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Immunotherapy in Cancer



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From the Desk of Director Research

Immunotherapy is a new class of cancer treatment that directs the body's own immune system to fight cancer cells. The clinical goal of tumor immunotherapy is to provide either passive or active immunity against malignancies by harnessing the immune system to target tumors. Passive immunotherapy is administration of an immunologically active agent made outside the body such as administration of monoclonal antibodies or adoptive cell therapy. Active immunotherapy focuses on stimulating the host's own immune system to eradicate cancer, by vaccination with tumor antigens, non specific immunomodulation, or targeting negative regulatory receptors that prevent the development of the tumor immune response.

The pillars of human cancer therapy have historically been surgery, radiotherapy, and chemotherapy, but a fourth modality of immunotherapy has been well documented since 1890. The addition of immunotherapy to the oncologist's armamentarium represents a paradigm shift in the way cancer is treated. The increasing availability of immunotherapeutic agents is particularly encouraging for patients with tumor types that have historically been associated with poor survival. Monoclonal antibodies, cytokines, cellular immunotherapy, and vaccines have increasingly become successful therapeutic agents for the treatment of solid and hematological cancers in preclinical models, clinical trials, and practice. Because of the immune system's unique properties, these therapies may hold greater potential than current treatment approaches to fight cancer more powerfully by offering longer-term protection against the disease with fewer side effects.

The immunotherapy of cancer has made significant strides in the past few years due to improved understanding of the underlying principles of tumor biology and immunology. In 2010, sipuleucel-T received the first US Food and Drug Administration (FDA) approval of a cancer vaccine for the treatment of metastatic castration-resistant prostate cancer. It employs an adjuvant component to enhance the function of antigen presenting cells and immune effectors such as T cells. This was followed with the FDA approval in 2011 of the drug ipilimumab for the treatment of metastatic melanoma through potentiating T cell activity. This therapy is now considered as the third wave in cancer therapy after conventional treatments and targeted agents.

The field of cancer immunotherapy has seen many highs & lows and the myriad of approaches under development today. Indeed, it is opined that cancer immunotherapy is approaching a watershed moment where it transitions from the experimental to mainstream cancer treatment. However, critical to that transition is the need for understanding how to utilize all of the therapeutic tools becoming available to optimally manage patients. The future of immunotherapy will likely involve combination of immunotherapy such as vaccines and immunomodulators, and immunotherapy with poor potent inhibitors of tumor signaling pathways that remit disease in more than half of treated subjects.

The present issue of the Cancer News "Immunotherapy in Cancer" highlights newer advances in the field of immunotherapy and features the regular articles, such as 'Special Feature', 'Guest Article', 'Perspective', 'In Focus', 'Research and Development', 'New Technologies', 'Clinical Trial', 'Watch Out' and 'In Focus'. We are grateful for the contributions made by Dr Lalit Sehgal, PhD Postdoctoral Fellow, Dept of Lymphoma and Myeloma, University of Texas MD Anderson Cancer Center, USA, Dr Swgata Das of National Institute of Technology, Tiruchirappalli; Dr Dipanjan Ghosh of University of Texas MD Anderson Cancer Centre, USA and Dr Chaitanya Kumar, Associate Director, APAC Biotect, Gurgaon.

Suggestions/ comments from the readers are welcome.

Dr D C Doval

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SPECIAL FEATURE

CANCER IMMUNOTHERAPY: CONQUERING OVER SELF

After over two decades of untiring efforts to understand how tumors escape the body's immune system and how to use our own immunity to fight cancer cells, groundbreaking results are being seen in many small but landmark studies. The impact seems so promising that The SCIENCE magazine has labeled this approach as the BREAKTHROUGH OF THE YEAR 2013. The present issue of the Cancer News explores the new approaches which may radically change the way we treat cancer over the next decade.

The Past

The early steps were taken by cancer immunologist James Allison, now at the University of Texas MD Anderson Cancer Center in Houston. In the late 1980s, French researchers who weren't thinking about cancer at all identified a new protein receptor on the surface of T cells, called cytotoxic T-lymphocyte antigen 4 or CTLA-4. Allison found that CTLA-4 puts the brakes on T cells, and prevents them from launching all-out immune attacks. He wondered whether blocking the blocker, the CTLA-4 molecule, would set the immune system free to destroy cancer.

CTLA-4 was discovered in 1987. In 1996, Allison published a paper in the Science describing that antibodies against CTLA-4 erased tumors in mice. It took more than a decade to bring this approach from bench to bedside. Big pharma companies did not risk their revenue on this so the job of getting anti-CTLA-4 into people fell on a small biotechnology company, Medarex, in Princeton, New Jersey. In 1999, it acquired the rights to the antibody, taking the leap from biology to drug. In 2010, Bristol-Myers Squibb which had bought Medarex for more than \$2 billion, reported that patients with metastatic melanoma lived an average of 10 months on the antibody, compared with 6 months without it. It was the first time any treatment had extended life in advanced melanoma in a randomized trial. Nearly a quarter of participants survived at least 2 years.

The Japanese biologists working on immune system discovered a molecule expressed in dying T cells, which they called programmed death 1, or PD-1. Oncologist Drew Pardoll at the Johns Hopkins University, thought

of the approach of using own T cells against the cancer cells. He tied up with Medarex to test this antibody.

The first trial, with 39 patients and five different cancers, began in 2006. By 2008, doctors were amazed when they saw that in five of the volunteers, all of them with refractory disease, tumors were shrinking. Survival in a few stretched beyond what was imagined possible.

For years, Steven Rosenberg at the National Cancer Institute, had harvested T cells that had migrated into tumors, expanded them in the lab, and reinfused them into patients, saving some with dire prognoses. The technique worked only when doctors could access tumor tissue, though, limiting its application. In 2010, Rosenberg published encouraging results from so-called chimeric antigen receptor therapy, or CAR therapy, a personalized treatment that involved genetically modifying a patient's T cells to make them target tumor cells. Carl June and his team at the University of Pennsylvania, reported eye-catching responses to CAR therapy: patients with pounds of leukemia that melted away. At a meeting in New Orleans, June's team and researchers at Memorial Sloan-Kettering Cancer Center in New York reported that the T-cell therapy in their studies put 45 of 75 adults and children with leukemia into complete remission, although some later relapsed. CAR therapy is now the focus of numerous clinical trials. Researchers hope that it, like the antibodies, can target an assortment of cancers.

The Present Scenario

A brief overview of the various new immunotherapy approaches is given below:

Adoptive T Cell Therapy (ACT): It is a promising and rapidly advancing form of immunotherapy that overcomes tolerance by harnessing the natural ability of immune cells to recognize and eliminate target cells in order to generate durable anti-tumor immune responses. Adoptive T cell therapy involves the infusion of externally manipulated T cells. There are multiple sources and types of T cells used for adoptive therapy, such as expanded and activated tumor infiltrating lymphocytes (TILs), T cells modified *ex-vivo* to express a specific TCR, and T cells engineered to express a receptor that is a fusion between an antibody and the T cell receptor's intracellular machinery, a so-called chimeric antigen receptor, or CAR.

TIL Therapy: It involves extracting lymphocytes from tumor tissue, *ex-vivo* expansion with IL-2 followed by reinfusion. A recent pooled analysis of TIL protocols reported a 20% complete response rate and a 70%

overall objective response rate in patients with melanoma. Although expanded TILs are thought to be among the least labor-intensive ACT strategies, several limitations preclude widespread adoption at present. These include the need for appropriately equipped cell processing facilities as well as the need for patients to have moderately bulky tumors for TIL isolation.

Chimeric Antigen Receptors (CARs): CARs offer one of the most promising strategies that includes an extracellular antibody single-chain variable region joined with the intracellular portion of a TCR. CARs are unique in that they combine the cytotoxic activity of a CD8⁺ T cell with the highly avid and MHC-independent antigen recognition capacity of high-affinity monoclonal antibodies. To help overcome tolerance mechanisms, second generation CARs include expression of co-stimulatory signaling domains in addition to the CAR. There have been promising clinical results with refractory chronic lymphocytic leukemia (CLL), using a lentiviral derived vector expressing a CAR with specificity for CD19 (a B cell antigen). This CAR is coupled with two signaling domains, including the cytoplasmic domain of 4-1BB receptor (CD137), which serves as a co-stimulatory receptor in T cells, and CD3-zeta, a signal-transduction component of the T cell antigen receptor. Two of three patients with CLL treated with this regimen demonstrated a complete remission, and a portion of the transformed T cells expressing the CAR persisted as memory T cells that retained CD19 effector functionality.

Unlike TIL therapy, which often leads to widespread systemic toxicity, the grade 3 or 4 toxicities experienced in this clinical series were tumor lysis syndrome with associated cytokine release and lymphopenia. However, not unexpectedly, patients experienced chronic B cell aplasia and hypogammaglobulinemia. Adoptive T cell therapy represents an advancement for personalized medicine in the form of customized cellular therapies.

