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MESOTELIOMA PLEURICO

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Immunotherapy and Malignant Pleural Mesothelioma

Introduction

Tumors have developed multiple mechanisms to avoid destruction by the **immune system**(1).
There are, in fact, various inhibitory pathways in the immune system that allow this complex system to tolerate cells and antigens physiologically present in the body, and not to mount an excessive immune response. These inhibitory pathways are essential because they allow the immune T-cells to block the growth of cancer cells; however, tumors use mechanisms to escape the control of the immune system, preventing the T-cells from using their cytotoxic activity against the tumor. Both the innate and adaptive immune responses can act against tumors.
A deficiency in cytotoxic T lymphocytes and natural killer cells can, in fact, increase tumor incidence.
One of the mechanisms by which tumors can escape the immune system is the expression of ligands that inhibit T-cell expression, such as CTLA-4 (cytotoxic T-lymphocyte antigen 4), PD-L1 (programmed death-ligand 1) and PD-1 (programmed death-1)(2). **Malignant Pleural Mesothelioma** (MPM) is considered an "inflammatory" tumor because it is often characterized by a prominent infiltration of lymphocytes, macrophages and T-cells(3 4).
The continuous, chronic inflammation of the mesothelial cells helps at first to promote, progress and transform these healthy cells into cancer cells. Similarly, the escape mechanisms from the immune system allow the tumor to evade the immune response from the host.
The role of the immune system in the biogenesis of MPM is also complex and multifaceted and appears to involve both the innate and adaptive immune responses(5 6). This is why research is being conducted in immune mechanisms that allow tumors to grow by escaping the body's control, with the goal of identifying suitable targets for an effective immunotherapy for MPM.
PD-L1 expression in MPM appears to be associated with a greater extent of disease at the time of presentation and with a greater incidence of sarcomatoid histology(7). This could be a plausible explanation for the poor prognosis seen in these cases.
Immunotherapy represents a new frontier in the treatment of cancer. The significant progress made in our understanding of the immune system has led to the development of new molecules that can increase the immune response of patients. Regardless of their genetic or metabolic disorders, many cancer patients may benefit from treatment because it is the immune response itself of the patient that is targeted and not the cancer cell.

Immune Check-Points

The term **immune-checkpoints** refers to a series of inhibitory pathways in the immune system that are crucial for maintaining self-tolerance and preventing the excessive, prolonged and potentially harmful activity of T-cells in peripheral tissues(8).
It is now clear that tumors can use these immune-checkpoints to evade the antitumor immune response, such as through the loss of expression of tumor-associated antigens (TAA) and/or major histocompatibility complex (MHC) antigens, or through the production of cytokines and the expression of new, inhibitory membrane molecules.
This continuous molecular remodeling phenomenon is known as **"cancer immunoediting"**, and consists of three main, consecutive phases:

- Elimination (complete destruction of the tumor cells by the host's immune system)
- Equilibrium (tumor cells selected by the T-cells become resistant to the immune system)
- Escape (the tumor cells give rise to clinically detectable lesions)(9)

The immune-checkpoints currently known to be involved in the development of lung cancer are the cytotoxic T-lymphocyte antigen-4 receptor **(CTLA-4)** and the programmed cell death-1 **(PD-1)**/programmed cell death-ligand 1 **(PD-L1)**.

Cytotoxic T lymphocyte antigen-4

Because immune-checkpoints are activated in most cases by a ligand-receptor interaction, they can be inhibited by antagonist antibodies or recombinant forms of ligands/receptors.
The CTLA-4 receptor, known also as CD152, is a member of the immunoglobulin (Ig) superfamily that is expressed on cytotoxic T lymphocytes(10 11 12). After binding with one of its ligands, B7-1 or B7-2 expressed on the antigen-presenting cells (APC), it transmits an inhibitory signal to the lymphocyte, thus helping to homeostatically regulate the immune response(13).
CTLA-4 plays a vital role in the maintenance of immune tolerance to the tumor(14). Specifically, the CTLA-4 receptor acts on the CD80 and CD86 costimulatory immune signals activated by the antigen-presenting cells, so they increase the activation threshold of the T lymphocytes. The systemic administration of anti-CTLA-4 inhibitors as monotherapy or in combination with other therapeutic cancer vaccines has been shown to induce a regression of melanoma and colon cancer in murine models(15 16).
In a Phase II study evaluating the activity of an anti-CTLA-4 antibody (tremelimumab) that enrolled 29 patients with chemotherapy-resistant MPM (28 pleural and 1 peritoneal)(17 18 19), objective clinical responses were observed only in 29 patients, however, stable disease was seen in 9 patients, or approximately 31% of patients with epithelial histology. The median overall survival rate at one year was 48% and 37% at two years.

Programmed death-1 receptor

PD-1 is a cell surface receptor belonging to the Ig superfamily that is expressed on T-cells and pro-B cells and binds two ligands, PD-L1 and PD-L2. PD-L1 is a transmembrane protein that binds to its PD-1 and B7.1 receptors on the surface of T-cells, deactivating them(20).
The PD-1 receptor stimulates the cells into inactivation by allowing the tumor cell to escape the surveillance of the immune system(21).
This receptor is activated by binding with its ligand: programmed death-ligand 1 (PD-L1), which is usually found in the tumor microenvironment on the surface of cancer cells(22).
Researchers have shown that PD-L1 is present on rat murine cells in vivo(23). PD-L1 expression increases in response to an increase in the concentration of interferon y (IFN)-? and the draining of T-cells into the tumor-draining lymph nodes, supporting the hypothesis that this is an important local immunosuppressive pathway.
The inhibitory action of PD-L1 on the different subpopulations of T-cells produces opposite effects on tumor progression and suggests that the immune suppression of the tumor is mediated by a specific subclass of T-cells.
Researchers have demonstrated that PD-L1 was expressed in approximately 40% of 106 samples of mesothelioma analyzed, all of which were tumors with sarcomatoid histology with a poor prognosis (5.0 months versus 14.5).

