

Adopting Bodily Defenses to Cure Cancer

Steven Rosenberg, M.D., Ph.D., Chief of CCR's Surgery Branch since 1974, is a genuine pioneer in the development of immunotherapies for cancer. In 1985, he was the first to demonstrate that an immunotherapy—specifically, the administration of interleukin-2 (IL-2)—could cure certain patients with metastatic disease. A few years later, he opened the doors to cell-based immunotherapies by showing that tumor-infiltrating T lymphocytes (TILs) could be isolated from melanomas, stimulated to proliferate, and reintroduced into patients to promote cancer regression. Since that time, Rosenberg and his colleagues have discovered and developed innovative ways to improve upon cell transfer therapies. He was the first to insert foreign genes into humans in 1990 and the first to demonstrate that genetic modification of T cells could mediate cancer regression in patients with melanoma, sarcomas, and lymphomas. Rosenberg has written more than 1,100 scientific articles, as well as eight books, and was the most cited clinician in the world in the field of oncology between 1981 and 1998.

(Photo: R. Baer)



Steven Rosenberg, M.D., Ph.D.

My colleagues and I are trying to develop curative treatments for patients with metastatic cancer. While I was still a surgical resident in Boston, I came across a patient who had spontaneously recovered from an untreatable, aggressive stomach cancer. The patient's own body had cured his disease. Since that time, I've seen the immune system as our best source of untapped therapeutic potential.

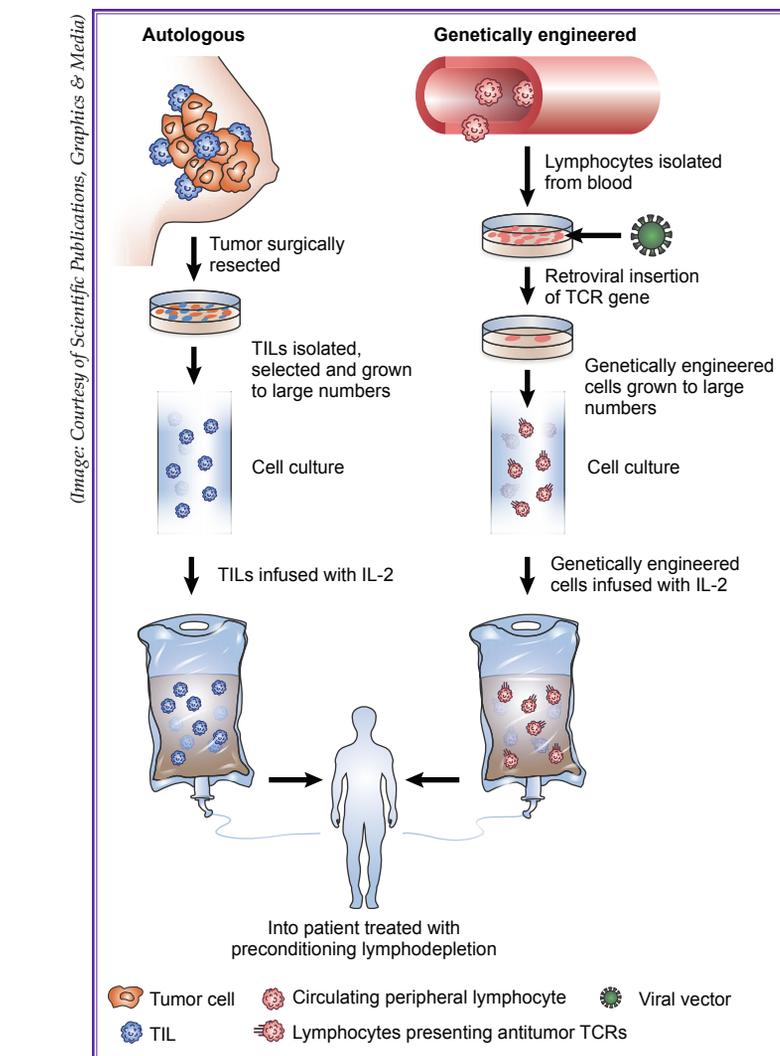
After several years of research, we developed IL-2 as a systemic treatment for metastatic disease in the 1980s, and found that it could cure a small fraction (seven to eight percent) of patients with metastatic melanoma and renal cancer. IL-2 is a cytokine that stimulates the growth

and proliferation of lymphocytes. We have also worked with other systemic immunomodulators and were the first to demonstrate the cancer regression properties of an anti-CTLA-4 antibody in humans with metastatic melanoma.

However, most of our current research is based on the development of anticancer T cells that can recognize and destroy melanomas and other cancers. TILs are naturally occurring T lymphocytes that are capable of attacking tumors but have apparently been unsuccessful in fending them off unaided in our patients. In 1988, we described a procedure for extracting TILs from the surgically resected tumors of cancer patients, allowing the cells to proliferate outside the body, and then reinfusing them in sufficient numbers to successfully eliminate tumors. Since then, we have worked on a number of strategies for optimizing this adoptive cell transfer immunotherapy (ACT) approach.

