Adoptive Cell Transfer

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Adoptive Cell Transfer is a technique whereby Tumor Infiltrating Lymphocytes, usually T cells, are collected from a tumor, enhanced ex vivo and replaced with the intent of enhancing the immune system. Work in this area has progressed and we examine herein multiple approaches as well as some history of the ACT, We also argue for further consideration of the innate system including NK cells as well as complement.

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1 INTRODUCTION

As understanding of the immune system has progressed its use as a therapeutic for various cancers has also moved forward. This hand in glove approach has allowed one to go from observation to utilization to modification and then cycle again. The area of tumor infiltrating lymphocytes and their application in the area of adoptive cell transfer has been a topic of interest for almost three decades. The presence of T cells around tumors is a common occurrence. For example in malignant melanoma there is often such a proliferation seen upon biopsy. The question then is; if the immune system acts accordingly then why does it not follow through and destroy the tumor? We now understand some of the basics of this process and one suspects a great deal more will be learned. However the proliferation of the T cells, called Tumor Infiltrating Lymphocytes, TILs, led early investigators to try and utilize them as a therapeutic. This was done by ex vivo acceleration and proliferation of the cells and implanting them back in the patient. This is adoptive cell transfer. This we shall focus on in this brief note.

Rosenberg and his Lab have for nearly 40 years been investigating this area. In a 1985 NEJM paper he wrote about his work with autologous lymphokine-activated killer (LAK) cells:

The administration of LAK cells in conjunction with interleukin-2 as reported in this paper represents a possible new approach to the treatment of cancer, with potential applicability to a wide variety of tumors. A major advantage of this approach is its broad antitumor specificity. It should be emphasized, however, that this study involved a limited number of patients and that the frequency and duration of the clinical responses have yet to be determined.

The practicality and safety of administering this therapy to large numbers of patients also remain to be fully defined. The similarity of our initial experience in patients to our prior experience in mice, however, offers hope that this therapy can be made effective against human cancer.

1.1 WHAT IS ACT

As noted, this examination of TILs and their function has been examine for decades. For example, in 1991 Jicha et al (in Rosenberg's Lab) had noted:

Interleukin 7 (IL:7) is a 25-kD cytokine that was initially described as a pre-B cell growth factor. This cytokine has also been shown to have T cell proliferative and differentiation effects. In this report, we demonstrate that antitumor cytotoxic T lymphocytes (CTL) generated by secondary in vitro sensitization of draining lymph node cells in IL7 are effective in treating 3-day syngeneic methylcholanthrene (MCA) sarcoma pulmonary metastases in mice.

In vivo titrations comparing IL7 to Ib2 antitumor CTL show that they have equivalent potency in adoptive immunotherapy. IL+-7 antitumor CTL generated against MCA sarcomas of weak immunogeneity are also tumor specific in their in vivo efficacy. This study represents the first successful use of a cytokine other than IL-2 for the generation of cells with in vivo efficacy in cellular adoptive transfer.

Earlier Belldegrun et al (also from Rosenberg's Lab) noted:

The identification, isolation, and adoptive transfer of selected subsets of immune cells with specific antitumor reactivity into tumor bearing patients to mediate cancer regression in vivo is a prime goal of tumor immunology. Currently, however, there are no available techniques for generating such lymphoid cells with reactivity against specific tumor antigens in the human.

Recent experiments have demonstrated that the adoptive transfer of lymphokine-activated killer cells plus IL-2 can mediate tumor regression in a variety of animal models and human tumors as well. This approach, however, requires the transfer of large numbers of sensitized fresh lymphocytes, i.e., more than 10^{11} immune cells, into tumor bearing humans, along with the systemic administration of relatively high doses of RIL-2 (100,000 units/kg body weight i.e. every 8 h).

Many human tumors are infiltrated with chronic inflammatory cells, including lymphocytes. We have recently identified a population of lymphoid cells infiltrating murine tumors that could be expanded in vitro in IL-2 and, when adoptively transferred, were capable of totally eliminating 3-day established pulmonary metastases. When compared to LAK cells, these TIL cells were at least 50 times more potent in mediating the therapy of established micrometastases. The simultaneous administration of IL-2 enhanced the in vivo therapeutic effective ness of the adoptive transfer of TIL, although high doses of TIL alone were also effective. The greater therapeutic efficacy of TIL compared to LAK cells in the treatment of established metastases in mice raises the possibility that TIL isolated from human tumors and expanded in vitro in IL-2 may similarly be effective for the treatment of human cancer.

Now in our current understanding these have again attracted attention as Horton and Gajewski (2018) note:

Tumours from multiple cancer types can be infiltrated by CD8+ T cells (TILs). TILs are thought to be suppressed by multiple immune inhibitory molecules in the tumour microenvironment, and this suppression has been associated with tumour progression.

Therefore, despite tumour infiltration, almost all tumours containing TILs will progress if not treated. While several immune inhibitory mechanisms have been identified, immune inhibitory receptors expressed on activated T cells, like CTLA-4 and PD-1, have received the most attention over recent years owing to the immense clinical success of PD-1 and CTLA-4 neutralizing antibodies. The engagement of inhibitory receptors expressed by TILs is thought to render TILs dysfunctional.

However, evidence from both human tumour samples and mouse models has suggested that, despite inhibitory receptor expression, TILs are not functionally inert and actually retain the ability to proliferate, produce IFN-g and show ex vivo cytotoxicity.