Treatments

Targeted immunotherapies

The first **immunopotentiation** study dates back to 1975, investigating the intrapleural administration of a vaccine consisting of irradiated and sterilized BCG, which led to a reduction in tumor growth, secondary to the activation of the immune system(25 26). Other studies followed the same line of research using Mycobacterium vaccae in combination with chemotherapy, for example (27 28 29). Another attempt at

potentiating the immune response is the use of cytokines, such as interferon(30 31 32 33). Viral vectors have also been used to increase the efficacy of therapy(34 35 36). Interleukin-2 has also been tested in this disease to activate the immune system against the tumor(37 38 39 40). Another cytokine used in immunopotentiation studies is GM-CSF (granulocyte/macrophage colony-stimulating factor)(41 42 43 44). Several studies have evaluated the possibility of increasing the expression of major histocompatibility molecules (MHCs) to increase T-cell clones with antitumor effect. Other studies have evaluated coadjuvant therapies such as CpGm oligodeoxynucleotide, CpG ODNs, Toll-like receptor 3 agonist, Toll-like receptor-7 agonist(47 48 49).
Immunomodulation involves modifying the lack of cell immunity within tumor sites(50 51).
The first **immunomodulation** studies were conducted in the 1990s, evaluating therapies such as autologous and allogeneic LAK cells to reduce pleural effusions(52). Other studies demonstrated the cytotoxic action of CTLs against MPM cells(53). More recently, research has been conducted on the activity of dendritic cells and their ability to protect antitumor immunity(54). Other studies have investigated CD40-activated follicular B-cells and autologous T-cells expressing a chimeric antigen receptor (CAR) (55 56).
Immunodepletion, often known as lymphodepletion, is another area of immunotherapy that involves eliminating the cells that have infiltrated the tumor foci(57). Promising studies have investigated the depletion of tumor-associated macrophages (TAMs) from the tumor mass and the possibility of reversing these cells from TAMs to M1 status(58 59). Similarly, other studies have demonstrated the utility of activating the TAMs infiltrating MPM tissue, promoting the release of cytokines and chemokines such as TNF-a, IFN-inducible protein 10 and IL6(60 61).

Vaccines

The discovery of mesothelin in the 1990s was very important because researchers hoped it could be used as a specific marker for MPM(62). Indeed, since this protein is also expressed in other tumor types, promising vaccines against these cancers were developed(63 64).
Notwithstanding the controversy surrounding the possible role of the SV40 virus in the biogenesis of MPM, SV40 antigens have been tested as potential immunological targets for the disease(65 66 67 68).
Wilms' tumor 1 protein (WT1) has also been investigated as a potential diagnostic marker specifically for MPM(69 70 71 72).
One of the treatment strategies for this disease is developing cellular vaccines and a number of studies have evaluated the effects of cancer cells transfected with IL-4, IL-2, GM-CSF, B7-1, as vaccine cells(73 74 75 76).
Dendritic-cell based vaccines have also been investigated to activate both Th1 cells and CTLs by activating phagocytosis and apoptosis(77 78). Researchers have also reported that MPM cells in apoptosis could be used as potent inducers of anticancer CTLS(79).

Monoclonal Antibodies

Monoclonal antibodies have long been used in immunohistochemical techniques to obtain a differential diagnosis between MPM and other tumors(80).
The first approach in this area was to test a monoclonal antibody and in combination with a toxin as a potential immunotherapy(81). Other studies evaluated monoclonal antibody reagents in combination with the 7D3 transferrin receptor, ricin A or doxorubicin(82 83).
The monoclonal antibody K1 targeting mesothelin has been investigated as a potential immunotherapy for MPM(84).
Various biomedical engineering methods have been used to design monoclonal antibodies combined with toxins to construct chimeric agents(85 86 87 88).
Fusion proteins that can target cancer cells when subjected to radiotherapy have also been developed, and

whose results suggest greater cytotoxic effects with fewer side effects(89).
Researchers have developed an anti-MPM immunotoxin by combining Pseudomonas exotoxin with a fragment of the anti-mesothelin antibody(90). Human mesothelin has also been combined with an antibody targeting a dendritic cell receptor, increasing the potential for vaccination with mesothelin(91).
Wingless-type (Wnt) protein, which is involved in various cancers, is another important antigen against which monoclonal antibodies have been developed(92 93 94).
Antibodies against the surface antigen CD26, which is involved in tumor growth, have also been developed(95).
Other examples of monoclonal antibodies described above include CTLA-4 inhibitors (ipilimumab and tremelimumab), and PD-L1 (pembrolizumab) and PD-1 receptor (nivolumab) inhibitors(96 97 98 99).
Tremelimumab was investigated in a single-arm Phase II study of pretreated patients but the primary endpoint of objective response rate was not achieved(100). The disease control rate of patients treated with tremelimumab was around 31%, progression-free survival (PFS) was 6.2 months (95% CI 1.3–11.1), and the mean survival time (mST) was 10.7 months (0.0–21.9) (101). Studies of anti-PD-L1 and PD-1 monoclonal antibodies are also currently underway(102).

Recent clinical trials

Below is a short list of recent clinical studies that have been completed or currently ongoing. Please see www.clinicaltrials.gov for further details about these studies.