However, multiple challenges will have to be addressed prior to these technologies become commercially available and are offered as a standard of care. Efforts are currently underway to demonstrate that adoptive T cell therapy is clinically efficacious, safe, reproducible, perhaps most importantly, exportable beyond a limited range of academic centers.

Anti-tumor Antibodies

Monoclonal antibodies (mAb) directed against tumor associated antigens like CD20 and HER-2, are a standard of care treatment in many malignancies. This technology was facilitated by the simultaneous understanding of

antibody structure and the application of hybridoma technology, leading to a Nobel Prize for Jerne, Kohler, and Milstein in 1984.

Over the past few years, several modified antibody technologies have emerged, including radioimmunotherapies, TRAP molecules, antibody-drug conjugates (ADCs), single chain dual specificity bi-specific T cell engagers (BiTEs), and chimeric antigen receptors (CARs). Indeed, as early as 2002, the FDA approved radioimmunoisotopes to treat refractory non-Hodgkin's lymphoma with agents, such as ibritumomab (anti-CD20+ Yttrium-90) and in 2003 tositumomab (anti-CD20+ Iodine 131).

In 2012, the FDA approved the use of aflibercept (a TRAP molecule combining 2 separate regions mimicking VEGFR1 and VEGFR2 bound to an IgG1 Fc region) for metastatic colorectal carcinoma. The development of antibody-drug conjugates has incited great interest as these agents are designed to improve local delivery of highly toxic chemotherapeutics while simultaneously attempting to minimize systemic toxicity.

In 2011, the FDA approved brentuximab vedotin (anti-CD30-MMAE [monomethyl auristatin E]) for relapsed or refractory Hodgkin lymphoma or anaplastic large cell lymphoma and in 2013 approved trastuzumab emtansine (TDM-1) for patients with metastatic HER2 positive breast cancer.

Another interesting technology has been the development of engineered bi-specific antibodies, in which one fragment arm binds the CD3 portion of the T cell receptor (TCR) on T lymphocytes, and the other fragment arm carries specificity for a tumor antigen. These constructs aim to co-localize T lymphocytes to tumor cells and thus induce anti-tumor immune responses. Blinatumomab (a BiTE specific for the B cell surface marker CD19) is currently undergoing phase I and II clinical trials in non-Hodgkin's lymphoma and acute lymphoblastic leukemia (ALL).

Cancer Vaccines: In the setting of cancer, oncogenic viruses are an ideal target for preventative cancer vaccines, and the HPV vaccine has been shown in large clinical trials to drastically reduce the chances of developing cervical cancer. Other examples of preventative cancer vaccines include the HBV vaccine which can significantly reduce the incidence of hepatocellular carcinoma. Therapeutic cancer vaccines, on the other hand, aim to treat cancer after diagnosis.

They include peptide based vaccine, cell-based vaccines, virus-based vaccine and vaccines based on *ex-vivo* generated dendritic cells.

Immune Checkpoint Blockade: Probably due primarily to recent clinical success, a great deal of excitement in immunotherapy has surrounded further understanding and modulating immune checkpoints for cancer immunotherapy. As described earlier, when a T cell interacts with an antigen presenting cell (APC) through the TCR-antigen/MHC complex, there are both costimulatory and coinhibitory signals occurring simultaneously that ultimately affect downstream T cell responses. Co-stimulation classically involves the interaction of B7 with CD28, and disruption of this interaction by the presence of CTLA-4 on the surface of T cells is one example of coinhibition. Early work in the field led by Allison and colleagues showed that in preclinical models blockade of CTLA-4 induces an anti-tumor immune response. This initial body of work culminated in a phase III trial in which CTLA-4 blockade with an anti-CTLA-4 mAb improved overall survival in patients with metastatic melanoma compared to patients receiving a tumor vaccine, and to subsequent approval of the anti-CTLA-4 antibody ipilimumab for metastatic melanoma.

PD-1 inhibition appears to have clinical activity in a variety of cancers, showing durable responses in a proportion of patients, many of whom failed other therapies. In addition, combining CTLA-4 blockade with PD-1 blockade (ipilimumab+nivolumab) in metastatic or advanced melanoma patients showed a large proportion of patients with dramatic and rapid responses in their disease burden with a proportion of patients maintaining a durable response.

These checkpoint inhibitors are also being tested in a variety of tumor types, including non-small cell lung carcinoma (NSCLC), small cell lung carcinoma (SCLC), renal cell carcinoma (RCC), prostate cancer, and hematological malignancies. Further emphasizing interest in combined checkpoint blockade, a phase I trial combining PD-1 and LAG-3 inhibition has recently commenced. Other combination approaches include combining immune checkpoint blockade with chemotherapy (cytotoxic or low dose regimens), or other immune-modulating therapies (including cytokines such as IL-2 or IL-21, cell based vaccines, peptide vaccines, or indolamine 2–3 dioxygenase inhibitors), molecularly targeted therapies (JAK/STAT inhibitors, BRAF inhibitors), and radiation therapy (stereotactic radiation versus conventional or intensity modulated

radiation therapy). In particular, combining radiation therapy with immunotherapy is an area of intense clinical and pre-clinical research activity, incited to some degree by a recent case report of a potential abscopal effect in a patient with metastatic melanoma. Continued delineation of the most effective combinatorial approaches for patients is important, as optimal combinations will likely be different for various tumor types.

Outlook

The immune system is extremely potent, as evidenced by auto-immune disease or cytokine storm. These encouraging results have brought faith in our own immune system as well as human "Never say die attitude" in the battle against cancer and the need to win over the self. Today's immune therapies don't help everyone, and researchers are largely clueless as to why more don't benefit.

For physicians accustomed to losing every patient with advanced disease, the numbers bring a hope they couldn't have fathomed a few years ago. For those with metastatic cancer, the odds remain long. Researchers are working globally to identify biomarkers that might offer answers and experimenting with ways to make therapies more potent. It's likely that some cancers will not yield to immunotherapy for many years, if ever.

With all these interesting results, one thing is certain that immunotherapy is the new and extremely powerful weapon in our armamentarium in the battle against Cancer.

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GUEST ARTICLE

TUMOR INFILTRATING LYMPHOCYTE (TIL) THERAPY: HITTING THE TARGET

The rapid advancement of sequencing technologies has provided insight into the neoplastic process, which includes accumulated mutations of genes that are involved in crucial cellular signaling pathways. This has led to clinical success of targeted therapies aiming to correct aberrant cellular signaling. However, clinical responses with targeted therapies are often short-lived due to the rapid development of resistance. Enhancing the cell-mediated immune response against tumor cells offers several advantages over targeted therapies, notably the generation of a long-term memory lymphocyte population patrolling the body to attack metastases before metastatic lesions are visible by traditional imaging modalities.

An effective immune response requires sufficient numbers of activated T cells capable of recognizing tumor antigens. It also requires appropriate engagement of positive co-stimulatory molecules on lymphocytes while limiting signaling through inhibitory "immune checkpoint" receptors.

Promoting T-Cell Function by Modulating Co-Stimulation or Co-Inhibition: Immune activation is tightly regulated by co-receptors expressed on T cells. Co-stimulatory receptors include CD28 and ICOS (inducible T cell co-stimulator) of the Ig superfamily, as well as 4-1BB, OX40, CD27, CD30, CD40, GITR (glucocorticoid inducible TNF receptor-related protein), and HVEM (herpes-virus entry mediator) of the TNFR superfamily. These co-stimulatory signals are counterbalanced by co-inhibitory members of the Ig superfamily, including CTLA-4, PD-1, BTLA (B and T lymphocyte attenuator), lymphocyte activation gene-3 (LAG-3), TIM3 (T cell immunoglobulin and mucin domain-containing protein 3), and VISTA (V-domain immunoglobulin suppressor of T cell activation) on T cells. The idea of blocking the immune co-inhibitors as a therapeutic anticancer strategy was suggested by James Allison over a decade ago. Anti-CTLA-4 was used as a prototype but antibodies that either stimulate co-stimulatory T cell receptors or block other inhibitory immune-checkpoint molecules have been examined more recently.

