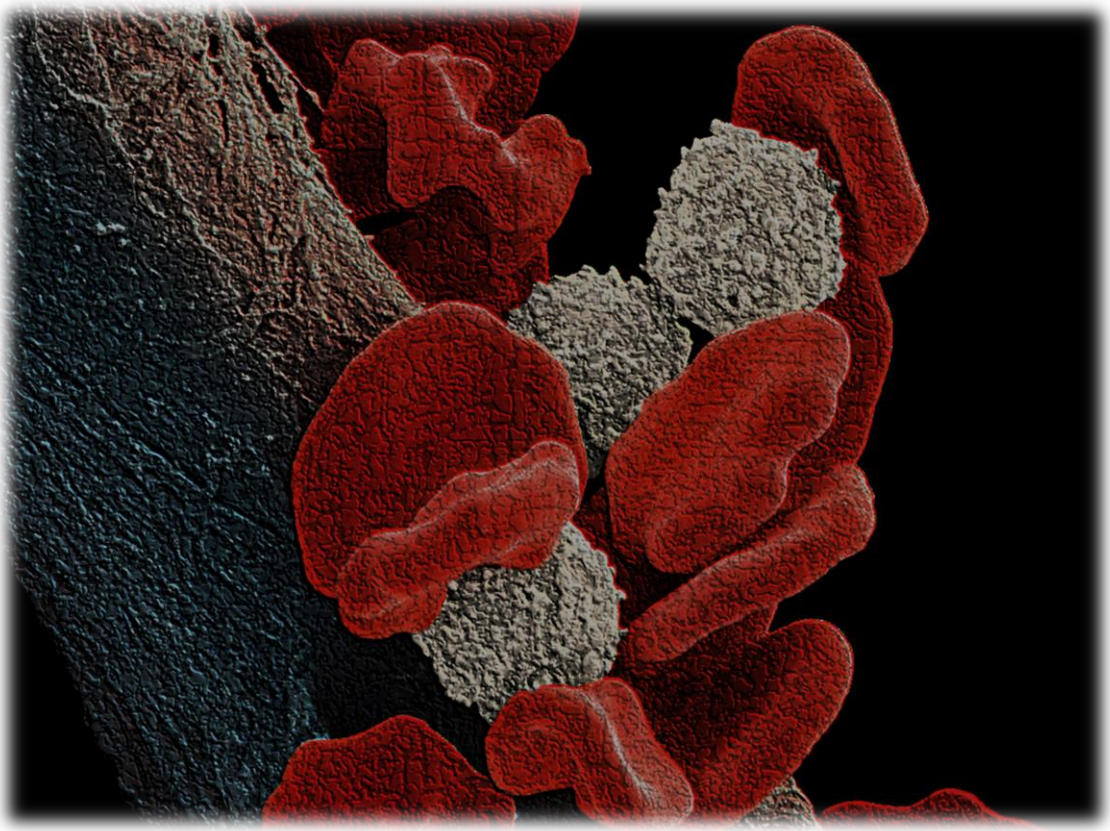
IMMUNOTHERAPY FOR THE TREATMENT OF CANCER



STEVEN A. ROSENBERG

1968: Steven A. Rosenberg, then a young surgeon, faced an unusual medical fact: a patient's immune system destroyed a cancer in an advanced stage. It was an extraordinary observation. After obtaining the necessary permission, blood was transfused from this patient to another who was suffering from stomach cancer in its terminal phase. The attempt to cure him failed, but Steven Rosenberg pursued his interest in finding an explanation for this exceptional event. Take into account the date: 1968!

Half a century later, Steven A. Rosenberg, now 76, chief surgeon of the National Cancer Institute, belongs to an increasingly small group of researchers who continue to pursue a dream: to destroy tumours by means of the patient's own immune system.

Steven Rosenberg, Carl H. June and Michel Sadelain are at the forefront of decades of research, working, sometimes in cooperation, sometimes in competition, on the pursuit of a strategy by which a patient's immune system reacts against T-cells in the body that have undergone a cancerous drift. At present, some versions of this therapeutic strategy,

known as "cell therapy", are in the final stages of preclinical development as a treatment for a few hematologic cancers. This development has not been easy.

