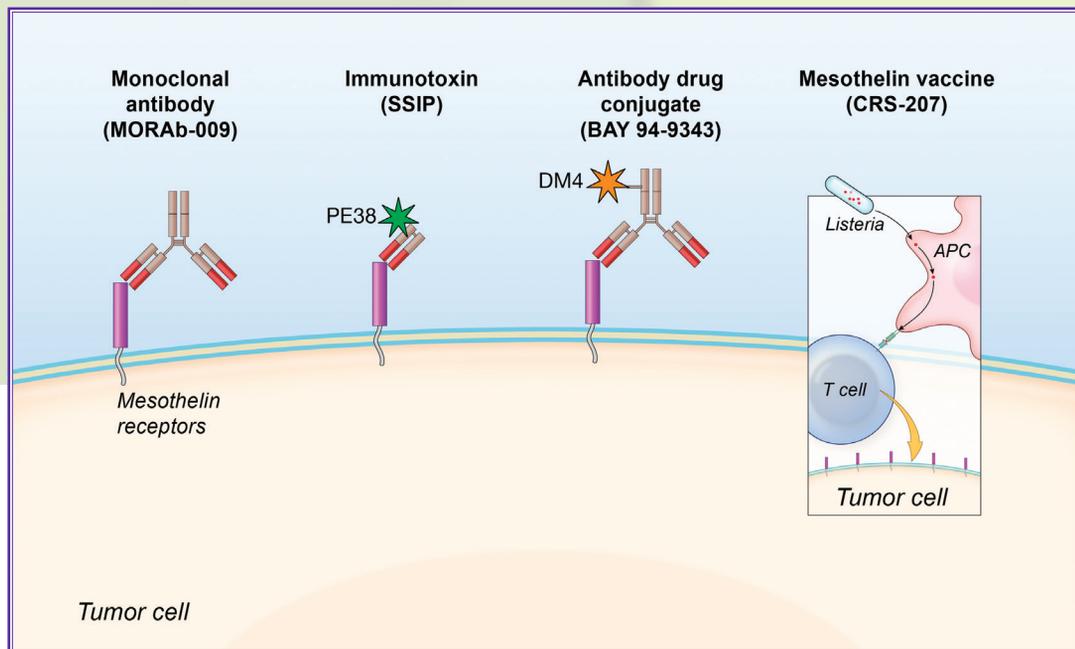


# Precision in Targeting with Anti-Mesothelin Therapies

For two decades, Raffit Hassan, M.D., Co-Chief of CCR's Thoracic and Gastrointestinal Oncology Branch, and Ira Pastan, M.D., Co-Chief of CCR's Laboratory of Molecular Biology (LMB) have been systematically plotting a new era of targeted cancer therapeutics. Building on Pastan's discovery of mesothelin, a protein that is expressed almost exclusively by cancer cells in the adult, Hassan has led efforts to capitalize on this rare selectivity by exploiting mesothelin-directed "smart bombs" developed in the Pastan lab to attack solid tumors, while sparing healthy tissue.

Early clinical trials are validating mesothelin's potential as a selective cancer target. These trials are increasingly showing that mesothelin-directed agents can be used effectively in mesothelioma and have the potential to treat other malignancies, including ovarian, lung, and pancreatic cancers. Hassan and Pastan hope that these new agents will do for solid tumors what precision therapies are already achieving in various blood cancers.



Different strategies to target mesothelin for treatment of mesothelioma

## A New Target for Solid Tumors

Pastan discovered mesothelin in 1994 during a search for new drug targets in ovarian cancer. At the time, he was collaborating with Mark Willingham, M.D., previously a

Senior Investigator in CCR and now at the Wake Forest Baptist Medical Center's Comprehensive Cancer Center, in N.C. While studying ovarian cancer cells in immunized mice, the team encountered a unique antibody. Though it bound readily

to cancer cells, this antibody—now called mAB K1—ignored normal cells from almost all healthy tissues. In fact, the only normal cells expressing the antibody's target antigen—a 40-kilodalton cell surface glycoprotein—were mesothelial

(Photo: R. Baer)



Raffit Hassan, M.D., and Ira Pastan, M.D.

cells from the pleura (which lines the lungs), the pericardium (which lines the heart), and the peritoneum (which lines the abdominal cavity). That finding got Pastan's attention, given that patients can lose mesothelial cells during cancer treatment without experiencing life-threatening consequences. (See "A Better Immunotoxin," *CCR connections* Vol. 2, No. 1).

Pastan and colleagues sequenced the target gene, now called mesothelin, but even today, much remains to be discovered about its biology. Deleting mesothelin has no apparent effect in mice or their offspring, so the protein does not appear to be necessary for normal growth and development. Yet, according to Pastan, many tumors express mesothelin, and mounting evidence implicates it in cancer progression and metastatic spread.