Completed studies:

- **Dendritic Cell-based Immunotherapy in Mesothelioma** (tumor lysate-loaded autologous dendritic cells).
- **Dendritic Cell-based Immunotherapy Combined With Low-dose Cyclophosphamide in Patients With Malignant Mesothelioma** (DC+CTX)(103)
(Allogeneic Tumor Cell Vaccine (K562); Drug: Celecoxib; Drug: cyclophosphamide)(104 105 106).
- **Pilot Study of Allogeneic Tumor Cell Vaccine With Metronomic Oral Cyclophosphamide and Celecoxib in Patients Undergoing Resection of Lung and Esophageal Cancers, Thymic Neoplasms, and Malignant Pleural Mesotheliomas** (Allogeneic Tumor Cell Vaccine (K562); Drug: Celecoxib; Drug: cyclophosphamide).(104 105 106).
- **Cyclophosphamide Plus Vaccine Therapy in Treating Patients With Advanced Cancer** (allogeneic tumor cell vaccine; Biological:autologous tumor cell vaccine; Biological: recombinant interferon alfa; Biological: recombinant interferon gamma; Biological:sargramostim; Drug:cyclophosphamide).
- **Study of Safety and Tolerability of Intravenous CRS-207 in Adults With Selected Advanced Solid Tumors Who Have Failed or Who Are Not Candidates for Standard Treatment** (Biological: CRS-207, Live-attenuated Listeria monocytogenes expressing human Mesothelin).
- **Safety and Immune Response to a Multi-component Immune Based Therapy (MKC1106-PP) for Patients With Advanced Cancer** (PSMA/PRAME).

Ongoing studies:

- **Safety and Efficacy of Listeria in Combination With Chemotherapy as Front-line Treatment for Malignant Pleural Mesothelioma** (Immunotherapy plus chemotherapy; Biological: Immunotherapy with cyclophosphamide plus chemotherapy).
- **Dendritic Cells Loaded With Allogeneous Cell Lysate in Mesothelioma Patients (MesoCancerVac).**
- **CAR T Cell Receptor Immunotherapy Targeting Mesothelin for Patients With Metastatic Cancer** (Fludarabine; Biological:Anti-mesothelin CAR; Drug:Cycolphosphamide; Drug:Aldesleukin).
- **Genetically Modified T Cells in Treating Patients With Stage III-IV Non-small Cell Lung Cancer or Mesothelioma** (Aldesleukin; Biological: Autologous WT1-TCRc4 Gene-transduced CD8-positive

Tcm/TnLymphocytes; Drug:Cyclophosphamide; Other: Laboratory Biomarker Analysis; Procedure: Therapeutic Conventional Surgery).
- **The Anti-CTLA-4 Monoclonal Antibody Tremelimumab in Malignant Mesothelioma (Tremelimumab).**
- **Adjuvant Tumor Lysate Vaccine and Iscomatrix With or Without Metronomic Oral Cyclophosphamide and Celecoxib in Patients With Malignancies Involving Lungs, Esophagus, Pleura, or Mediastinum** (H1299 Lysate Vaccine; Drug:Cyclophosphamide; Drug:Celecoxib).
- **A Study of Tremelimumab Combined With the Anti-PD-L1 MEDI4736 Antibody in Malignant Mesothelioma (NIBIT-MESO-1)** (tremelimumab plus MEDI4736).
- **A Clinical Study With Tremelimumab as Monotherapy in Malignant Mesothelioma** (Tremelimumab).
- **Phase II Study of Adjuvant WT-1 Analog Peptide Vaccine in MPM Patients After MSK10-134** (WT-1-vaccine Montanide+GM-CSF; Biological:Montanide adjuvant + GM-CSF).
- **Randomized Study of Adjuvant WT-1 Analog Peptide Vaccine in Patients With Malignant Pleural Mesothelioma (MPM) After Completion of Combined Modality Therapy** (WT-1-vaccine Montanide +GM-CSF; Biological: Montanide adjuvant + GM-CSF (This arm is closed))
- **Nivolumab in Patients With Recurrent Malignant Mesothelioma**.
- **Gene Therapy for Pleural Malignancies** (Adenoviral-mediated Interferon-beta; Biological:SCH721015).
- **Four Versus Six Cycles of Pemetrexed/Platinum for MPM** (Pemetrexed/platinum chemotherapy).
- **Intrapleural AdV-tk Therapy in Patients With Malignant Pleural Effusion** (AdV-tk+ valacyclovir).

Response to Immunotherapy

Immunotherapy has changed the way in which we measure objective responses in both clinical studies as well as clinical practice. Melanoma immunotherapy studies have shown that the antitumor response is not seen until weeks or months after the start of treatment, with a survival gain seen after several months. This is because immunotherapy drugs activate the immune system, which in turn elicits a cell-mediated response.
The response to treatment is measured by using **RECIST or WHO criteria**. During immunotherapy, these conventional criteria are not capable of adequately measuring the presence of peritumoral inflammatory infiltrate which may mimic a pseudoprogression, a phenomenon typically encountered during this type of treatment. To avoid this problem, **immune-related response criteria (irRC)** have been developed, in which an initial radiographic progression, in other words the appearance of new lesions and/or an increase in the size of existing lesions, in the absence of clinical progression, must be confirmed by another evaluation(107). The correct use of irRC can identify long-term survivors, including patients who would be considered by conventional criteria to be progressing and so may not continue to benefit from targeted treatment(108).
Another aspect evidenced by immunotherapy is the need to understand whether this treatment is suitable for everyone or only certain patients who are most likely to benefit from immunotherapy.

Conclusions

The association between the immune system and MPM is complex and multifaceted. Immunity certainly plays a key role in inducing damage to the DNA of mesothelial cells, which is strongly and pathogenically linked to exposure to asbestos.
Dividing the immune system into innate response and adaptive response also helps balance the inhibition and activation of the cells involved in this complex mechanism.
Although the results from immunotherapy studies have not yet been earth-shattering, they are nevertheless extremely promising and provide a new view of this disease in anticipation that it will lead to new therapeutic approaches.

