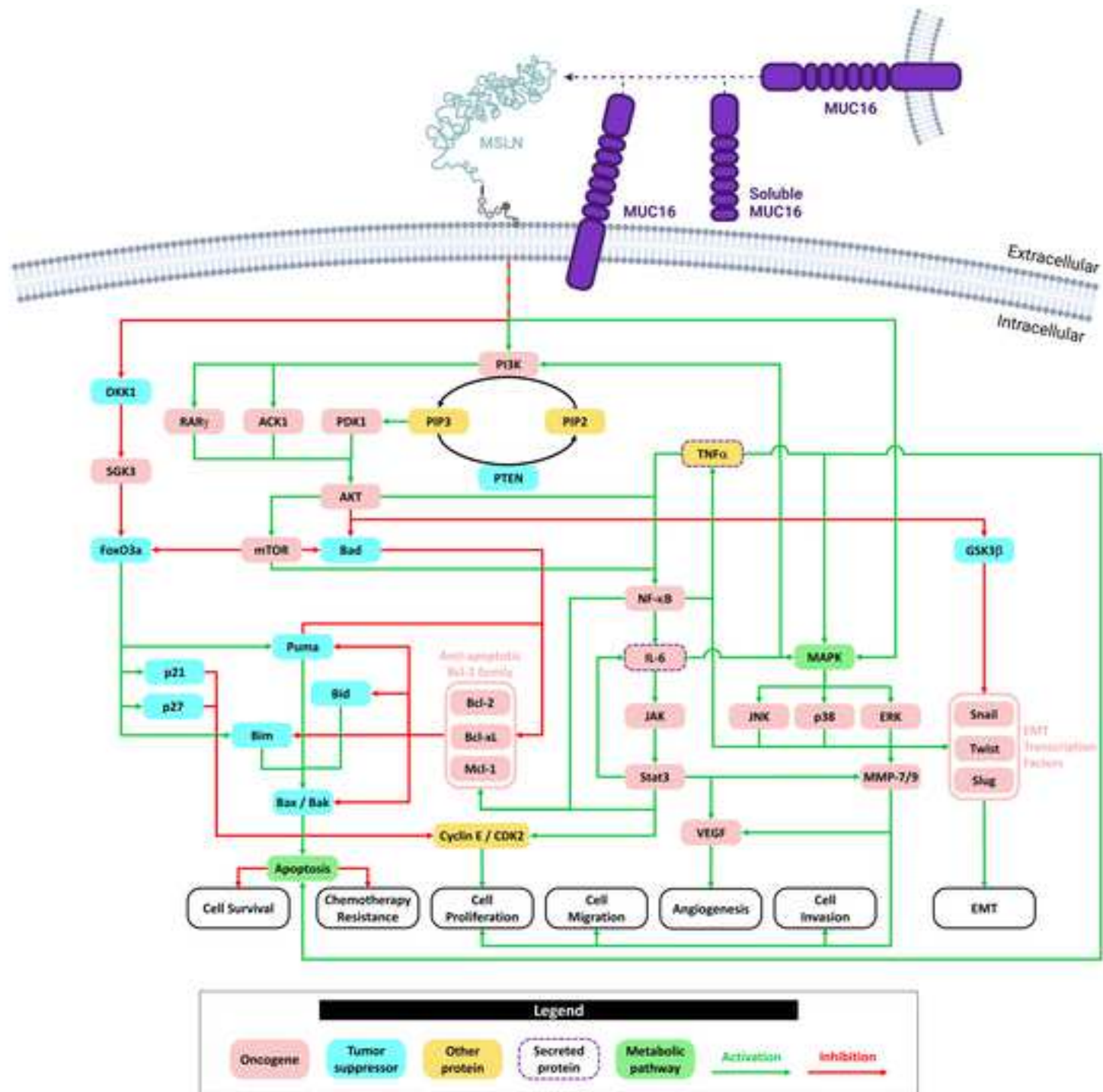
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1319 **Figure 4.** Schematic representation of antibody-based immunotherapies targeting MSLN that have advanced to the clinical
 1320 trial phase, along with the duration of their clinical study. (A) The molecules were separated based on the targeting epitope on
 1321 MSLN. Regarding molecules that compete with MUC16, based on SS1 three molecules were engineered: SS1P (immunotoxin,
 1322 dsFv conjugated to PE38), LMB-100 (immunotoxin, Fab conjugated to PE24), and amatuximab (ADCC and ADCP mediator,
 1323 chimeric human antibody). Regarding molecules that do not compete with MUC16, based on BAY 86-1903 two molecules

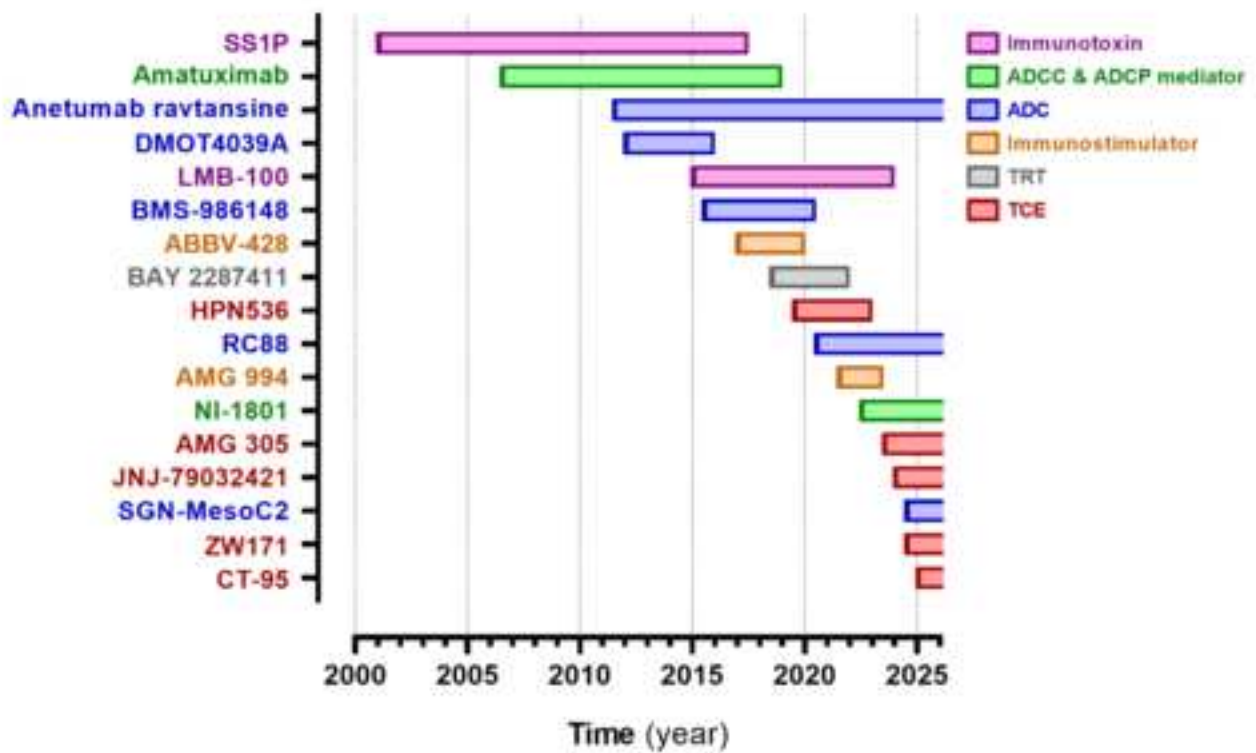
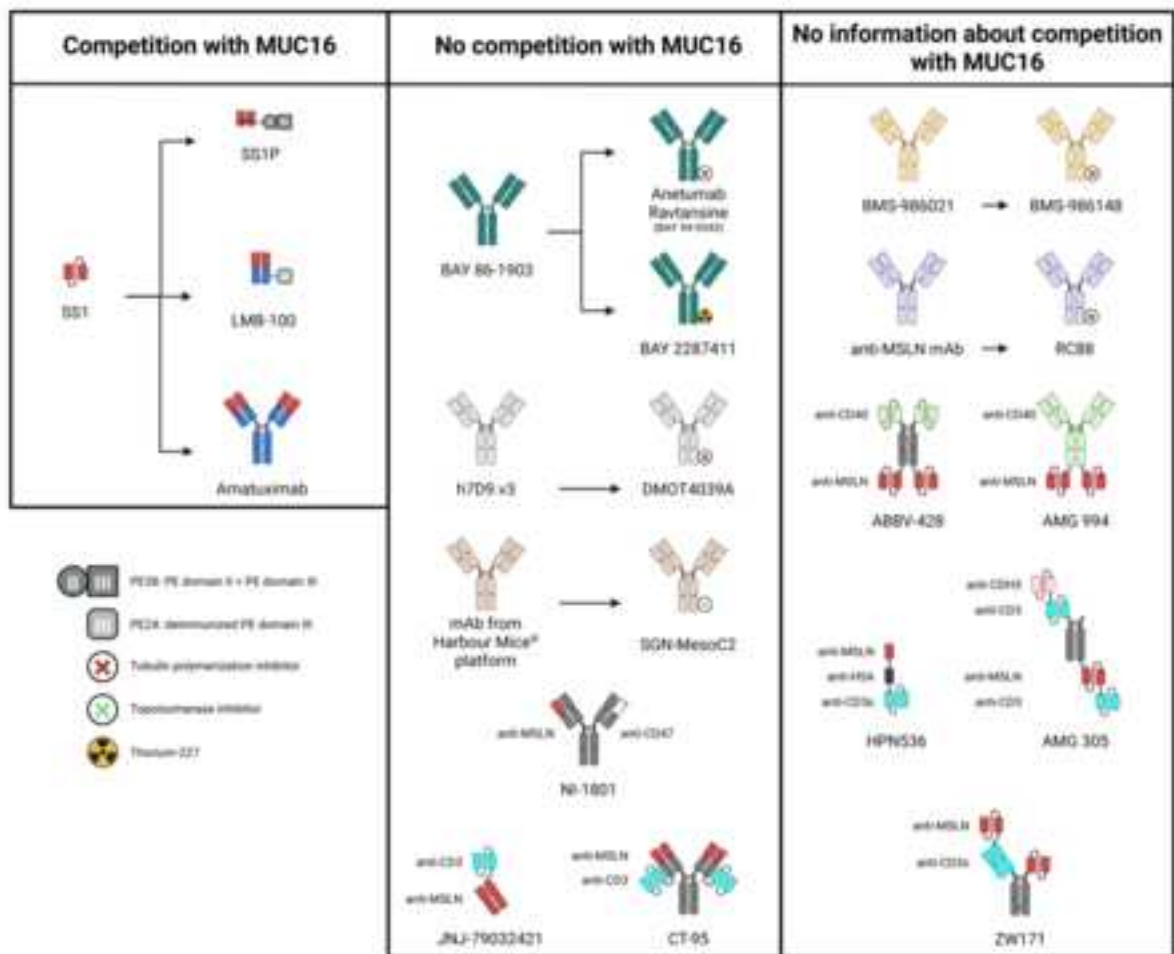
1324 were engineered: BAY 94-9343 (ADC, payload: DM4) and BAY 2287 (TRT, radionuclide: ²²⁷Th). Based on h7D9.v3,