Conceptually, "cell therapy" consists of the genetic manipulation of the patient's T-cells, modifying them to express on their membrane a specific antibody (“marker”) which acts against a specific kind of tumour. T-cells modified in this way multiply exponentially *in vitro*, and once modified they are reinjected into the patient. Once inside the patient, they act as if they were “serial killers”, with impressive efficiency: a single genetically modified T-cell can destroy 10^5 cancer cells. Furthermore, modified T-cells multiply within the body. Imagine a drug that, once administered, could be multiplied inside the body increasing its effectiveness exponentially.

"Cell therapy" (“Cell Transfer Technique”, as Steven Rosenberg commonly referred to it) should not be confused with another relatively novel anti-cancer strategy, named PL1-ligand inhibitors" (1) (PDL, acronym of Programmed Death Ligand) which act "releasing" the brake that many cancerous processes impose on the immune system. While "PD-1 ligand inhibitors" de-repress the immune system so that it can cope with tumour cells, "cell therapy" redesigns the patient's T-cells to specifically target those cancer cells expressed on the outer membrane (tumour “marker”).

So far, "cell therapy" experiments have focussed on hematologic cancers, not solid tumours. In addition, it is important to avoid a massive attack on tumour cells which, in the manner of the Jarisch-Herxheimer reaction with antibiotics, can endanger the patient's life. In fact, a recent clinical trial has been disrupted due to an overreaction of T-cells used against patients’ healthy tissues. Three of these developed fatal encephalitis.

The pharmaceutical industry has shown interest in "cell therapy", although the early stages have been modestly successful, but there have also been notorious failures.

The US Food and Drug Administration has received a favourable report (10 votes in favour, none against) from the Oncologic Drugs Advisory Committee for the approval of a gene therapy developed by Carl H. June’s team at the University of Pennsylvania, This “cell therapy” has been marketed by Novartis AG. It is a “gene therapy” known as CTL019 (Tisagenlecleucel) and indicated for the treatment of acute lymphoblastic leukaemia in children and adults up to 25 years of age. (2).

Steven Rosenberg is the son of orthodox Polish Jews who migrated to New York. When he was a child he learned that many of his relatives, including his six siblings, had been killed during the Holocaust. He graduated in medicine from Johns Hopkins University, and also obtained a doctorate in biophysics from Harvard University. Like many of those who wish to flee from their past, he is an obsessive worker. In 1992 he published a very interesting book: "The Transformed Cell" (3).

He did his first immunotherapy trials in 1974, then he became a fellow of the National Cancer Institute. Initial attempts to administer T-cells extracted from pigs to patients failed dramatically. Everything changed with Robert Gallo's discovery at that time, when he was working at the National Cancer Institute, of a molecule, originally called "T-cell growth factor", but today known as interleukin-2 (IL2). [Robert Gallo was known for the isolation of the HIV virus, *ex aequo Luc Montaigner*]. It was soon discovered that IL2 is synthesized in a T-cell strain, called T-helper cells. Among the functions of interleukin-2 is the differentiation of some T-helper cells, turning them into T-cytotoxic cells.

Some experiments were carried out where T-cells, previously cultured *in vitro,* were then injected into a culture medium enriched with interleukin-2. The results of these injections were frustrating, in part because the immune response was so drastic that many patients had to be placed in Intensive Care Units.

From 1980 to 1984, Steven Rosenberg treated 66 patients, to no avail. However, in 1984 he came upon a patient who, along with others later, catapulted him to fame even beyond the academic field. This patient was Linda Taylor, a Navy officer diagnosed with melanoma with an observation in her medical history that indicated "imminent death." This success in curing Mrs Taylor put his name on the pages of newspapers and the general information magazine Newsweek. The procedure was as follows: after the surgical removal of the main tumour, he isolated T-cells that had already infiltrated the tumour tissue. He cultured these T-cells *in vitro*, and then, along with intraleukin-2 intravenous bolus, they were reinjected into patients.

When lymphocytes from healthy subjects were treated with interleukin-2, a wide range of cancer cells from melanomas, sarcomas, and colon cancer were lysed *in vitro*. These lymphocytes were named with the appropriate acronym LAK (Lymphokine-Activated-Killer). LAK cells did not come from cytotoxic T-lymphocytes but from a cell line known as 'null cells'. These constitute approximately 5% of all circulating lymphocytes.

They are reminiscent of a primordial non-specific immunological surveillance system. Their lack of specificity was attractive because of their potential use against various types of tumours. Interleukin-2 is a "growth factor" for LAK cells just as it was for T-cells.