Shortly after mesothelin's discovery, Hassan—then a Clinical Fellow in CCR's Medical Oncology Branch—joined Pastan's laboratory and began to investigate if mesothelin expression in tumor cells could be exploited for cancer therapy. Hassan knew that mAB K1 homed in on mesothelin-expressing tumors in mice. So he chemically attached the antibody to a potent bacterial poison,

called PE38, to build an immunotoxin that kills malignant cells by inhibiting their ability to synthesize proteins. The new therapy eliminated cancer cells in mice, but using mAB K1 also posed clinical drawbacks: it bound poorly to mesothelin, and with its large size, it did not penetrate easily into the murky interiors of solid tumors.

To solve the issue of size, Pastan's Postdoctoral Fellow, Partha Chowdhury, Ph.D., isolated a smaller protein fragment with much higher mesothelin binding affinity. Dubbed SS1, the fragment was linked to PE38 to create a new immunotoxin that proved to be highly effective against cancer cells in preclinical studies. This complex, which they called SS1(dsFv)PE38, or SS1P, has since been the focus of extensive clinical research.

### Better Results with Combination Treatments

In 2002, Hassan launched a mesothelioma clinic at the NIH Clinical Center. Now one of the largest mesothelioma clinics in the U.S., it offers care to approximately 100 new patients each year, most of them are enrolled in clinical trials investigating mesothelin-directed treatments.

Hassan chose mesothelioma as a "proof-of-concept" disease for evaluating mesothelin-directed therapy, in part because new treatments are badly needed for this rare and aggressive malignancy. "Findings from mesothelioma studies can be easily translated into other cancers that also express mesothelin," he explained. "Our ultimate goal is to treat a range of solid tumors with mesothelin-targeted approaches."

Hassan's first published phase 1 study showed that SS1P monotherapy is safe, but only minimally effective in human patients. Robert Kreitman, M.D., Senior Investigator in LMB, conducted a phase 1 clinical trial of SS1P using a slightly different schedule of administration that produced similar results. According to Pastan, SS1P monotherapy is less effective than desired because after just a few doses, the patient's immune system recognizes and then inactivates SS1P's foreign bacterial toxin. "So we started asking 'What are we going to do with this agent that by itself has low efficacy in solid tumors?'" Pastan recalled. The answer, Pastan and Hassan concluded, was to give SS1P in combination with other drugs.

In ongoing research, the two scientists have shown that SS1P in combination with chemotherapy completely eliminates mesothelin-positive tumors in mice. Based on these findings, they designed a new clinical trial that enrolls newly diagnosed mesothelioma patients who are also given standard chemotherapy for their disease: pemetrexed and cisplatin. Results for the trial, which is now closed, will soon be published. "The data look promising," Hassan reported. "Patients treated with the combination do better than what you would expect from chemotherapy alone." Chemotherapy breaks up the tumor cell's exterior layer to facilitate SS1P's access to the tumor's interior.

(Photo: R. Baer)



Raffit Hassan, M.D., and his team meet with Kristi Lescalleet and her husband, Roy Lescalleet, to discuss her case. From left to right: Jeannette Nashed, N.P., Andrew Miller, M.D., Raffit Hassan, M.D., Kristi Lescalleet, Roy Lescalleet, Yvonne Mallory, R.N., and Lisa Bengtson, R.N.

While more effective than SS1P alone, this combination regime leaves untouched the immune response generated against the foreign toxin at the heart of SS1P. But Daniel Fowler, M.D., Senior Investigator in CCR's Experimental Transplantation and Immunology Branch, has found a novel way to suppress this immune response, which Hassan and Pastan have incorporated into their therapeutic strategy.

## Delaying Immune Responses

Fowler developed his immunosuppressive method while working with allogeneic stem cell transplants, which are procedures that deliver bone marrow-derived stem cells into patients whose own marrow has been damaged by disease. Immune reactions against foreign stem cells (obtained from donors), also complicate these procedures. But in preclinical and clinical studies, Fowler has found that pretreating with pentostatin and cyclophosphamide suppresses and delays the body's immune response to perceived threats. Pioneered at NCI and used in treating different leukemias, pentostatin primes T cells to commit suicide by

apoptosis in response to fairly minor challenges, including low doses of cyclophosphamide. Fowler's method is to give pentostatin first and then to follow with cyclophosphamide over several days. "That combination gives us a safe and effective way to bring the immune system down over time," he explained. Moreover, the two drugs preserve the neutrophil and monocyte arms of the immune system, so that patients can avoid near-term risks of infection arising from T cell depletion.

Pastan reasoned that pentostatin and cyclophosphamide would also delay immunity against the SS1P toxin.

The results of their pilot study to test Fowler's immune suppression technique on the development of SS1P antibodies surpassed the team's expectations and were recently published in the journal *Science Translational Medicine*. "The results were very exciting and unexpected in this group of heavily pretreated patients who had failed all standard therapy," said Hassan. "Three of the 10 patients had major tumor regressions and are alive more than two years later, which is unprecedented for this tumor type."