1325 DMOT4039A was engineered (ADC, payload: MMAE). Concerning SGN-MesoC2 (ADC, payload: SGD-12280) and NI-1801
1326 (ADCC and ADCP mediator, bispecific), it is likely that the MSLN arm targets a membrane-proximal epitope on MSLN. JNJ-
1327 79032421 (TCE, bispecific) and CT-95 (TCE, bispecific) were engineered to target a membrane-proximal epitope on MSLN.
1328 For seven molecules no information was found about their competition with MUC16: BMS-986148 (ADC, payload: tubulysin),
1329 RC88 (ADC, payload: MMAE), ABBV-428 (immunostimulator, bispecific), AMG 994 (immunostimulator, bispecific),
1330 HPN536 (TCE, trispecific), AMG 305 (TCE, trispecific), and ZW171 (TCE, trispecific). **(B)** Representation of the clinical trial
1331 periods for the 17 anti-MSLN antibody-based immunotherapies. *ADC: antibody-drug conjugate, ADCC: Antibody-Dependent*
1332 *Cell-mediated Cytotoxicity, ADCP: Antibody-Dependent Cell-mediated Phagocytosis, DM4: ravtansine, dsFv: disulfide-*
1333 *stabilized Fv, HSA: Human Serum Albumin, ICE: immune cell-engager, MMAE: monomethyl auristatin E, MSLN: mesothelin,*
1334 *MUC16: mucin 16, PE: Pseudomonas exotoxin A, SGD-12280: novel topoisomerase 1 inhibitor, TCE: T-cell engager, TRT:*
1335 *targeted radionuclide therapy.*







A



1 Tumor-restricted overexpression of mesothelin, contrasting with limited normal tissue expression make
2 it a compelling target in oncology. Boisgard, Kerfelec and colleagues provide an up-to-date synthesis of
3 established and underdiscussed aspects of mesothelin biology, and clinical trial details. They also
4 underscore several questions critical for the future optimization of mesothelin-targeted therapies.