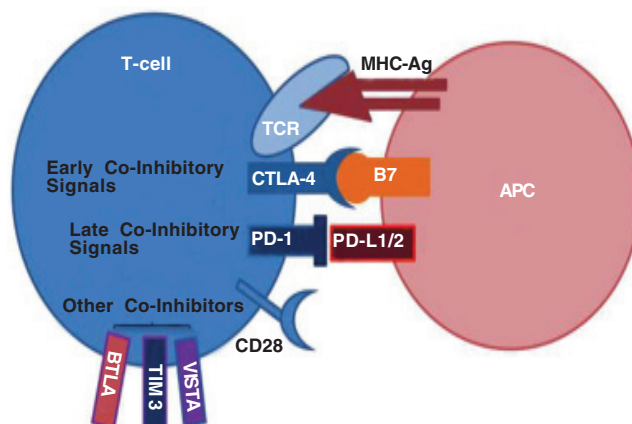


Figure 1: T cell APC interaction and role of Co-Inhibitory Signals in inducing energy to tumor antigens

Amplifying Existing Tumor Reactive T Cells: Adoptive T Cell Therapy for the Treatment of Cancer

Cancer-reactive T cells recognizing antigens from solid tumors are found at low frequency in the peripheral blood of patients and can be isolated and cloned by limiting dilution for eventual amplification and re-infusion to the patient. However, this process requires 3-5 months to generate a product sufficient for infusion. Clinical trials show modest clinical responses using cloned T cells recognizing MART-1 antigen for metastatic melanoma and poor persistence of the cells post transfer. The limited clinical efficacy may be due to targeting a unique antigen that may not be uniformly expressed by all tumor cells leaving the growth of antigen negative cells unaffected. In rare cases, therapy with T cell clones has been shown efficacious. For example, in a recent clinical trial with adoptively transferred NY-ESO-1-specific CD4 T cell clone, one patient experienced complete tumor regression associated with persistence of the transferred cells for more than 80 days and with antigen spreading, or the appearance of specific immune responses to tumor antigens unrelated to NY-ESO-1 post transfer.

An alternative approach to find cancer fighting immune cells is to isolate and expand T cells found in the tumor itself, or Tumor Infiltrating Lymphocytes (TIL), naturally enriched in tumor-specific T cells. Besides, recognizing shared tumor antigens melanoma, TILs also recognize unique mutated antigens, such as mutated β -catenin. The number and localization of TIL within the tumor have been correlated with clinical outcome for different malignancies. Isolation and *ex-vivo* rapid expansion of TILs from melanoma and other

malignancies are now readily achievable. Infusion of large numbers of expanded autologous TIL (up to 10^{11}) to metastatic melanoma patients, followed by one course of high dose IL-2 as growth factor to sustain the persistence of TIL *in vivo*, resulted in 34% clinical response. When pre-conditioning of the patients with cytoxan and fludarabine lymphodepleting regimen was introduced, half of the treated patients responded to the therapy, including 10% of the patients that had complete disappearance of their tumors. Clinical response rate was even increased to 70% in a small cohort of patients undergoing an additional pre-conditioning with total body irradiation (TBI) of 12Gy. Durable clinical responses have been observed and persistence of infused cells in responder patients have been measured for several years following therapy. However, randomized clinical trials are needed to compare this therapy with standard approaches.

Lympho-depletion of the patient prior to TIL cells transfer extends the persistence and frequency of infused T cells in the blood and is crucial to the success of TIL adoptive cell therapy for melanoma. The mechanisms involved are unclear. In animal models, lympho-depletion induces compensatory homeostatic expansion that together with regulatory CD4+ T cell (Treg) depletion can induce strong anti-tumor activity. In patients, cytoxan and fludarabine lympho-depleting regimen cause an increase in the levels of the homeostatic cytokines IL-7 and IL-15 while transiently ablating white blood cells, including Treg. Addition of total body irradiation to the regimen results in lymphoablation requiring autologous stem cell transfer to ensure the repopulation of the lymphoid compartment. The more profound lympho-depletion occurring with TBI may delay the reconstitution of the endogenous Treg compartment and favor better anti-tumor T-cell activity. Cytoxan/cyclophosphamide

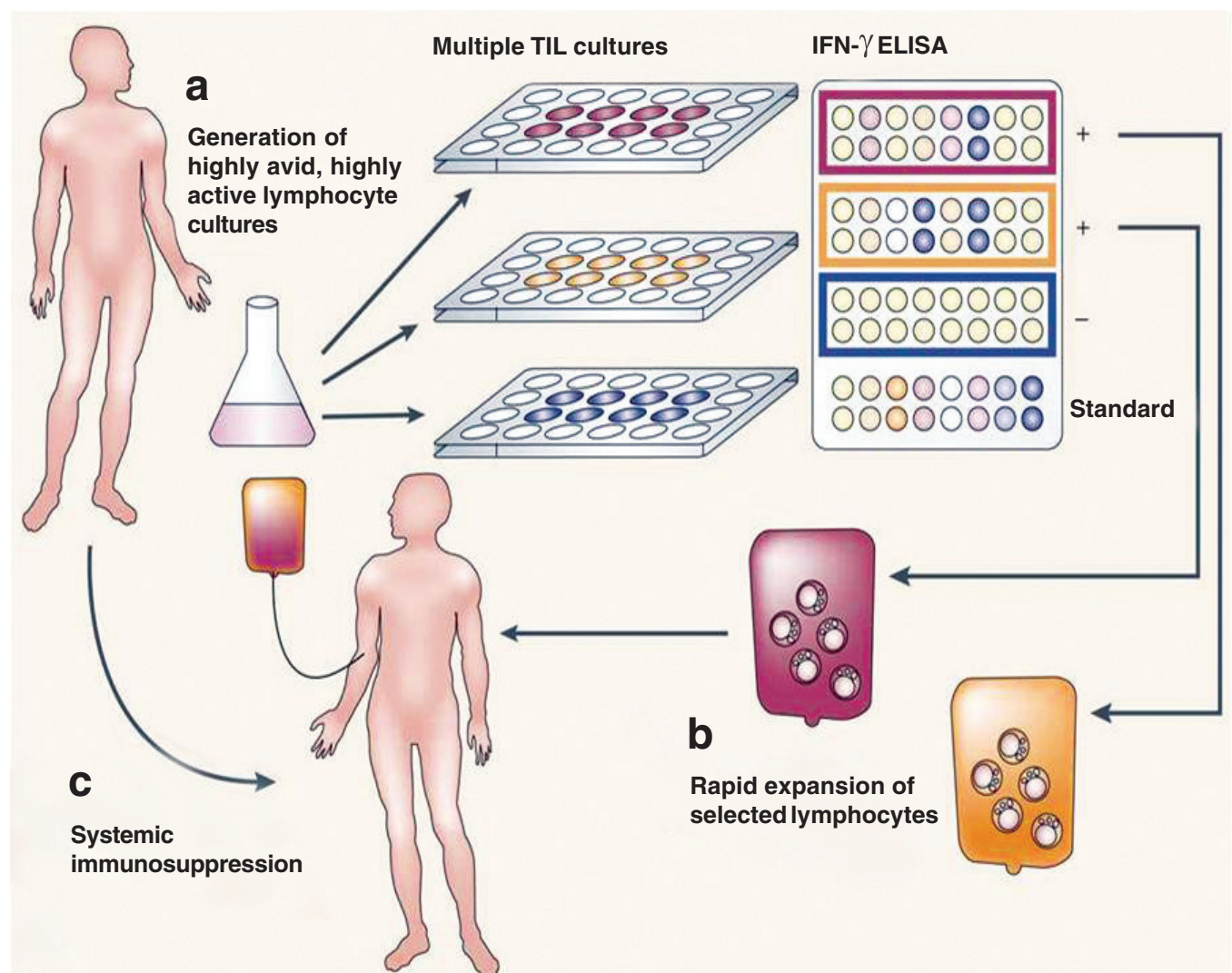


Figure 2: The process of TIL therapy

and fludarabine also affect the myeloid compartment and cause rapid colonization of the spleen with a suppressive population of immature CD11b+ GR-1+ myeloid cells in mice (myeloid derived suppressor cells or MDSC), inhibiting T cells through nitric oxide production. How TBI regimen impacts MDSC colonization of the spleen in patients is unclear, but potential clearance of MDSCs by TBI could contribute to the benefit of irradiation in the pre-conditioning regimen. Unraveling exactly how lympho-depletion potentiates TILACT requires further study.

Recent clinical success with TIL therapy is motivating additional investigation of this treatment modality. Substantial improvements in clinical outcomes for patients can be obtained by improvements in generating T cells for transfer and in manipulating the host immune environment to enhance *in vivo* antitumour activities. The generation of active, tumor-specific lymphocyte cultures with the characteristics necessary for *in vivo* effectiveness remains a considerable obstacle to the application of TIL therapy. The molecular characterization of tumor antigens has created the possibility of using these defined tumor antigens to generate tumor-specific lymphocyte cultures for patient treatment by repetitive *in vitro* stimulations. Another approach involves the genetic engineering of T cells to express a defined antigen specificity. Several investigators have reported that the transfer to PBLs of a gene encoding a tumour-antigen-specific T-cell receptor conferred specific recognition of tumor cells to the T cells. Clinical trials with these genetically engineered lymphocytes will soon begin to assess the utility of this approach.

Concurrently, a growing appreciation of lymphocyte characteristics other than antigen specificity that can impact lymphocyte behaviour *in vivo* is influencing the practice of TIL therapy. For instance, the discovery that the maturation state of CD8+ T lymphocytes determines their *in vivo* transport and persistence during an immune response indicates new avenues for manipulating T-cell behaviour. These might include the alteration of *in vitro* T-cell cultures to maintain a central memory phenotype—for instance by altering the cytokine milieu during T-cell growth—or genetically engineering lymphocytes to constitutively express their own growth factors that would enhance survival after cytokine withdrawal *in vivo*⁹⁵. A more detailed understanding of these and other factors influencing CD8+ T-cell persistence *in vivo* could improve TIL therapy.