References

1. Hanahan D., Weinberg R.A., Hallmarkis of cancer: the next generation. Cell 2011;144:646-74
2. Currie AJ, Prosser A, McDonnell A, Cleaver AL, Robinson BW, Freeman GJ, et al. Dual control of antitumor CD8 T cells through the programmed death-1/programmed death-ligand 1 pathway and immunosuppressive CD4 T cells: regulation and counterregulation. J Immunol 2009;183:7898–908.
3. Gr.goire M. What's the place of immunotherapy in malignant mesothelioma treatments? Cell Adh Migr 2010; 4: 153–61.
4. Cornelissen R, Heuvers ME, Maat AP, et al. New roads open up for implementing immunotherapy in mesothelioma. Clin Dev Immunol 2012; 2012: 927240.
5. H. Bielefeldt-Ohmann, A.G. Jarnicki, D.R. Fitzpatrick, Molecular pathobiology and immunology of malignant mesothelioma, J. Pathol. 178 (1996) 369–378.
6. M.J. Garlepp, C.C. Leong, Biological and immunological aspects of malignant mesothelioma, Eur. Respir. J. 8 (1995) 643–650.
7. Mansfield AS, Roden A, Peikert T, Sheinin YM, Harrington SM, Krco CJ, et al. B7–H1 expression in malignant pleural mesothelioma is associated with sarcomatoid histology and poor prognosis. J Thorac Oncol 2014;9:1036–40.
8. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012; 12:252-264.
9. Dun GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol 2004; 22:329-360.
10. Calabr. L, Danielli R, Sigalotti L, Maio M. Clinical studies with anti-CTLA-4 antibodies in non-melanoma indications. Semin Oncol 2010; 37: 460–67.
11. Hoos A, Ibrahim R, Korman A, et al. Development of ipilimumab: contribution to a new paradigm for cancer immunotherapy. Semin Oncol 2010; 37: 53–46.
12. Lynch TJ, Bondarenko I, Luft A, et al. Ipilimumab in combination with paclitaxel and carboplatin as fi rst-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J Clin Oncol 2012; 30: 2046–54.
13. Reck M, Paz-Ares L. Immunologic checkpoint blockade in lung cancer. Semin Oncol 2015; 42:402-417.
14. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immu¬nity by CTLA-4 blockade. Science. 1996;271(5256):1734–1736.
15. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immu¬nity by CTLA-4 blockade. Science. 1996;271(5256):1734–1736.
16. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med. 1999;190(3):355–366.
17. Ribas A, Keff ord R, Marshall MA, et al. Phase III randomized, clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol 2013; 31: 616–22.
18. Tarhini AA, Cherian J, Moschos SJ, et al. Safety and effi cacy of combination immunotherapy with interferon alfa-2b and tremelimumab in patients with stage IV melanoma. J Clin Oncol 2012; 30: 322–28.
19. Reck M, Paz-Ares L. Immunologic checkpoint blockade in lung cancer. Semin Oncol 2015; 42:402-417.
20. Reck M, Paz-Ares L. Immunologic checkpoint blockade in lung cancer. Semin Oncol 2015; 42:402-417.
21. Currie AJ, Prosser A, McDonnell A, et al. Dual control of antitumor CD8 T cells through the programmed death-1/programmed death-ligand 1 pathway and immunosuppressive CD4 T cells: regulation and counterregulation. J Immunol. 2009;183(12):7898–7908.
22. Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. Nat Rev Immunol. 2008;8(6):467–477.
23. Currie AJ, Prosser A, McDonnell A, et al. Dual control of antitumor CD8 T cells through the programmed death-1/programmed death-ligand 1 pathway and immunosuppressive CD4 T cells: regulation and counterregulation. J Immunol. 2009;183(12):7898–7908.
24. Mansfield AS, Peikert T, Roden A, et al. Programmed cell death 1 ligand 1 expression and association with survival in mesothelioma. European Lung Cancer Conference 2014; 2014 Mar 26–29; Geneva, Switzerland. J Thorac Oncol. 2014;9(Supplement 9):S7–S52. Abstract #1270.
25. M.V. Pimm, R.W. Baldwin, BCG therapy of pleural and peritoneal growth of transplanted rat tumours, Int. J. Cancer 15 (1975) 260–269.
26. I. Webster, J.W. Cochrane, K.R. Burkhardt, Immunotherapy with BCG vaccine in 30 cases of mesothelioma, S. Afr. Med. J. 61 (1982) 277–278.
27. M.E. O'Brien, A. Saini, I.E. Smith, A. Webb, K. Gregory, R. Mendes, C. Ryan, K. Priest, K.V. Bromelow, R.D. Palmer, N. Tuckwell, D.A. Kennard, B.E. Souberbielle, A randomized phase II study of SRL172 (Mycobacterium vaccae) combined with chemotherapy in patients with advanced inoperable nonsmall- cell lung cancer and mesothelioma, Br. J. Cancer 83 (2000) 853–857
28. M.E. O'Brien, A. Saini, I.E. Smith, A. Webb, K. Gregory, R. Mendes, C. Ryan, K. Priest, K.V. Bromelow, R.D. Palmer, N. Tuckwell, D.A. Kennard, B.E. Souberbielle, A randomized phase II study of SRL172 (Mycobacterium vaccae) combined with chemotherapy in patients with advanced inoperable nonsmall- cell lung cancer and mesothelioma, Br. J. Cancer 83 (2000) 853–857
29. H.S. Pandha, P. Mortimer, B. Souberbeille, P. McCoubrie, M.E. O'Brien, Cutaneous toxicity after intradermal vaccination with Mycobacterium vaccae against lung cancer and malignant mesothelioma, Br. J. Dermatol. 144 (2001) 648–649
30. R. Mendes, M.E. O'Brien, A. Mitra, A. Norton, R.K. Gregory, A.R. Padhani, K.V. Bromelow, A.R. Winkley, S. Ashley, I.E. Smith, B.E. Souberbielle, Clinical and immunological assessment of Mycobacterium vaccae (SRL172) with chemotherapy in patients with malignant mesothelioma, Br. J. Cancer 86 (2002) 336–341.
31. R. Rosso, R. Rimoldi, F. Salvati, M. De Palma, A. Cinquegrana, G. Nicoló, A. Ardizzoni, U. Fusco, A. Capaccio, R. Centofanti, M. Neri, A.R. Cruciani, L. Maisto, Intrapleural natural beta interferon in the treatment of malignant pleural effusions, Oncology 45 (1988) 253–256..
32. A. Hand, K. Pelin, K. Mattson, K. Linnainmaa, Interferon (IFN)-alpha and IFNgamma in combination with methotrexate: in vitro sensitivity studies in four human mesothelioma cell lines, Anticancer Drugs 6 (1995) 77–82
33. F. Gattacceca, Y. Pilatte, C. Billard, I. Monnet, S. Moritz, J. Le Carrou, M. Eloit, M.C. Jaurand, Ad-IFN gamma induces antiproliferative and antitumoral responses in malignant mesothelioma, Clin. Cancer Res. 8 (2002) 3298–3304
34. D.H. Sterman, A. Recio, A.R. Haas, A. Vachani, S.I. Katz, C.T. Gillespie, G. Cheng, J. Sun, E. Moon, L. Pereira, X. Wang, D.F. Heitjan, L. Litzky, C.H. June, R.H. Vonderheide, R.G. Carroll, S.M. Albelda, A phase I trial of repeated intrapleural adenoviral-mediated interferon-beta gene transfer for mesothelioma and metastatic pleural effusions, Mol. Ther. 18 (2010) 852–860.
35. C.L. Willmon, V. Saloura, Z.G. Fridlender, P. Wongthida, R.M. Diaz, J. Thompson, T. Kottke, M. Federspiel, G. Barber, S.M. Albelda, R.G. Vile, Expression of IFN-beta enhances both efficacy and safety of oncolytic vesicular stomatitis virus for therapy of mesothelioma, Cancer Res. 69 (2009) 7713–7720.
36. H. Li, K.W. Peng, D. Dingli, R.A. Kratzke, S.J. Russell, Oncolytic measles viruses encoding interferon beta and the thyroidal sodium iodide symporter gene for mesothelioma virotherapy, Cancer Gene. Ther. 17 (2010) 550–558.
37. P. Lissoni, S. Barni, A. Ardizzoia, F. Paolorossi, E. Tisi, S. Crispino, G. Tancini, Intracavitary administration of Interleukin-2 as palliative therapy for neoplastic effusions, Tumori 30 (1992) 118–120.
38. R. Nano, E. Capelli, M. Civallero, G. Terzuolo, E. Volpini, C. Nascimbene, P. Cremaschi, Effects of Interleukin-2 for the treatment of malignant mesothelioma, Oncol. Rep. 5 (1998) 489–492.
39. S.H. Goey, A.M. Eggermont, C.J. Punt, R. Slingerland, J.W. Gratama, R. Oosterom, R. Oskam, R.L. Bolhuis, G. Stoter, Intrapleural administration of Interleukin 2 in pleural mesothelioma: a phase I-II study, Br. J. Cancer 72 (1995) 1283–1288.
40. C. Jackaman, C.S. Bundell, B.F. Kinnear, A.M. Smith, P. Filion, D. van Hagen, B.W. Robinson, D.J. Nelson, IL-2 intratumoral immunotherapy enhances CD8+ T cells