Destroying the Competition

In 2002, we demonstrated that we could increase the therapeutic efficacy of ACT dramatically, by first extracting TILs, then depleting the patient's remaining lymphocytes with a combination of drugs (cyclophosphamide and fludarabine) before reinfusing the expanded population of TILs into the patient. We recently reported that among the first 93 patients with metastatic melanoma who were treated in this way, 20 had complete regressions. Of those 20, 19 maintained their tumor-free status for more than six years and some have been followed for more than 10 years. We reported these data from three successive pilot trials; in the last trial, 40 percent of patients experienced complete cancer regression.



Adoptive cell transfer immunotherapy using autologous tumor-infiltrating T lymphocytes (TILs) extracted from patients' tumors or using lymphocytes isolated from blood and genetically engineered to express antitumor T-cell receptors (TCR). After cells are stimulated to proliferate *in vitro*, they are reintroduced into patients whose remaining lymphocytes have been depleted.

We don't know why some patients respond completely to the therapy and others only respond partially or not at all. There is no relationship between the size of the tumors, where they are located, or any prior treatments and the likelihood of regression.

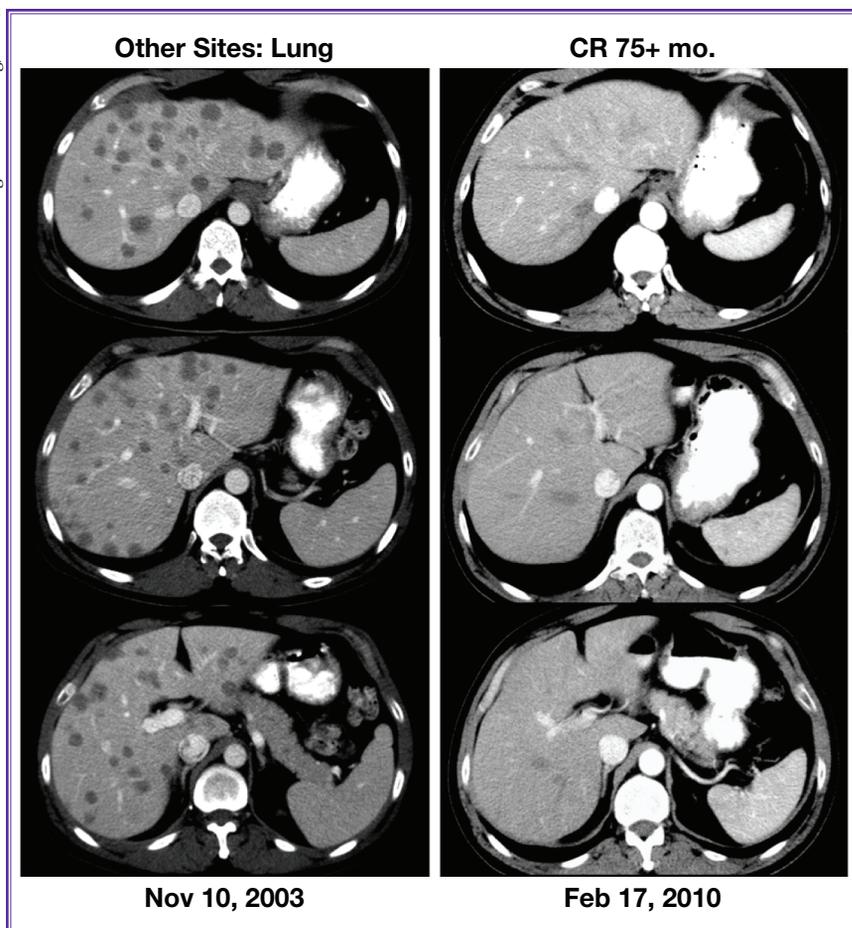
We have studied the molecular and cellular mechanisms underlying the enhancement of ACT therapy by depletion of lymphocytes, both in our clinical work and in animal models. There are many types of lymphocytes in the body and some, such as regulatory T cells

and myeloid-derived suppressor cells, actually dampen the immune response. Moreover, without other lymphocytes competing for and depleting circulating cytokines like IL-7 and IL-15, TILs can benefit from exclusive access to their growth-promoting abilities. In fact, IL-15 is not normally found in the blood of patients, but is found in high levels after the chemical depletion of lymphocytes.

Better, Stronger T Cells

We're working hard now to extend the development of our cell transfer

(Image: S. Rosenberg, CCR)



Computed tomography (CT) images of a melanoma patient before and after treatment with adoptive cell transfer immunotherapy. The patient has an ongoing complete regression for more than 10 years.

therapies from melanoma to other cancer types. Melanoma is one of the few tumors that give rise to TILs naturally. In order to treat other kinds of solid tumor metastases, we have developed methods for genetically engineering a patient's own circulating lymphocytes to recognize the cancer.