Outlook Future

Rapid progress in understanding the role of the host immune environment on tumor therapy is also likely to impact on the practice of cell-based therapies. For instance, the dual role of CD4+ T cells in tumor immunity has important clinical implications. The recent reports describing a requirement of CD4+ T cells for persistence of CD8+ T cells after an immune response underscores a potential role of CD4+ T cells in TIL cultures for TIL therapy. By contrast, the demonstration that CD4+CD25+ T cells suppress autoimmunity and might be potent inhibitors of antitumour effects in mice indicates a rationale for additional investigation of lymphodepleting conditioning for TIL therapy. These opposing effects of CD4+ T cells highlight the need for additional investigation of CD4+ T-cell-mediated antitumor immunity. A better understanding of the homeostatic regulation of T-cell number and activation, and the role of concurrent vaccination after T-cell transfer has similar potential for improving TIL therapy. Each of these manipulations of the host immune environment is being translated for clinical investigation in ACT clinical protocols. These technologies can be combined with emerging strategies and new biological therapeutics for systemic immune stimulation to improve and enhance the scope and efficacy of TIL Therapy.

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PERSPECTIVE

INDIAN SCENARIO IN CELLULAR IMMUNOTHERAPY

Introduction

Despite numerous advances in medicine, cancer is the second leading cause of death in the world. There are approximately 7.6 million new cancer cases globally, of which 52% occur in developing countries every year. According to 1999 report on cancer occurrence in India, it was estimated that nearly 6.5 lakhs new cases of cancer occur in India per year with more than 50% of the total cancer occurring in women. The crude incidence of cancer in India is approximately 100 per 100,000 population. Recent epidemiological studies done at the National Cancer Registry programme in India report that cancer burden in the country is 2.5 to 3 million and number of new cases diagnosed every year is around 700,000 - 900,000.

Although the common treatment modalities, such as surgery and/or chemo and radiotherapies, play major roles in bringing down the mortality and morbidity to a significant extent, complete cure is still uncertain and the prognosis varies depending upon the stage and the type of the disease. Even when patients experience tumor regression immediately after therapy, recurrence or metastasis (spreading to other parts of the body) can occur later. Immunotherapies have the potential to be used to fight cancer by either applying an external stimulus to the immune system to make it act more 'forcefully' or 'smarter', or by providing the immune system with man-made or naturally-derived tumor specific proteins made outside of the body so that the immune system can recognize the tumor as a foreign entity and destroy it.

Cellular Therapies

The different types of cellular immunotherapies are briefly described below

1. Dendritic Cell Therapy: Dendritic cell therapy comprises a group of methods that provoke anti-tumor responses by causing dendritic cells to present tumor antigens. Sipuleucel-T (first cell based immunotherapy), was approved in 2010 for the treatment of prostate cancer.

2. Monoclonal Antibodies Therapy: Many immunotherapeutic approaches involve the use of antibodies which are raised against specific antigens, such as the unusual antigens that are presented on the surfaces of tumors.

Types of monoclonal antibodies are naked monoclonal, and conjugated monoclonal antibodies. Some of the approved mAbs therapies are: Alemtuzumab, Bevacizumab, Brentuximab vedotin, Cetuximab, etc.

3. Cytokines Therapy: Two commonly used groups of cytokines are the interferons and interleukins.

4. Adoptive T-Cell Therapy: T-cells are activated by the presence of APCs, such as dendritic cells that present tumor antigens to the T-cells. As of 2014, several clinical trials for ACT were underway.

It is well known fact now that chemotherapy of cancer has toxic side effects and limitations in efficacy. Radiotherapy is also a very effective mode of treatment in certain types of cancer, with its own adverse effects as well. These two modalities affect not only the cancer affected cells, but also the normal cells. The latest scientific advancement cancer treatment includes Immune therapy and cancer vaccines. Consequently, many patients with advanced cancer opt for less toxic therapies like immuno cell therapy and cancer vaccine which are collectively known as biological therapy.

Immuno cell therapy is a promising new addition to the family of cancer treatments that includes surgery, chemotherapy, radiotherapy and cancer vaccines. This mode of treatment uses the body's immune system, either directly or indirectly, to fight cancer by enhancing the immune mechanisms of the body. As this therapy uses only the patients own cells for treatment, it is very safe and doesn't result in any allergy and combining this with the conventional therapy(ies) appropriately as per the patients condition, type of cancer and regimen of chemo/radio therapies, improves the outcome. As the immune cells of the patient upon transfusion do not affect the normal functioning cells of the body, it is furthermore safe.

Although drug discovery in India is two decades old, global pharma market is still looking forward to a drug discovered in the country. But cancer drug discovery is not absent in India. In the last five years, Aurigene in India has spent good amount of money on internal cancer drug discovery programmes and cancer drug discovery, and has seven molecules that are likely to enter clinical trials in a year or two. They are in a hot new area: immunotherapy. This emerging area, which has seen the launch of the first few products recently, is expected to change the direction of cancer treatment. Aurigene is the only company in the world developing peptides against immune checkpoints natural mechanisms that cancers hijack to evade the immune system. No one knows what kind of molecules and in what combination, will be effective in cancer immunotherapy. But Aurigene's

pipeline and the recent deal have brought it to the notice of the international drug discovery community.

Piramal Group has four molecules in clinical trials but none in areas that are completely novel. Invictus APAC Biotech Pvt. Ltd. a Gurgaon-based biotechnology company is involved in dendritic cell based immunotherapy and is preparing the formulation to impede the growth of cancer cells. Their prime objective is to boost the immune system of the cancer patients naturally through their novel dendritic cell based therapy and help them fight against malignant tumors. They are also involved in T-cell therapy. Invictus, set up in 2011, is the first cancer-focussed drug discovery company in India. A large drug complex can evade the healthy tissues, which have small pores, and go directly to the cancer cells.

Another Delhi-based firm is also readying an anti-cancer molecule. Curadev, set up in 2010 to do drug discovery, has a few molecules under development, including for cancer immunotherapy, for which it has filed patent applications. With all these programmes now accelerating, no one should be surprised if an Indian anti-cancer molecule hits the market in a decade.

NCRM (Nichi-In Centre for Regenerative Medicine) have signed a collaborative agreement with Biotherapy Institute of Japan (BIJ) and has brought Autologous Immune Enhancement Therapy (AIET) to India that would benefit patients suffering from cancers. This treatment uses the patient's own cells taken from the peripheral blood and doesn't have any rejection problem or any other side effects. The focus presently of Autologous Immune Enhancement therapy (AIET) at NCRM is based on reported clinical experiences well accepted by the scientific community.

Conclusion

Immunotherapy is a very active area of cancer research wherein the treatment makes the immune system fight against cancer. Scientists around the world are studying new ways to use immunotherapy to treat cancer. The immune system basically works by recognizing and fighting out any foreign substance invading or found in the body. But the immune system's normal ability to fight cancer is limited, because the cancer cells are not different enough from that of normal cells. To overcome this, researchers have designed ways to help the immune system recognize cancer cells and strengthen its response so that it will destroy them. Biological therapy, also known as immunotherapy (and as biotherapy or biological

response modifier therapy), is a type of treatment that works with persons' own immune system to help fight cancer or help control side effects from other cancer treatments like chemotherapy.

To date, there is no "magic bullet" in the treatment of cancer, and because of the complexity of cancer biology, it perhaps may not be attainable. It is now generally agreed that the future of cancer therapy lies in the combination of therapies with different mechanisms of action. Immunotherapy holds a bright future in the treatment of cancer. Many companies and research institutes in India and abroad are focusing in the development and in clinical trials of many immunotherapies for cancer treatment.

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RESEARCH & DEVELOPMENT

Antitumor Immunity

Optimal tumor cell surface expression of human leukocyte antigen (HLA) class I molecules is essential for the presentation of tumor-associated peptides to T-lymphocytes. However, a hallmark of many types of tumor is the loss or downregulation of HLA class I expression associated with ineffective tumor antigen presentation to T cells. Frequently, HLA loss can be caused by structural alterations in genes coding for HLA class I complex, including the light chain of the complex, β 2-microglobulin (β 2m). Scientists have characterized a replication-deficient adenoviral vector carrying human β 2m gene, which is efficient in recovering proper tumor cell surface HLA class I expression in β 2m-negative tumor cells without compromising the antigen presentation machinery. Tumor cells transduced with β 2m induce strong activation of T cells in a peptide-specific HLA-restricted manner. Gene therapy using recombinant adenoviral vectors encoding HLA genes increases tumor antigen presentation and should be considered as part of cancer treatment in combination with immunotherapy.