that mediate destruction of tumor cells and tumor-associated vasculature: a novel mechanism for IL-2, J. Immunol. 171 (2003) 5051–5063.
41. G. Alì, L. Boldrini, M. Lucchi, A. Picchi, M. Dell'Omodarme, M.C. Prati, A. Mussi, V. Corsi, G. Fontanini, Treatment with Interleukin-2 in malignant pleural mesothelioma: immunological and angiogenetic assessment and prognostic impact, Br. J. Cancer 101 (2009) 1869–1875.
42. J.A. Davidson, A.W. Musk, B.R. Wood, S. Morey, M. Ilton, L.L. Yu, P. Drury, K. Shilkin, B.W. Robinson, Intralesional cytokine therapy in cancer: a pilot study of GM-CSF infusion in mesothelioma, J. Immunother. 21 (1998) 389–398.
43. P.L. Triozzi, W. Aldrich, K.O. Allen, J. Lima, D.R. Shaw, T.V. Strong, Antitumor activity of the intratumoral injection of fowlpox vectors expressing a triad of costimulatory molecules and granulocyte/macrophage colony stimulating factor in mesothelioma, Int. J. Cancer 113 (2005) 406–414.
44. A. Yoshimoto, K. Kasahara, K. Saito, M. Fujimura, S. Nakao, Granulocyte colony-stimulating factor-producing malignant pleural mesothelioma with the expression of other cytokines, Int. J. Clin. Oncol. 10 (2005) 58–62.
45. A. Powell, J. Creaney, S. Broomfield, I. Van Bruggen, B. Robinson, Recombinant GM-CSF plus autologous tumor cells as a vaccine for patients with mesothelioma, Lung. Cancer 52 (2006) 189–197
46. C. Leong, J. Marley, S. Loh, B. Robinson, M. Garlepp, Induction and maintenance of T-cell response to a nonimmunogenic murine mesothelioma cell line requires expression of B7-1 and the capacity to upregulate class II major histocompatibility complex expression, Cancer Gene. Ther. 3 (1996) 321–330.
47. C.C. Leong, J.V. Marley, S. Loh, N. Milech, B.W. Robinson, M.J. Garlepp, Transfection of the gene for B7-1 but not B7-2 can induce immunity to murine malignant mesothelioma, Int. J. Cancer 71 (1997) 476–482.
48. R.G. van der Most, H. Himbeck, S. Aarons, S.J. Carter, I. Larma, C. Robinson, A. Currie, R.A. Lake, Antitumor efficacy of the novel chemotherapeutic agent coramsine is potentiated by cotreatment with CpG-containing oligodeoxynucleotides, J. Immunother. 29 (2006) 134–142.
49. G.W. Stone, S. Barzee, V. Snarsky, C. Santucci, B. Tran, R.S. Kornbluth, Regression of established AB1 murine mesothelioma induced by peritumorale injections of CpG oligodeoxynucleotide either alone or in combination with poly(I:C) and CD40 ligand plasmid DNA, J. Thorac. Oncol. 4 (2009) 802–808.
50. M. Mamede, T. Saga, T. Ishimori, Y. Nakamoto, N. Sato, T. Higashi, T. Mukai, H. Kobayashi, J. Konishi, Differential uptake of (18)F-fluorodeoxyglucose by experimental tumors xenografted into immunocompetent and immunode ficient mice and the effect of immunomodification, Neoplasia 5 (2003) 179–183.
51. C.J. Bradish, K. Allner, R. Fitzgeorge, Immunomodification and the expression nof virulence in mice by defined strains of semliki forest virus: the effects of cyclophosphamide, J. Gen. Virol. 28 (1975) 225–237.
52. H. Yanagawa, S. Sone, K. Fukuta, Y. Nishioka, T. Ogura, Local adoptive immunotherapy using lymphokine-activated killer cells and Interleukin-2 against malignant pleural mesothelioma: report of two cases, Jpn. J. Clin. Oncol. 21 (1991) 377–383
53. M. Tani, H. Tanimura, H. Yamaue, S. Mizobata, M. Yamamoto, M. Iwahashi, K. Ura, Y. Nagai, T. Tsunoda, H. Wakasaki, K. Nanjo, K. Fujino, S. Yukawa, Successful immunochemotherapy for patients with malignant mesothelioma: report of two cases, Surg. Today 28 (1998) 647–651.
54. J.P. Hegmans, A. Hemmes, J.G. Aerts, H.C. Hoogsteden, B.N. Lambrecht, Immunotherapy of murine malignant mesothelioma using tumor lysate
55. Y. Zhao, E. Moon, C. Carpenito, C.M. Paulos, X. Liu, A.L. Brennan, A. Chew, R.G. Carroll, J. Scholler, B.L. Levine, S.M. Albelda, C.H. June, Multiple injections of electroporated autologous T cells expressing a chimeric antigen receptor mediate regression of human disseminated tumor, Cancer Res. 70 (2010) 9053–9061.
56. G.E. Plautz, P.A. Cohen, S. Shu, Considerations on clinical use of T cell immunotherapy for cancer, Arch. Immunol. Ther. Exp. 51 (2003) 245–257.
57. N.R. Miselis, Z.J. Wu, N. Van Rooijen, A.B. Kane, Targeting tumor-associated macrophages in an orthotopic murine model of diffuse malignant mesothelioma, Mol. Cancer Ther. 7 (2008) 788–799.