In 2009, Kristi Lescalleet, a 55-year-old wife and mother of two grown children, was diagnosed with pleural mesothelioma, which is a cancer of the lining of the lungs. For Lescalleet and her family, the diagnosis was unexpected. "I had never even heard of mesothelioma," said Lescalleet, who attributed her initial symptoms—shortness of breath, a cough, and back pain—to bronchitis or pneumonia. Lescalleet underwent treatment and did not develop anti-SS1P antibodies. "This was a very good result—something we have never seen before," Hassan said. Kristi continues to be in partial remission more than two years later. She has received no treatment since she went on the protocol and continues to maintain the partial response without any additional therapy. "I have been feeling just great," said Lescalleet, who has once again resumed an active lifestyle.

Mary Hesdorffer, Executive Director of the Mesothelioma Applied Research Foundation, referred Lescalleet to CCR. She agrees that Lescalleet and other patients have enjoyed "unexpectedly positive responses" to mesothelin-directed treatments. "You would not expect to see this type of response in patients

with advanced disease,” she said. “That is what makes these treatments so exciting. But we have to be cautious—we still do not know if the findings will translate to the general mesothelioma population.”

Based on the results of their pilot study, Hassan and his colleagues have moved directly into a phase 2 clinical trial to evaluate the efficacy of this regimen in patients with pleural and peritoneal mesothelioma.

### Other Approaches to Target Mesothelin

In addition to SS1P, Hassan is evaluating other mesothelin-directed agents that also look promising. A chimeric antibody called MORAb-009, or Amatuximab, for instance, which was developed by the biotech firm Morphotek, in collaboration with LMB, is now in phase 2 clinical trials for mesothelioma in combination with pemetrexed and cisplatin, with Hassan as Principal Investigator. Hassan also collaborates on a mesothelin-directed cancer vaccine, CRS-207, developed by scientists at Aduro Biotech, Inc. This vaccine is being evaluated in a phase 1 clinical trial in combination with chemotherapy for newly diagnosed patients with mesothelioma. Finally, Hassan is also working on a new antibody-drug conjugate called BAY 94-9394. Developed by Bayer Healthcare, this compound is currently in a phase 1 clinical trial at



(Photo: R. Bauer)

The Hassan laboratory staff: Neetu Kalra, Ph.D., and Jingli Zhang, Research Biologist

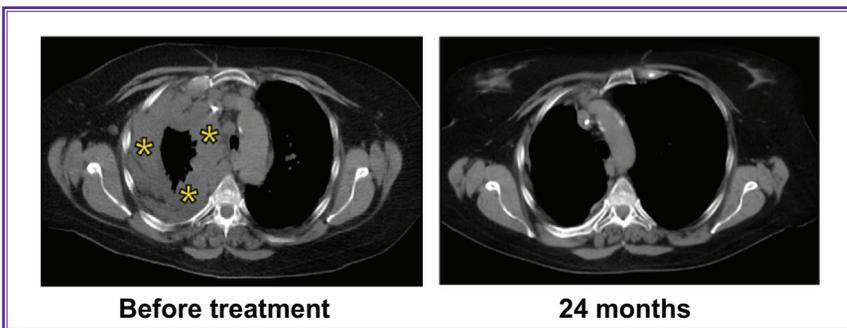
NCI, Sarah Cannon Research Institute in Nashville, Tenn., and the University of Texas M.D. Anderson Cancer Center in Houston. BAY94-9394 links a mesothelin-directed antibody with a potent chemotherapy agent called DM4. Preclinical research showed the drug was highly active against mesothelin-expressing cell lines and tumors. Now the phase 1 study will assess safety, pharmacokinetics, and maximum tolerated doses in patients with advanced solid tumors. In addition, Steven Rosenberg, M.D., Ph.D., Chief of CCR’s Surgery Branch, in collaboration with Pastan and Hassan, has initiated a clinical trial of adoptive T cell therapy in patients with mesothelin-expressing cancer, providing yet another

treatment option for patients whose tumors express this protein antigen.

“We’re looking at mesothelin in a very broad, holistic manner,” Hassan said. “There are very few antigens that can be targeted for cancer therapy, and the attraction in this case is that mesothelin is not expressed by important human tissues. Even if treatment damages healthy mesothelial cells, this is not a life-threatening problem. I am heartened that the work done by Dr. Pastan and me, in addition to our many collaborators, is now leading to new treatment options for patients with cancer.”

Meanwhile, Lescalleet’s tumor continues to show good responses. “Every three months I go back for another CT scan (computed tomography scan), and so far, SS1P is keeping the tumor under control,” she said.

(Image: R. Hassan, CCR)



A computed tomography (CT) scan of a patient with pleural mesothelioma before and after treatment with SS1P.

To learn more about Dr. Hassan’s research, please visit his CCR website at <http://ccr.cancer.gov/staff/staff.asp?name=hassan>.