(*Cancer Gene Ther*, June 27, 2014)

Combination Therapy

A new study has shown that targeting toll like receptors 7, 8 and 9 eliminates large established tumors. The TLR7/8 agonist 3M-052 and the TLR9 agonist CpG ODN both trigger innate immune responses that support the induction of tumor-specific immunity. Normal mice were challenged with syngeneic tumors. Once these tumors reached clinically detectable size (500-800 mm³), they were treated by intra-tumoral injection with 3M-052 and/or CpG ODN. Anti-tumor immunity and tumor growth were evaluated. The co-delivery of agonists targeting TLRs 7, 8 and 9 increased the number and tumoricidal activity of tumor infiltrating CTL and NK cells while reducing the frequency of immunosuppressive MDSC. The combination of 3M-052 and CpG ODN (but not each agent alone) eradicated large primary tumors and established long-term protective immunity. The combination of agonists targeting TLRs 7/8 and 9 represents a significant improvement in cancer immunotherapy.

(*J Immunother Cancer*, May 2014)

Immunotherapy and Gene Therapy

The antitumor activity of lymphokine activated killer (LAK) cells immunotherapy is not always effective in all patients, especially when used alone. A new study has highlighted the role of LAK cell immunotherapy and adenovirus-p53 gene therapy for head and neck squamous cell carcinoma. The *in vitro* cytotoxicity of LAK cells was tested in H891 cells infected with or without Ad-p53, and the mRNA expression levels of natural killer group 2D ligands [UL16 binding protein (ULBP) 1 to 5] and tumor necrosis factor (TNF- α) in these cells were measured by real-time reverse transcription polymerase chain reaction. Ad-p53 infection increased the cytotoxicity of LAK cells against H891 cells, and also increased the mRNA expression levels of the ULBPs in H891 cells and TNF- α in the LAK cells. The antitumor activities of LAK cells in H891 cells were enhanced by Ad-p53. The combinational therapy of LAK immunotherapy and Ad-p53 gene therapy may represent a new paradigm for the treatment of head and neck cancer.

(*Anticancer Res*, July 2014)

Immunotherapy of Acute Lymphoblastic Leukemia

Disease relapse or progression is a major cause of death following umbilical cord blood (UCB) transplantation (UCBT) in patients with high risk, relapsed or refractory acute lymphoblastic leukemia (ALL). Adoptive transfer of donor-derived T cells modified to express a tumor-targeted chimeric antigen receptor (CAR) may eradicate persistent disease after transplantation. Scientists have developed a novel strategy to expand UCB T cells to clinically relevant numbers in the context of exogenous cytokines. UCB derived T cells cultured with IL-12 and IL-15 generated >150-fold expansion with a unique central memory/effector phenotype. Moreover, UCB T cells were modified to both express the CD19-specific CAR, 1928z, and secrete IL-12. 1928z/IL-12 UCB T cells retained a central memory-effector phenotype and had increased anti-tumor efficacy *in vitro*. Furthermore, adoptive transfer of 1928z/IL-12 UCB T cells resulted in significantly enhanced survival of CD19⁺ tumor-bearing SCID-Beige mice. Clinical translation of CAR modified UCB T cells could augment the graft-versus-leukemia effect after UCBT and thus further improve disease-free survival of transplant patients with B cell ALL.

(*Leukemia*, July 09, 2014)

NEW TECHNOLOGIES

New Immunotherapy for Melanoma

A new study published by researchers at the University of California, San Francisco, United States showed that the deadly skin cancers in mice reduced in size in response to a new treatment that may complement other “immunotherapies” developed recently to boost the body’s own defenses against disease threats. The researchers have discovered a way to manipulate the thymus gland that makes some specialized immune cells passing through it to fight cancer cells. The mice that normally would be dead in one or two weeks, were observed to reject tumors and survived. The thymus gland plays an important role in educating T cells of the immune system about which molecules encountered in the body should be attacked and which are normal parts of the body and must be tolerated. The researchers marked these thymus cells for destruction by targeting a protein molecule called RANK-L. The treatment that inhibited RANK-L reduced their numbers by more than 90%. As a result of the elimination of the thymus cells, T cells that target tumors survived and escaped central tolerance. The treatment of just two was sufficient to generate enough tumor-specific T cells to destroy deadly melanoma skin cancers in the mice. A theoretical concern with the anti RANK-L strategy was autoimmunity where the immune system used to destroy normal tissue due to the process of learning to tolerate “self” molecules was disrupted by treatment. Hence, the significant autoimmune reactions in treated mice were not observed and immune responses were back to normal within ten weeks of stopping the treatment.

(Science Newslines, May 13, 2014)

Novel Immunotherapeutic Tool

A team of tumor immunology scientists and oncologists from the Winship Cancer Institute of Emory University, Atlanta, have discovered an innovative approach to cancer immunotherapy exploiting the power of B-cells. The novel fusion cytokine generated by N-terminal coupling of GM-CSF to IL4, generating a fusokine has been termed as GIFT4. The B-cells are best known for their ability to make antibodies to fight off viruses and bacteria, however, the use of a novel engineered cytokine

named GIFT4 leads to conversions of normal B-cells to potent anti-cancer promoting cells, as verified in a mouse model of melanoma. This new technology provides an opportunity to harness a component of immunity which has never been utilized in cancer cell immunotherapy. The researchers intend to transform this discovery made in mice into a first-in-human clinical trial. In this approach, a blood sample would be treated with GIFT4 fusokine in a specialized lab. This personalized B-cell product would serve as a transfusion medicine and companion to cancer therapy. The results of the study demonstrated that GIFT4 could mediate expansion of B-cells with potent antigen-specific effector function, thereby suppressing melanoma growth. The product of new technology GIFT4 may offer a novel immunotherapeutic tool and define a previously unrecognized potential for B-cells in melanoma immunotherapy.

(Cancer Research, June 17, 2014)

Target for Cancer Immunotherapy

Scientists at A*STAR’s Singapore Immunology Network (SIgN) have discovered a new class of the lipids in leukemia cells that are detected by a unique group of immune cells. By recognizing the lipids, the immune cells stimulate an immune response to destroy the leukemia cells and suppress their growth. This new class of lipids, ie, methyl-lysophosphatidic acids (mLPA), is accumulated in leukemia cells. The research team had also identified a specific group of immune cells described as mLPA-specific T-cells, capable of recognizing the mLPA in the leukemia cells. mLPA-specific T cells efficiently kill CD1c(+) acute leukemia cells, poorly recognize non transformed CD1c-expressing cells and protect the immunodeficient mice against CD1c(+) human leukemia cells and thereby limits cancer progression. The efficacy of the T cells in killing leukemia cells was also demonstrated in a mouse model of human leukemia. Thus far, only proteins in cancer cells have been known to activate T cells. This study pioneers the discovery of mLPA and the specific T cells that can identify lipids expressed by cancer cells. Since current treatments run the risk of failure due to the regrowth of residual leukemia cells that survive after stem cell transplants, the T cell immunotherapy may serve as a complementary treatment for more effective and safer therapeutic approach towards leukemia.

(J Exp Med, June 30, 2014)

CLINICAL TRIAL

Combination Immunotherapy for Melanoma Patients

A phase 2 clinical trial was conducted at Advocate Cancer Institute, Ames in patients with advanced melanoma to see the effects of combination immunotherapy. Hyper Acute Melanoma (HAM) vaccine (NLG-12036, NewLink Genetics) combined with pegylated interferon (Sylatron, Merck). Study cohort consisted of 25 patients with median age of 60. Patients were treated for total 12 weeks with the initial induction phase of 4 weekly treatments of HAM alone, administered intradermally followed by 8 additional treatments of HAM and Sylatron, given subcutaneously. Of 25 patients, 21 completed the trial and 4 stopped because of progressive disease (PD). Results showed that of the 16 stage 4 patients, 2 had a complete response, 1 had stable disease, and 4 had no evidence of disease (NED) after resection. For stage 2/3 patients, 3 of 9 remained NED, and the 1 stage 2C patient had slow progressive disease. The study concluded that combination therapy using the HAM vaccine and Sylatron is feasible, safe, and shows promising efficacy data. This combination is also capable of inducing complete and durable clinical responses with regression of bulky metastatic disease.

(*Br J Cancer, April 2014*)

CIK Cells for Pancreatic Cancer

Patients with advanced pancreatic cancer refractory for second line chemotherapy Gemcitabine show poor disease outcome due to rapid disease progression. A phase II trial was done by the researchers of South Korea to evaluate the efficacy and safety of immunotherapy using *ex vivo*-expanding cytokine induced killer (CIK) cells in pancreatic cancer patients. Twenty patients were enrolled in the study and CIK cells were given intravenously, every week 10 times for 5 weeks. The disease control rate was observed to be 25%. The median progression free survival (PFS) was 11 weeks and overall survival (OS) was 26.6 weeks. Grade 4 toxicity was not found in any patients. Overall results showed encouraging results in terms of quality of life, ie, improvement in pancreatic pain, gastrointestinal distress jaundice and altered bowel habits. PFS and OS

time was also found to be comparable with the data of previous trial using conventional chemotherapeutic.