In 2006, we showed for the first time that genetically modified lymphocytes could mediate tumor regression. In patients with melanoma, we used a retrovirus to introduce the gene for a tumor-specific T-cell receptor into patients' T cells. The receptor bound a molecule found on the surface of melanoma cells known as "melanoma antigen recognized by T cells 1" or MART-1. There are

a series of known cancer-related antigens that can be used. Some mutations are shared among cancers; others like CD19 are present on normal B cells as well as lymphomas. We've gone on to successfully treat patients with synovial cell sarcomas with genetically modified T cells targeting the cancer-testis antigen NY-ESO-1, which is present on many common epithelial cancers. The NY-ESO-1 testis antigen is expressed during fetal development but has very little expression in adults. It is, however, re-expressed in many adult cancers.

T cells can be engineered with receptors that are not found in the naturally occurring repertoire of immune responses. Chimeric antigen receptors (CARs) can be designed that not only bind to tumor

antigens, but also contain additional components that stimulate the T cell to respond and proliferate. In 2010, we demonstrated that we could successfully treat patients with B-cell lymphomas and leukemias by engineering their own T cells to produce a CAR that recognized the B-cell antigen CD19. Some patients from that trial have remained progression free of lymphoma for over four years.

We're also looking at ways to genetically enhance T cells, beyond their ability to identify tumor antigens. For example, T cells could be engineered to produce their own source of IL-2 or IL-12, subverting the requirement for supplemental cytokines.

We have over a dozen trials underway to use ACT for a variety of cancers and T-cell strategies. A full list is available here: https://bethesdaclinicaltrials.cancer.gov/clinical-trials-search-physician?field_investigator_name_value=Rosenberg.

Identifying New Targets

For the immune system to recognize cancer as different from "self," it must recognize and attack unique protein features. We know some of those targets, but the majority still eludes us. The future of ACT lies in finding ways to engineer cells to attack the unique mutations on an individual's cancer. Melanomas are distinct among cancers by virtue of having a much larger number of mutations, i.e. several hundreds, than most cancers, which have on the order of 20 to 70 mutations. Tumors with larger numbers of mutations are likely to give rise to immune reactions that are capable of affecting a growing cancer, which may explain the ready availability of TILs in melanomas.

However, just because a protein contains a mutation, that does not mean it is going to be recognized

and attacked by the immune system. One of the major efforts in our laboratory is to identify mutations in individual cancers that are capable of eliciting a T-cell response. Last year, we published a paper in *Nature Medicine* in which we described a new technique—combining whole-exome sequencing and immunology—to identify mutations in melanoma samples, which generate a T-cell response. We used a bioinformatics approach to predict which mutations would generate protein fragments that would be presented to T cells as a threat. Then we tested those predictions using TILs from patients whose tumors had completely regressed after ACT. More recently, we have developed a new technique to identify unique immunogenic mutations in patients with common epithelial cancers. We published in *Science*, the successful use of this approach in a patient with a metastatic bile duct cancer. We believe this approach may be a generally applicable method for identifying mutated antigens expressed in a variety of tumor types.

Commercial Development

The immune reaction against cancer is very complex, but the fact that TILs can be used to cure patients with metastatic melanoma shows that this kind of therapy is capable of eliminating every last tumor cell. The challenge is enormous. Last year 580,000 Americans died of cancer; but, I believe these immunotherapies have unlimited potential if we can develop clever ways to create T cells that identify cancer mutations.

There are many companies that are involved in developing adoptive cell transfer strategies to treat cancer; NCI has a Cooperative Research and Development Agreement (CRADA) with Kite Pharma to develop our gene modification



(Photo: R. Baer)

Patient Jay Lake and Steven Rosenberg, M.D., Ph.D., discuss Jay's case the day before his surgery.

approaches for ACT. Kite was founded in 2009, specifically to commercialize cancer immunotherapy approaches. NCI also has a CRADA with Lion Biotechnologies to commercialize TIL therapy. Many other larger pharmaceutical companies are also becoming very interested in these cell transfer therapies. The Seattle-based biotechnology company Juno Therapeutics just raised one of the largest early private investments to develop CAR-based immunotherapies.

Our Patients

My own laboratory has about eight to ten scientists at any given time. But our Tumor Immunology Section in CCR has 30 to 40 people that are all working towards better cancer immunotherapies. I make rounds every day, visiting all the patients we are treating. We often have about a dozen patients with advanced cancer at any one time, each receiving these new immunotherapy approaches. I spend probably a third of my time on clinical work and most of the rest of the time on laboratory research.

When information is published

about new treatments for patients who have otherwise untreatable diseases, we are besieged by patients asking to be included in trials. Whereas 20 years ago, all our referrals came from oncologists, now half of our inquiries come from the patients themselves. Many who call us have been through other treatments and are desperately seeking something that might be of use. Some we can treat, but some are not eligible given the criteria we have.

Every patient we treat at CCR has a disease that cannot be successfully treated by today's standard-of-care medicine. All have advanced cancer that is refractory to existing treatments. The patients we see have a very limited life expectancy. Our goal is not to administer today's treatments; we are here to create the treatments of tomorrow. But tomorrow no longer seems so far away.

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