58. J.D. Veltman, M.E. Lambers, M. van Nimwegen, R.W. Hendriks, H.C. Hoogsteden, J.P. Hegmans, J.G. Aerts, Zoledronic acid impairs myeloid differentiation to tumour-associated macrophages in mesothelioma, Br. J. Cancer 103 (2010) 629–641.
59. A.S. Jassar, E. Suzuki, V. Kapoor, J. Sun, M.B. Silverberg, L. Cheung, M.D. Burdick, R.M. Strieter, L.M. Ching, L.R. Kaiser, S.M. Albelda, Activation of tumor-associated macrophages by the vascular disrupting agent 5,6- dimethylxanthenone-4-acetic acid induces an effective CD8+ T-cellmediated antitumor immune response in murine models of lung cancer and mesothelioma, Cancer Res. 65 (2005) 11752–11761.
60. K. Chang, I. Pastan, Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers, Proc. Natl. Acad. Sci. USA 93 (1996) 136–140.
61. K. Chang, I. Pastan, Molecular cloning and expression of a cDNA encoding a protein detected by the K1 antibody from an ovarian carcinoma (OVCAR-3)cell line, Int. J. Cancer 57 (1994) 90–97.
62. P. Argani, C. Iacobuzio-Donahue, B. Ryu, C. Rosty, M. Goggins, R.E. Wilentz, S.R. Murugesan, S.D. Leach, E. Jaffee, C.J. Yeo, J.L. Cameron, S.E. Kern, R.H. Hruban, Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE), Clin. Cancer Res. 7 (2001) 3862–3868.
63. J. Yokokawa, C. Palena, P. Arlen, R. Hassan, M. Ho, I. Pastan, J. Schlom, K.Y. Tsang, Identification of novel human CTL epitopes and their agonist epitopes of mesothelin, Clin. Cancer Res. 11 (2005) 6342–6351.
64. Adams, A. Gibbs, Simian virus 40 detection in human mesothelioma: reliability and significance of the available molecular evidence, Front. Biosci. 6 (2001) E12–E22.
65. A.G. Elmishad, M. Bocchetta, H.I. Pass, M. Carbone, Polio vaccines, SV40 and human tumours, an update on false positive and false negative results, Dev. Biol. 123 (2006) 109–117.
66. Y.C. Xie, C. Hwang, W. Overwijk, Z. Zeng, M.H. Eng, J.J. Mulé, M.J. Imperiale, N.P. Restifo, M.G. Sanda, Induction of tumor antigen-specific immunity in vivo by a novel vaccinia vector encoding safety-modified simian virus 40 T antigen, J. Natl. Cancer Inst. 91 (1999) 169–175.
67. R.K. Bright, E.T. Kimchi, M.H. Shearer, R.C. Kennedy, H.I. Pass, SV40 Tagspecific cytotoxic T lymphocytes generated from the peripheral blood of malignant pleural mesothelioma patients, Cancer Immunol. Immunother. 50 (2002) 682–690.
68. N.G. Ordóñez, Immunohistochemical diagnosis of epithelioid mesothelioma: an update, Arch. Pathol. Lab. Med. 129 (2005) 1407–1414.
69. R. Bei, A. Scardino, TAA polyepitope DNA-based vaccines: a potential tool for cancer therapy, J. Biomed. Biotechnol. 2010 (2010) 102758.
70. R.J. May, T. Dao, J. Pinilla-Ibarz, T. Korontsvit, V. Zakhaleva, R.H. Zhang, P. Maslak, D.A. Scheinberg, Peptide epitopes from the Wilms' tumor 1 oncoprotein stimulate CD4+ and CD8+ T cells that recognize and kill human malignant mesothelioma tumor cells, Clin. Cancer Res. 13 (2007) 4547–4555.
71. L.M. Krug, T. Dao, A.B. Brown, P. Maslak, W. Travis, S. Bekele, T. Korontsvit, V. Zakhaleva, J. Wolchok, J. Yuan, H. Li, L. Tyson, D.A. Scheinberg, WT1 peptide vaccinations induce CD4 and CD8 T cell immune responses in patients with mesothelioma and non-small cell lung cancer, Cancer Immunol. Immunother. 59 (2010) 1467–1479.
72. C. Palumbo, R. Bei, A. Procopio, A. Modesti, Molecular targets and targeted therapies for malignant mesothelioma, Curr. Med. Chem. 15 (2008) 855–867.
73. A.K. Nowak, R.A. Lake, H.L. Kindler, B.W. Robinson, New approaches for mesothelioma: biologics, vaccines, gene therapy, and other novel agents, Semin. Oncol. 29 (2002) 82–96.
74. C.L. Chang, T.C. Wu, C.F. Hung, Control of human mesothelin-expressing tumors by DNA vaccines, Gene. Ther. 14 (2007) 1189–1198.
75. S. Mukherjee, D. Nelson, S. Loh, I. van Bruggen, L.J. Palmer, C. Leong, M.J. Garlepp, B.W. Robinson, The immune anti-tumor effects of GM-CSF and B7–1 gene transfection are enhanced by surgical debulking of tumor, Cancer Gene. Ther. 8 (2001) 580–588.
76. M. Grégoire, C. Ligeza-Poisson, N. Juge-Morineau, R. Spisek, Anti-cancer therapy using dendritic cells and apoptotic tumour cells: pre-clinical data in human