(*Cancer Immunol Immunother, June 12, 2014*)

Ipilimumab Retreatment for Advanced Melanoma

According to the randomized phase III trial, ipilimumab improves survival in pretreated patients of advanced melanoma. Researchers of Napoli, Italy, recruited 855 patients in the trial who had previously failed or were intolerant to the systemic therapy, had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2, and no other therapeutic option. Patients were given intravenous ipilimumab 3 mg/kg every 3 weeks and total of four doses. Of 855 patients treated with ipilimumab, 51 were retreated upon disease progression. Of these 28 (55%) regained disease control upon retreatment. Eleven patients (22%) had a treatment related adverse event of any grade. Median PFS among all treated patients was 3.7 months and median OS was 7.2 months. Overall results showed that retreatment with ipilimumab is generally well tolerated and can accrue clinical benefit.

(*Br J Cancer, April 2014*)

Multiple Peptide Vaccine for Esophageal Cancer

Patients of unresectable esophageal squamous cell carcinoma (ESCC) often develop local recurrence after chemoradiation therapy (CRT). A phase I trial was done in the ESCC patients where, with immunotherapy by peptide vaccine was given to the patients along with CRT. The peptide vaccine included the 5 peptides as: TTK protein kinase (TTK), up-regulated lung cancer 10 (URLC10), insulin-like growth factor-II mRNA binding protein 3 (KOC1), vascular endothelial growth factor receptor 1 (VEGFR1), and 2 (VEGFR2). CRT consisted of radiotherapy (60 Gy) with concurrent cisplatin (40 mg/m²) and 5-fluorouracil (400 mg/m²). Total 11 unresectable chemo-naïve ESCC patients were recruited in the study. Six of these patients showed complete response (CR) and 5 progressive disease (PD) after the 8th vaccination. CR for 2.0, 2.9, 4.5 and 4.6 years is seen in the 4 CR patients who continued the peptide vaccination. Very few grade 3 toxicities, leucopenia, neutropenia, anemia and grade 1 local skin reactions in the injection sites of vaccination were observed. Results of the study show that multi-peptide vaccine combined with CRT has satisfactory the safety levels and long CR period in unresectable ESCC patients.

(*J Transl Med, April 2014*)

WATCHOUT

Compositions for Immunotherapy

McKenzie, et al. of MacFarlane Burnet Institute for Medical Research and Public Health Ltd (Melbourne, AU), have been awarded US Patent No. 8,771,701 on July 8, 2014. The present invention relates to a method of eliciting a cytotoxic T lymphocyte response to an antigen in an animal, the method comprising pulsing mannose receptor-bearing antigen presenting cells *in vitro* or *ex vivo* with a conjugate comprising an antigen and a carbohydrate polymer comprising mannose, wherein said carbohydrate polymer is a fully oxidized carbohydrate polymer comprising free aldehydes; and administering the pulsed antigen presenting cells to an animal. The invention is particularly advantageous in that it regulates T cell responses by increasing the uptake of an antigen: carbohydrate polymer conjugate of the present invention by inducing receptors for mannose on cells capable of stimulating T cells reactive to the antigen of the conjugate. In addition, the invention enables an antigen, for example a mucin: carbohydrate polymer conjugate of the present invention, to be administered to an animal in such a manner that binding of the antigen, e.g., mucin, by naturally occurring antibodies directed against or crossreactive with the antigen in the animal is avoided. The patent grant protects the method of composition and the method of use of CVac, which is formulated in a patient's own dendritic cells and then reinjected back into the patient to generate a cytotoxic T cell response against the mucin 1 antigen. CVac is a personalised immunotherapy composed of a patient's own dendritic cells pulsed with the cancer antigen mucin 1, conjugated to oxidised mannan.

(www.uspto.org, July 15, 2014)

Determining PD-1 Activity using Gene Expression Profiles

Dr. Rafick-Pierre Sékaly, PhD and Dr. Elias Haddad, PhD U.S. of The Vaccine and Gene Institute of Florida (VGTFI Florida), have been awarded the US Patent No. 8,647,822 for their invention titled Determining whether a test compound modulates PD-1 activity in activated immune cells using gene expression profiles. The PD-1 pathway (programmed cell death) is being actively pursued by most of the big pharmaceutical companies

due to its critical role as a checkpoint in our immune system's response to cancer and chronic inflammation. This therapeutic strategy is actively being evaluated in multiple clinical trials, such as melanoma, non-small cell lung carcinoma, and HIV. Phase I trials are also under way for breast cancer, metastatic bladder cancer, and head and neck cancer. Modulation of PD-1 activity in the presence or absence of an agent as measured by a gene expression profile of at least two genes is provided. Reagents, kits, methods and uses thereof for the modulation of immune function comprise the identification of modulators of PD-1 activity. This patent represents a body of research that has been very useful to drug developers working on the PD-1 blockbuster pathway. Using gene expression profiles of cells allows drug developers to evaluate the cellular pathways that are operating in response to drug candidates. The patent, issued on February 11, 2014 was also awarded to the scientists' co-inventors at the Massachusetts General Hospital. A Canadian Patent Application No. 2,742,926 remains in review.

(www.vgtifl.org, July 10, 2014)

Immunotherapies Derived from Viruses

Qu Biologics, Vancouver, Canada has been issued New Zealand Patent No. 601232. The granted patent involves using components of viruses to stimulate an immune response to treat a range of cancers. It is a biotechnology company developing site specific immunomodulators (SSIs) that aim to "reboot" the body's immune system. SSIs, derived from inactivated bacterial and viral components, activate an innate immune response in the specific organ or tissue commonly infected by the pathogen. Since innate immune system defect or deficiency plays an important underlying role in cancer or other immune-related diseases, this site-directed immunostimulation represents a promising new approach to engaging the body's own immune response for the treatment of these important diseases. The company's SSI platform represents a promising new approach to the treatment of cancer and immune-related disease by addressing the underlying immunological factors. Qu Biologics has developed multiple SSIs, each of which targets a specific tissue/organ system. SSIs aim to 'reboot' the body's immune response to cancer, using immunostimulative bacterial components, representing a novel treatment approach.

(www.qubiologics.com, July 5, 2014)

INFOCUS

CANCER IMMUNOTHERAPY WITH CHIMERIC ANTIGEN RECEPTOR (CAR)-T-CELLS

Immunotherapy in Cancer

Cancer cells are the altered self-cells and for malignant growth they require to manifest six traits- self-sufficiency from external growth signals, insensitivity to negative growth signals, resistance to apoptosis, limitless replicative potential, sustained angiogenesis and acquisition to tissue invasiveness.

Cancer immunotherapy relies on the immune component to rule out cancer in patient. Immunotherapies have the potential to be used to fight cancer by either applying an external stimulus to the immune system to make it act more 'forcefully' or 'smarter', or by providing the immune system with man-made or naturally-derived tumor specific proteins made outside of the body so that the immune system can recognize the tumor as a foreign entity and destroy it. The main premise is stimulating or educating the patient's immune system to attack the malignant tumor cells responsible for the disease. There are two ways to do it, either through immunization of cancer vaccine, in which case the patient's own immune system is trained to recognize tumor cells as targets to be destroyed, or through the administration of humanized therapeutic antibody as drugs, in which case the patient's immune system is recruited to destroy tumor cells by the therapeutic antibodies.

Cell based immunotherapy is another major entity of cancer immunotherapy. This involves innate immune cells, such as the Natural Killer Cells (NK cells), Lymphocyte Activated Killer cell (LAK), Cytotoxic T Lymphocytes (CTLs), Dendritic Cells (DC), etc., which are either activated in vivo by administering certain cytokines, such as Interleukins or they are isolated, enriched and transfused to the patient to fight against cancer.

Immunotherapy in Cancer

To date, there is no "magic bullet" in the treatment of cancer, and because of the complexity of cancer biology, it may likely not be attainable. Until recently, chemotherapy was the only treatment that had been shown to improve overall survival in patients with metastatic castration-resistant prostate cancer. However,

in the last decade, a better understanding of the immune system, combined with innovative treatment approaches, has led to promising improvements in survival.

Immunotherapy seems to work better for some types of cancer than for others. It is used by itself to treat some cancers, but for many cancers it seems to work best when used along with other types of treatment.

T Cell Immunotherapy

T lymphocytes are powerful components of adaptive immunity, which essentially contribute to the elimination of tumors. Due to their cytotoxic capacity, T cells have emerged as attractive candidates for specific immunotherapy of cancer. Adoptive transfer of cytotoxic T cell-based immunotherapy is aimed to target cancer cells. T cells that have a natural or genetically engineered reactivity to a patient's cancer are generated in vitro and then transferred back into the cancer patient. This is done by taking T cells that are found with the tumor of the patient, which are trained to attack the cancerous cells. These T cells are referred to as tumor-infiltrating lymphocytes (TIL), are then encouraged to multiply in vitro using high concentrations of IL-2, anti-CD3 and allo-reactive feeder cells. These T cells are then transferred back into the patient along with exogenous administration of IL-2 to further boost their anti-cancer activity.