In 1985, a trial of 25 patients diagnosed with cancer (melanomas and renal cancers) with metastases who responded favourably to treatment with interleukin-2 and lymphocytes was published in The New England Journal of Medicine. A complete regression of the tumour was achieved in approximately 10% of patients, and a 50% reduction in tumour mass was observed in another 10% of the patients with melanoma. A similar reduction was observed in 25% of those affected by renal cancer. Similar improvements were achieved in patients with non-Hodgkin's lymphoma, colon cancers and cancer of the rectum.

The theoretical models suggested that tumour tissue would have the highest concentration of specific immune cells which fight against neoplastic tissue. Therefore, the research was focussed on isolating these 'tumour infiltration lymphocytes' (TILs). This research was justified because LAK cells (Lymphokine-Activated-Killer) stopped acting after about ten days. However, TIL continued to thrive until almost all tumour cells were digested, eventually forming a mass in which T-cells were predominant. These TIL cells were subsequently identified as 'cytotoxic T-cells'. TILs, unlike LAKs, are tumour-specific.

The efficacy of TIL (Tumour Infiltrating Lymphocytes) therapy was much greater than that observed with LAK cells. In all cases, injections (bolus) of interleukin-2 as a 'growth factor' were administered.

TIL cell assays were initially published in The New England Journal of Medicine in 1988, only three years after the publication of the LAK cell assays.

CARL H. JUNE

Carl H. June began his scientific career in the military field linked to the American Naval Academy. He was trained in the area of bone marrow transplants, and was also interested in the medical side effects of radiation. This matter was of great interest. Let us not forget that this was at the time of the "Cold War", and the possibility of a nuclear conflict was considered a real risk. As part of this work, carried out at the Naval Medical Research Centre, he perfected, along with Bruce Levine, the technology for the

massive multiplication of *in vitro* T-cells; systematic work that has not been bettered, and remains fully valid today. In the mid-1990s, while working for Cell Genesys, a gene therapy company, they attempted to genetically engineer T-cells as a possible strategy against HIV (Human Immunodeficiency Virus) infection, but without success.

The death of June's wife of ovarian cancer changed his scientific interest, and he began to focus on "gene therapy." In fact, he applied the rudimentary knowledge that existed in 1996 to save his wife. He did not succeed, and his wife died in 2001.

MICHEL SADELAIN

A third player of the triumvirate, Michel Sadelain, an immunology student at the University of Alberta during the 1980s, experimented with a technique to "load" T-cells in order to make them more efficient in the fight against cancer.

After moving to the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, he investigated the introduction of genes into the T-cell genome. He obtained the first results in 1992, using inactivated viruses to genetically modify mouse T-cells.

Michel Sadelain started working at the Memorial Sloan Kettering Cancer Center. In 2003, along with other colleagues, including Isabelle Riviere, who later became his wife, he demonstrated that genetically modified T-cells could eradicate certain types of cancer in rodents. How was this achieved? To fight cancer, T-cells must first recognize cancer cells as "non-own cells."

T-cells, derived from T-lymphocytes, patrol the body looking for specific protein fragments which, like metabolic waste, are expressed on the surface of cells infected by a micro-organism (bacteria, viruses) or by invading cells that do not belong to one's own body. When one of these fragments (antigenic determinants) is attached, the T-cell destroys the infected cell. [The sign that the cells belong to the organism is its molecular identity document; a set of proteins called the Major Histocompatibility Complex, better known by its acronym MHC.

Unfortunately, cancer cells are mutated versions of the body's own cells. In this way they evade their destruction by the immune system, which is unable to recognize them as "non-own" because they carry the "major histocompatibility complex".

The goal of Michel Sadelain's team was to chemically "signal" cancer cells as "non-self", to the point where the T-cells destroy them. However, chemically "marking" cancer cells has been shown to be insufficient. The T-cell needs to carry on its membrane some molecular structures which might behave as an "activator".

The strategy followed was to create a construct consisting of a T-cell carrying an antigen ('primer') and a specific antibody against a 'marker' expressed by the tumour cell. The construct (a chimeric structure) is called CAR (Chimeric Antigen Receptor).

Therapy with this T-cell construct (T-cells with their "initiator" and "specific anti-tumor antibody") is abbreviated as "CAR-T therapy" (Chimeric Antigen Receptor-T).