mesothelioma and acute myeloid leukaemia, Vaccine 21 (2003) 791–794.
77. F. Ebstein, C. Sapede, P.J. Royer, M. Marcq, C. Ligeza-Poisson, I. Barbieux, L. Cellerin, G. Dabouis, M. Grégoire, Cytotoxic T cell responses against mesothelioma by apoptotic cell-pulsed dendritic cells, Am. J. Respir. Crit. Care Med. 169 (2004) 1322–1330.
78. F. Ebstein, C. Sapede, P.J. Royer, M. Marcq, C. Ligeza-Poisson, I. Barbieux, L. Cellerin, G. Dabouis, M. Grégoire, Cytotoxic T cell responses against mesothelioma by apoptotic cell-pulsed dendritic cells, Am. J. Respir. Crit. Care Med. 169 (2004) 1322–1330.
79. J.E. King, N. Thatcher, C.A. Pickering, P.S. Hasleton, Sensitivity and specificity of immunohistochemical markers used in the diagnosis of epithelioid mesothelioma: a detailed systematic analysis using published data, Histopathology 48 (2006) 223–232.
80. S.M. Hsu, P.L. Hsu, X. Zhao, C.S. Kao-Shan, J. Whang-Peng, Establishment of human mesothelioma cell lines (MS-1, -2) and production of a monoclonal antibody (anti-MS) with diagnostic and therapeutic potential, Cancer Res. 48 (1988) 5228–5236.
81. T.W. Griffin, J. Collins, F. Bokhari, M. Stochl, A.B. Brill, T. Ito, G. Emond, H. Sands, Intraperitoneal immunoconjugates, Cancer Res. 50 (1990) 1031–1038.
82. T.W. Griffin, M. Stocl, J. Collins, J. Fernandes, V.E. Maher, Combined antitumor therapy with the chemotherapeutic drug doxorubicin and an anti-transferrin receptor immunotoxin: in vitro and in vivo studies, J. Immunother. 11 (1992) 12–18.
83. U. Brinkmann, K. Webber, A. Di Carlo, R. Beers, P. Chowdhury, K. Chang, V. Chaudhary, M. Gallo, I. Pastan, Cloning and expression of the recombinant FAb fragment of monoclonal antibody K1 that reacts with mesothelin present on mesotheliomas and ovarian cancers, Int. J. Cancer 71 (1997) 638–644.
84. P.S. Chowdhury, J.L. Viner, R. Beers, I. Pastan, Isolation of a high-affinity stable single-chain Fv specific for mesothelin from DNA-immunized mice by phage display and construction of a recombinant immunotoxin with anti-tumor activity, Proc. Natl. Acad. Sci. USA 95 (1998) 669–674.
85. P.S. Chowdhury, G. Vasmatzis, R. Beers, B. Lee, I. Pastan, Improved stability and yield of a Fv-toxin fusion protein by computer design and protein engineering of the Fv, J. Mol. Biol. 281 (1998) 917–928.
86. R. Hassan, W. Ebel, E.L. Routhier, R. Patel, J.B. Kline, J. Zhang, Q. Chao, S. Jacob, H. Turchin, L. Gibbs, M.D. Phillips, S. Mudali, C. Iacobuzio-Donahue, E.M. Jaffee, M. Moreno, I. Pastan, P.M. Sass, N.C. Nicolaides, L. Grasso, Preclinical evaluation of MORAb-009, a chimeric antibody targeting tumor-associated mesothelin, Cancer Immun. 7 (2007) 20–30.
87. R. Hassan, S.J. Cohen, M. Phillips, I. Pastan, E. Sharon, R.J. Kelly, C. Schweizer, S. Weil, D. Laheru, Phase I clinical trial of the chimeric anti-mesothelin monoclonal antibody MORAb-009 in patients with mesothelin-expressing cancers, Clin. Cancer Res. 16 (2010) 6132–6138.
88. N. Sato, R. Hassan, D.B. Axworthy, K.J. Wong, S. Yu, L.J. Theodore, Y. Lin, L. Park, M.W. Brechbiel, I. Pastan, C.H. Paik, J.A. Carrasquillo, Pretargeted radioimmunotherapy of mesothelin-expressing cancer using a tetravalent single-chain Fv-streptavidin fusion protein, J. Nucl. Med. 46 (2005) 1201– 1209.
89. T.K. Bera, J. Williams-Gould, R. Beers, P. Chowdhury, I. Pastan, Bivalent disulfide-stabilized fragment variable immunotoxin directed against mesotheliomas and ovarian cancer, Mol. Cancer Ther. 1 (2001) 79–84
90. B. Wang, J.M. Kuroiwa, L.Z. He, A. Charalambous, T. Keler, R.M. Steinman, The human cancer antigen mesothelin is more efficiently presented to the mouse immune system when targeted to the DEC-205/CD205 receptor on dendritic cells, Ann. N.Y. Acad. Sci. 1174 (2009) 6–17.