Clinical trials based on adoptive cell transfer of TILs for patients with metastatic melanoma are currently ongoing at the National Cancer Institute (Bethesda, MD, USA), Moffitt Cancer Center (Tampa, FL, USA), [31] MD Anderson Cancer Center (Houston, TX, USA), Sheba Medical Center (Tel Hashomer, Israel), Herlev University Hospital (Herlev, Denmark) and NKI Antonie van Leeuwenhoek (Amsterdam, Netherlands).

Recently, more improved version of T cell therapy has come in use. After removing from patients T cells are modified so that they express monoclonal antibody against specific tumor antigen. These T cells, which are referred as to chimeric antigen receptor (CAR) T cells, can then recognize and kill the cancer cells. Clinical studies of this approach have shown efficacy (Table 1).

CAR T Cells

Chimeric antigen receptors (CARs), the engineered receptors, are combination of the antigen binding site of a monoclonal antibody (more specifically the scFv) with the signal activating machinery of a T cell, freeing antigen recognition from major histocompatibility complex restriction. CARs expressing T (CAR-T) cells are highly

Table.1 List of Some Tumor Antigens and CARs in Vitro and in Vivo Trials

Target antigen	Associated malignancy	Receptor type	CARs generation
α -Folate receptor	Ovarian cancer	ScFv-Fc ϵ R1 γ CAIX	First
CAIX	Renal cell carcinoma	ScFv-Fc ϵ R1 γ	Second
CD19	B-ALL	ScFv-CD28-CD3 ζ	Second
CD19	ALL post-HSCT	ScFv-CD28-CD3 ζ	Second
CD19	Leukemia, lymphoma, CLL	ScFv-CD28-CD3 ζ vs. CD3 ζ	First and Second
CD19	B-cell malignancies	ScFv-CD28-CD3 ζ	Second
CD19	B-cell malignancies post-HSCT	ScFv-CD28-CD3 ζ	Second
CD19	Refractory Follicular Lymphoma	ScFv-CD3 ζ	First
CD19	B-NHL	ScFv-CD3 ζ	First
CD19	B-lineage lymphoid malignancies post-UCBT	ScFv-CD28-CD3 ζ	Second
CD19	CLL, B-NHL	ScFv-CD28-CD3 ζ	Second
CD19	B-cell malignancies, CLL, B-NHL	ScFv-CD28-CD3 ζ	Second
CD19	ALL, lymphoma	ScFv-41BB-CD3 ζ vs CD3 ζ	First and Second
CD19	ALL	ScFv-41BB-CD3 ζ	Second
CD19	B-cell malignancies	ScFv-CD3 ζ (Influenza MP-1)	First
CD19	B-cell malignancies	ScFv-CD3 ζ (VZV)	First
CD20	Lymphomas	ScFv-CD28-CD3 ζ	Second
CD20	B-cell malignancies	ScFv-CD4-CD3 ζ	Second
CD20	B-cell lymphomas	ScFv-CD3 ζ	First
CD20	Mantle cell lymphoma	ScFv-CD3 ζ	First
CD20	Mantle cell lymphoma, indolent B-NHL	CD3 ζ /CD137/CD28	Third
CD20	indolent B cell lymphomas	ScFv-CD28-CD3 ζ	Second
CD20	Indolent B cell lymphomas	ScFv-CD28-41BB-CD3 ζ	Third
CD22	B-cell malignancies	ScFv-CD4-CD3 ζ	Second
CD30	Lymphomas	ScFv-Fc ϵ R1 γ	First
CD30	Hodgkin lymphoma	ScFv-CD3 ζ (EBV)	First
CD33	AML	ScFv-CD28-CD3 ζ	Second
CD33	AML	ScFv-41BB-CD3 ζ	Second
CD44v7/8	Cervical carcinoma	ScFv-CD8-CD3 ζ	Second
CEA	Breast cancer	ScFv-CD28-CD3 ζ	Second
CEA	Colorectal cancer	ScFv-CD3 ζ	First
CEA	Colorectal cancer	ScFv-CD28-CD3 ζ	Second
erb-B2	Colorectal cancer	CD28/4-1BB-CD3 ζ	Third
erb-B2	Breast and others	ScFv-CD28mut-CD3 ζ	Second
erb-B2	Prostate cancer	ScFv-Fc ϵ R1 γ	First
erb-B 2,3,4	Breast and others	Heregulin-CD3 ζ	Second
FBP	Ovarian cancer	ScFv-Fc ϵ R1 γ	First
FBP	Ovarian cancer	ScFv-Fc ϵ R1 γ (alloantigen)	First
Fetal acetylcholine receptor	Rhabdomyosarcoma	ScFv-CD3 ζ	First
GD2	Neuroblastoma	ScFv-CD28-OX40-CD3 ζ	Third
GD3	Melanoma	ScFv-CD3 ζ	First
Her2/neu	Glioblastoma	ScFv-CD28-CD3 ζ	Second
IL-13R-a2	Glioma	IL-13-CD28-4-1BB-CD3 ζ	Third
IL-13R-a2	Medulloblastoma, Glioblastoma	IL-13-CD3 ζ	Second
k-light chain	(B-NHL, CLL)	ScFv-CD28-CD3 ζ vs CD3 ζ	Second
LeY	Epithelial derived tumors	ScFv-CD28-CD3 ζ	Second
L1 cell adhesion molecule	Neuroblastoma	ScFv-CD3 ζ	First
MAGE-A1	Melanoma	ScFv-CD28-Fc ϵ R1 γ	Second
Mesothelin	Various tumors	ScFv-CD28-41BB-CD3 ζ	Third
Murine CMV infected cells	Murine CMV	Ly49H-CD3 ζ	Second
MUC1	Breast, Ovary	ScFv-CD28-OX40-CD3 ζ	Third
NKG2D ligands	Various tumors	NKG2D-CD3 ζ	First
Oncofetal antigen (h5T4)	Various tumors	ScFv-CD3 ζ (vaccination)	First
PSCA	Prostate carcinoma	ScFv-b2c-CD3 ζ	Second
PSMA	Prostate/tumor vasculature	ScFv-CD28-CD3 ζ	Second
TAA targeted by mAbIgE	Various tumors	Fc ϵ R1-CD28-CD3 ζ (+ a-TAA IgEmAb)	Third

targeted, but additionally offer the potential benefits of active trafficking to the tumor sites, *in vivo* expansion and long term persistence. Moreover, genetic modification allows the introduction of counter measures to tumor immune evasion and of safety mechanisms.

The concept of doctoring T-cells genetically was first developed in the 1980s by Dr. Zelig Eshhar and colleagues at the Weizmann Institute of Science in Rehovot, Israel. By 1989, Eshhar and his colleagues had created in the lab the first CAR T cells that worked.

Basic Structure of CARs

A CAR constitutes two portions, viz, the Binding portion and the Signalling portion. The Binding portion comprises the binding site of a molecule that attaches strongly to the antigen being targeted, and the Signalling portion comprises of the cytoplasmic domains of conventional immune receptors responsible for initiating signal transduction that leads to lymphocyte activation. Therefore, the name CARs (Chimeric Antigen Receptors) is used as they combine portions of different molecules. Sometimes CARs are also known as T-Bodies since many of the original CARs systems attached an antibody fragment to a T cell.

The binding portion most commonly used is derived from the structure of the Fab (antigen binding) fragment of a monoclonal antibody (mAb) that has high affinity for the antigen being targeted. Now this is where a single-chain fragment variable (scFv) region comes into picture. Since the Fab is the product of two genes, the corresponding sequences are usually combined by a short linker fragment that allows the heavy-chain to fold over the light-chain derived peptides into their native configuration, thus giving rise to the scFv region. Some other antigen binding moieties that can be used include signalling portions of hormone or cytokine molecules, the extracellular domains of membrane receptors and peptides derived from screening of libraries (e.g. phage display).

The signalling portion of CARs usually contains the intracellular domains of the zeta (ζ) chain of the TCR/CD3 complex or, less commonly, the gamma (γ) chain of the immunoglobulin receptor Fc ϵ RI or the CD3-epsilon (ϵ) chain.

Since projection of the antigen recognition domains away from cell surface may be required for better binding to the antigen, it is advantageous if some degree of flexibility exists between the binding and the signalling portions of CARs. Therefore, a hinge region bridging

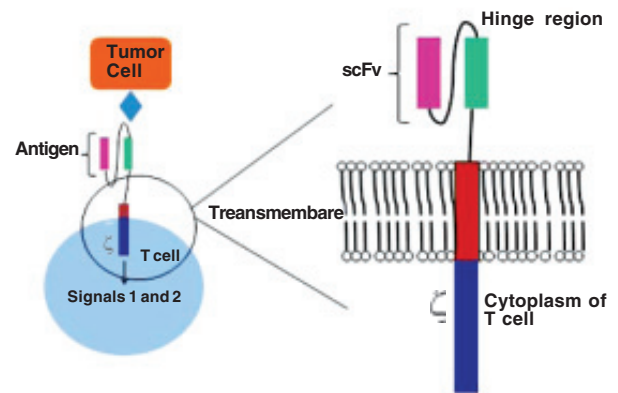


Fig 1: Basic structure of a CAR

these two portions is generally included in the construct. The importance of the hinge region has been illustrated by studies which demonstrated that, for the same targeting construct, optimal T cell activation depends on the relative length of this hinge region and the distance of the epitope from the target cell membrane. Examples of the hinge region include CH_2CH_3 portion of an immunoglobulin molecule such as IgG₁.