Other research groups designed these biological chimeras, too. Special mention should be given to the work teams led by the Israeli Zelig Eshhar, and Dario Campana's group, which was then associated with St. Jude Children's Research Hospital.

However, during the late twentieth and early twentieth first century, these investigations have been confined to the academic field, far from their clinical applications.

Initially the pharmaceutical industry showed little interest in "cell transfer therapy" as it was not easily accommodated to industrial production since it is a treatment that must be tailor-made for each patient.

Again, as in the case of Linda Taylor discussed above, a personal history had stimulated the further development of "cell therapy."

In 2001, Kimberly Lawrence Netter, a 44-year-old woman, died of breast cancer. Her father-in-law, Edward Netter, a wealthy financier and philanthropist, along with his wife, Barbara, founder of Alliance for Cancer Gene Therapy, donated a huge sum of money to the June and Sadelain's teams, adding the financial support of the Leukaemia and Lymphoma Society. Thanks to this funding, the research project went ahead.

In 2010 Steve Rosenberg's team published, in the journal Blood, the case of a patient with lymphoma, which attained favourable results, even though two cycles of treatment were required. Almost at the same time, Carl June reported other cases of chronic lymphocytic leukaemia which also gave favourable results. These findings aroused the interest of the Swiss multinational company, Novartis AG, which acquired the rights to Carl June's royalties.

Today there are three pharmaceutical laboratories at the forefront of 'cell therapy'. All three are expected to gain approval for their first treatments in 2017 or 2018.

The three pharmaceutical companies are linked to highly reputable academic institutions: Novartis to the University of Pennsylvania, Kite Pharma to the National Cancer Institute, and Juno Therapeutics to Sloan Kettering Cancer Center, The Fred Hutchinson Cancer Research Centre, and Seattle Children's Hospital.

The initial collaboration between the three researchers (Rosenberg, June and Sadelain) became a rivalry, especially when it is known that this technology could possibly be awarded the Nobel Prize for Physiology and Medicine.

PROSPECTIVE

However, these therapies should not be considered as a panacea. "Cell therapy" (perhaps we should say "chimeric gene therapy") has only been tested on leukaemia and lymphomas, which together account for about 5% of all diagnosed tumours. The results obtained in the case of haematological tumours are not reproducible in solid tumours. In addition, today, "cell therapy" costs hundreds of thousands of dollars per patient. A matter of transcendental importance is to make these therapies affordable for both public and private health systems. On the other hand, the process of the genetic modification of T-cells requires about 4 weeks, and many patients do not survive this period of time. In addition, the injection of genetically modified T-cells can give rise to an "excessive" immune reaction, which always jeopardizes the survival of a patient who is already seriously ill.

T-cells are powerful weapons. Their use for therapeutic purposes runs the risk of killing the patient as an adverse effect of their therapeutic action.

Another goal of "cell therapy" is to extend its efficacy to solid tumours. A classic "marker" in breast tumours is HER2 (Human Epithelial Receptor), however, this "marker" is also expressed in lung parenchyma cells. When Rosenberg’s team tested "T-cell therapy" on a patient with breast cancer, the reaction of the T-cells against healthy lung tissue was immediate. This adverse reaction was produced within 15 minutes of initiating intravenous infusion, and the patient died five days later.

For "cell therapy" to be effective, cancer cells must express the "markers" on their outer membrane. There is a stratagem that is now arousing interest: the patient's immune cells can be manipulated to make what is called TCRs (acronym for T-Cell-Receptors).

These can recognize proteins within the cancer cells. According to some experts, TCRs may be a good way of treating of solid tumours. In this line of research, some preliminary tests have been carried out on one type of sarcoma. But other improvements are on the way: Michael Sadelain and Carl June are working on shielded Chimeric Antigen Receptors that not only adhere to their molecular target, but are also capable of inducing an immune response. Cellectis, a French pharmaceutical company, has treated two very young children without needing to extract their T-cells.

Bellicum Pharmaceuticals is working on a “gene therapy” that slows the reaction down if it might endanger the patient's life.

We would like to end with a quote attributed to the French microbiologist Louis Pasteur: “luck favours those who seek it”. The cancerous drift of some, or many of our cells, seems to be an insurmountable problem. We are probably living with a number of neoplastic cells, any of them with the potential to multiply in a massive and disruptive way. Maybe we should "teach" our immune system to control the situation as best we can.

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