91. P. Polakis, Wnt signaling and cancer, Genes Dev. 14 (2000) 1837–1851.
92. B. He, L. You, K. Uematsu, Z. Xu, A.Y. Lee, M. Matsangou, F. McCormick, D.M. Jablons, A monoclonal antibody against Wnt-1 induces apoptosis in human cancer cells, Neoplasia 6 (2004) 7–14.
93. J. Mazieres, L. You, B. He, Z. Xu, S. Twogood, A.Y. Lee, N. Reguart, S. Batra, I. Mikami, D.M. Jablons, Wnt2 as a new therapeutic target in malignant pleural mesothelioma, Int. J. Cancer 117 (2005) 326–332.
94. T. Inamoto, T. Yamada, K. Ohnuma, S. Kina, N. Takahashi, T. Yamochi, S. Inamoto, Y. Katsuoka, O. Hosono, H. Tanaka, N.H. Dang, C. Morimoto, Humanized anti-CD26 monoclonal antibody as a treatment for malignant mesothelioma tumors, Clin. Cancer Res. 13 (2007) 4191–4200.
95. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011; 364: 2517–26.
96. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363: 711–23.
97. Lebbe C, Weber JS, Maio M, et al. Five-year survival rates for patients (pts) with metastatic melanoma (MM) treated with ipilimumab (ipi) in phase II trials. Ann Oncol 2012; 23: abstr 1116PD.
98. Maio M, Bondarenko I, Robert C, et al. Four-year survival update for metastatic melanoma (MM) patients (pts) treated with ipilimumab (ipi) plus dacarbazine (DTIC) on phase 3 study CA184-024.Ann Oncol 2012; 23: abstr 1127P.
99. Calabro` L, Morra A, Fonsatti E, Cutaia O, Amato G, Giannarelli D, et al. Tremelimumab for patients with chemotherapy-resistant advanced malignant mesothelioma: an open-label, single-arm, phase 2 trial. Lancet Oncol 2013;14: 1104–11.
100. Maio M, Scherpereel A, Di Pietro A, Vasey P, Tsao AS, Calabro` L, et al. Randomized, double-blind, placebo-controlled study of tremelimumab for second- and third-line treatment of unresectable pleural or peritoneal mesothelioma. J Clin Oncol 2014;32:5s (suppl; abstr TPS7609^).
101. Kindler HL, Zuo Z, Khattri A, Keck MK, Vigneswaran W, Husain AN, et al. T-cell inflamed phenotype and PDL1 expression in malignant mesothelioma. J Clin Oncol 2014;32 (suppl; abstr 7589).
102. Hegmans JP, Veltman JD, Lambers ME, de Vries IJ, Figdor CG, Hendriks RW, Hoogsteden HC, Lambrecht BN, Aerts JG. Consolidative dendritic cell-based immunotherapy elicits cytotoxicity against malignant mesothelioma. Am J Respir Crit Care Med. 2010 Jun 15;181(12):1383-90. doi: 10.1164/rccm.200909-1465OC. Epub 2010 Feb 18.
103. Hegmans JP, Hemmes A, Aerts JG, Hoogsteden HC, Lambrecht BN. Immunotherapy of murine malignant mesothelioma using tumor lysate-pulsed dendritic cells. Am J Respir Crit Care Med. 2005 May 15;171(10):1168-77. Epub 2005 Mar 11.
104. Flores RM, Zakowski M, Venkatraman E, Krug L, Rosenzweig K, Dycoco J, Lee C, Yeoh C, Bains M, Rusch V. Prognostic factors in the treatment of malignant pleural mesothelioma at a large tertiary referral center. J Thorac Oncol. 2007 Oct;2(10):957-65.
105. Kelsen DP, Winter KA, Gunderson LL, Mortimer J, Estes NC, Haller DG, Ajani JA, Kocha W, Minsky BD, Roth JA, Willett CG; Radiation Therapy Oncology Group; USA Intergroup. Long-term results of RTOG trial 8911 (USA Intergroup 113): a random assignment trial comparison of chemotherapy followed by surgery compared with surgery alone for esophageal cancer. J Clin Oncol. 2007 Aug 20;25(24):3719-25.
106. Manegold C, Thatcher N. Survival improvement in thoracic cancer: progress from the last decade and beyond. Lung Cancer. 2007 Aug;57 Suppl 2:S3-5.
107. Wolchok JD, Hoos A, O'Day S et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009; 15:7412-7415.
108. McCormick KA, Fleming B. Clinical practice guidelines. The Agency for Health Care Policy and Research fosters the development of evidence-based guidelines. Health Progress 1992; 73:30-40.