Generations of CARs

The initially designed CARs contained a single signalling domain. These 1st generation CARs exhibited feasibility, but had limited clinical benefit. The probable reasons for this could be ineffective or incomplete activation of these cells, leading to a very limited persistence.

In order to function, signal-1 needs to be generated in a T cell. Signal-1 is generated when a T cell binds through its TCR to its native antigen presented by an HLA molecule. However, a naïve T cell requires additional stimulatory events incited by neighbouring cells in order to function completely. These additional events lead to the generation of signal-2 and sometimes even signal-3. Examples of these include activating ligands displayed on the surface of the cells presenting the antigen, which bind co-stimulatory molecules in T cells (for signal-2) and stimulatory cytokines secreted by the same or other nearby cells (for signal-3).

Recently developed 2nd generation CARs have been engineered to include other stimulatory domains so as to provide T cells with additional activating signals. As these co-stimulatory domains are incorporated into the CAR, its activation by engagement with the respective antigen delivers both signal-1 and signal-2 to T cells, thus overcoming the problems that arise from a solitary signal-1. Many *in vitro* and preclinical studies have shown improved function of T cells bearing 2nd generation

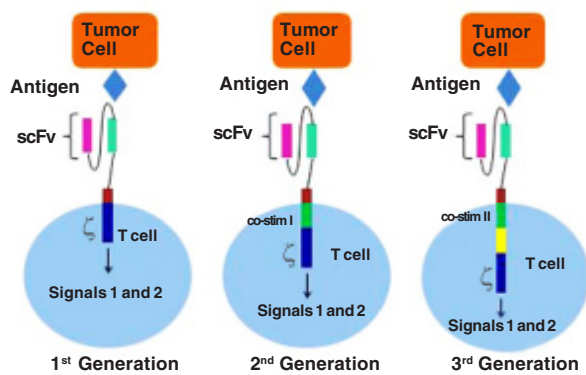


Fig 2: Generations of CARs

CARs than 1st generation ones. 3rd generation CARs incorporating 3 or more stimulatory domains have also been described but their effects are not yet apparent.

How to Embed CARs into T Cells (Gene Transfer Methods)

In most cases, the goal is to obtain constitutive expression of the CARs. Therefore, usually those techniques are utilised which lead to permanent genetic modification of the T cells rather than momentarily expressing CARs in the T cells. The most frequently employed strategy uses gammaretroviruses or lentiviruses (both members of the retrovirus family) that are engineered to encode the full length CAR molecule. Upon infection of the target cells, the viral genomic RNA gets retrotranscribed into DNA, which in turn gets randomly inserted into the host cell DNA through the action of a viral integrase, hence becoming part of that cell's genome. Since the retroviruses lack key genes like *gag*, *pol* and *env*, they are unable to complete their life cycle by proliferating and infecting other cells (replication-defective).

Gammaretroviruses require that their target cells are dividing for successful integration, unlike lentiviruses, which are capable of infecting resting cells. Two methods are used to obtain a population of T cells that have been activated *in vitro*.

One method is to use an activating monoclonal antibody anti-CD3 (OKT3) in order to obtain polyclonal stimulation of T cells. The "lymphoblasts" (OKT3-blasts) thus obtained are easily transducible by retroviruses. For further activating the T cells, stimulation of native CD28 receptors is done by another agonistic monoclonal antibody. After stimulation and transduction, which takes approximately 48 hours, the T cells are kept in culture for 2 to 3 weeks in IL-2 containing medium until sufficient numbers are reached.

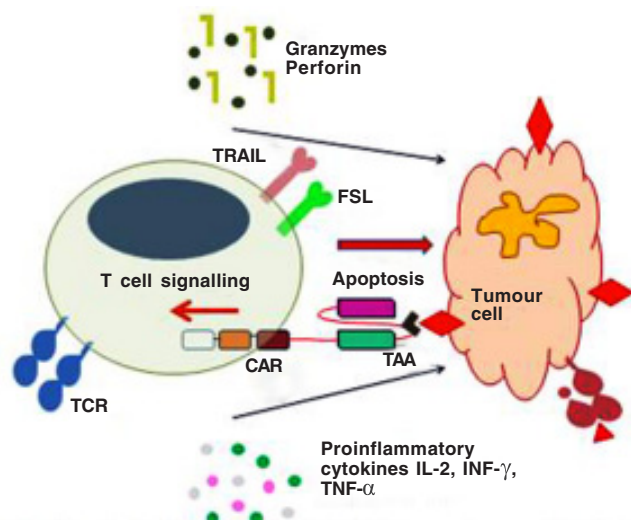
Another method is to selectively expand T cells with particular specificities in the presence of antigen presenting cells such as dendritic cells or lymphoblastoid cell lines. These antigen-specific T cells can then be transduced with a retrovirus encoding a CAR, generating bispecific cell lines.

Due to high costs of retrovirus production under good manufacturing practices, some non-viral methods are also used for permanent transduction, specifically transposon-based systems, including *Sleeping Beauty* and *PiggyBac*. These usually require double transfection with one plasmid containing the expression cassette for the desired CAR and another encoding a transposase. Once expressed in the target cells, the transposase catalyzes the integration of the CAR gene into specific sites throughout the genome and thus leads to stable integration of the gene of interest.

Depending on the final application and the overall efficiency of the transduction method used, a selectable marker (such as surface antigen or an antibiotic resistance gene) can be used to aid the purification of the transduced cells.

How CARs Work (Mechanism of Action)

Very little is known about the initial activation of CARs. It is commonly assumed that dimerization or multimerization of the receptors resulting from binding target antigen epitopes brings together their cytoplasmatic signalling domains, which then become targets for intracellular kinases, such as Lck. Phosphorylation of ITAM (immunoreceptor tyrosine based activation motives) contained in the signalling portion of the CARs then recruit adapter molecules, such as ZAP-70, which in turn stimulate downstream pathways that lead to activation of the transduced cells. Depending on the exact nature of the transduced cell, this leads to release of cytotoxic molecules (such as perforin or granzymes), expression of proapoptotic ligands (such as Fas ligand–FasL, and tumor necrosis factor-related apoptosis inducing ligand–TRAIL) or secretion of proinflammatory cytokines (such as IL-2, IFN- γ and TNF- α). Thus, tumor cells can be eliminated directly by the CAR-T cells or other immune cells can be recruited to the tumor microenvironment, which usually is hostile to effector T cells because of the presence of immunosuppressive cells (such as regulatory T cells, stromal cells and myeloid derived stromal cells) and inhibitory cytokines secreted



Pros and Cons of CARs

Advantages: The most prominent advantage of this strategy is that it avoids the requirement of antigen processing and presentation by the target cell and is applicable to non-classical T-cell targets like carbohydrates. Some other advantages of CARs are follows -

- Highly targeted
- Potential benefits of active trafficking to tumour sites
- *In vivo* expansion and long term persistence
- Have human leukocyte antigen (HLA)-unrestricted activity and can target non-protein antigens
- Since CARs are single molecules, they are not subject to mispairing with complementary chains (native TCR chains)

Disadvantages

- Only surface antigens can be recognized by CARs
- Presence of soluble antigen shed by tumours could

compete with binding and killing of the malignant cells (though its not a major issue)

Future Scope and Application of CARs

Till date, CAR modified T cells have been used in several clinical trials but the results have so far been unobtrusive. Some matters that have scope for development and could aid in making CARs a preferred method of cancer therapy are

- Incorporation of more potent costimulatory domains into the CAR-molecule
- Better gene transfer methods
- Transduction of specific memory populations
- Infusion of the modified T cells into a lympho-depleted host
- Provision of T-cell-specific cytokines

If the above-mentioned areas are addressed, then in the future CAR-modified T cells will most likely play an increasing role in cellular therapy of cancer, chronic infections and autoimmune disorders.

Concluding Remarks

The CAR-mediated recognition induces cytokine production and specific tumor-directed cytotoxicity of T cells. Second and third generation CARs include signal sequences from various co-stimulatory molecules resulting in enhanced T-cell persistence and sustained antitumor reaction. Clinical applications reveal that CAR T is going to be magic bullet of cancer patient's who suffered a failure from chemotherapy and other modalities of cancer treatment.

(Dr Swagata Das, National Institute of Technology, Tiruchirappalli; Dr Dipanjan Ghosh, University of Texas, M D Anderson Cancer Institute, Houston)



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