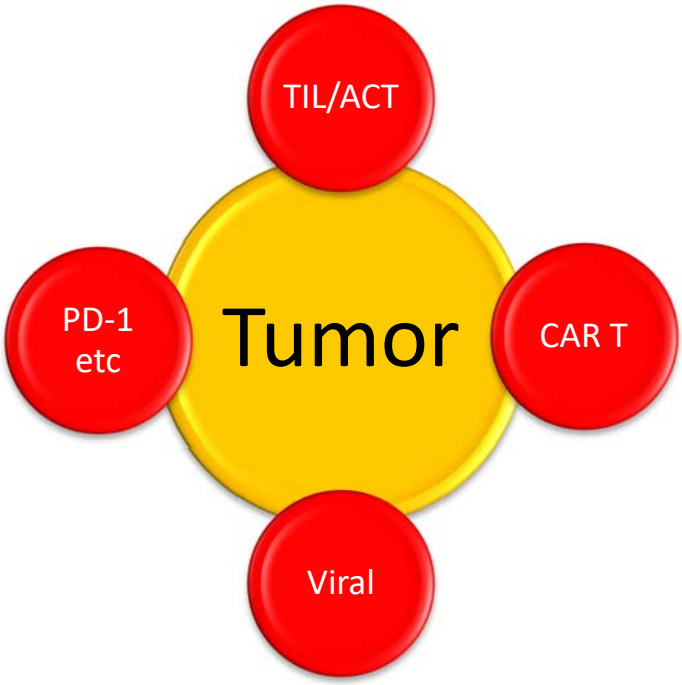
These observations raise the question of why activated TILs are not able to spontaneously control progressing tumours, and how tumours that contain TILs might sometimes be resistant to immunotherapies such as checkpoint blockade. Current immunotherapies can induce durable

tumour regression; however, they benefit a minority of patients: finding new strategies to increase the response rate to immunotherapies is of great interest to both researchers and clinicians.

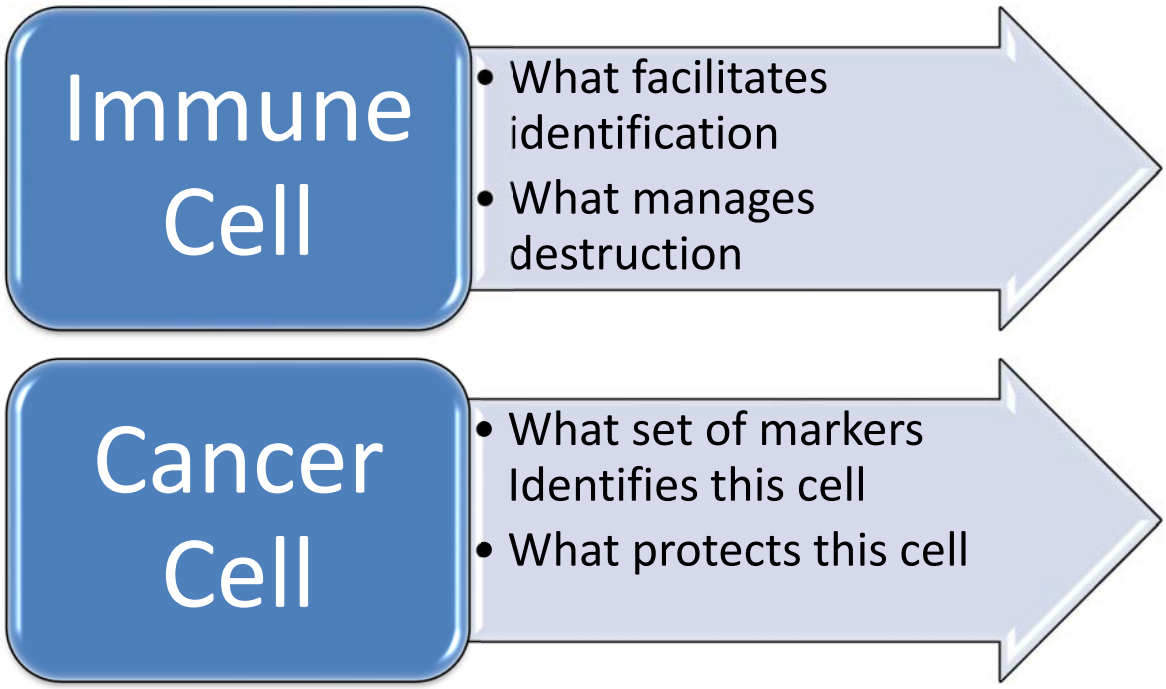
There are many dimensions available for employing the immune system. Many current foci relate to the T cell elements of the adaptive system. There are also a multiplicity relating to the innate system including the NK or natural killer cells. The overall approach requires an understanding of two things: (i) what makes a tumor cell different and how does it tend to protect itself, (ii) how do immune systems identify and attack aberrant cells. On the one hand we look at the malignant cell and how it expresses itself, which we know is arguably an ever changing process. The second element is how can we use and manipulate the bodies basic immune system, and here it should be both adaptive and innate.

1.2 MULTIPLICITY OF WAYS

There are a multiplicity of ways in which the immune system may attack cancer cells. We summarize this in the figure below. We have discussed checkpoint issues and CAR T cells previously and herein we focus on TIL and ACT mechanisms. All of these mechanisms shown below are somewhat variants of each other as we shall discuss. TIL/ACT mechanisms are the oldest in concept and are in many ways a brute force method of attacking the cancer cells in larger volume than they would have been in vivo.

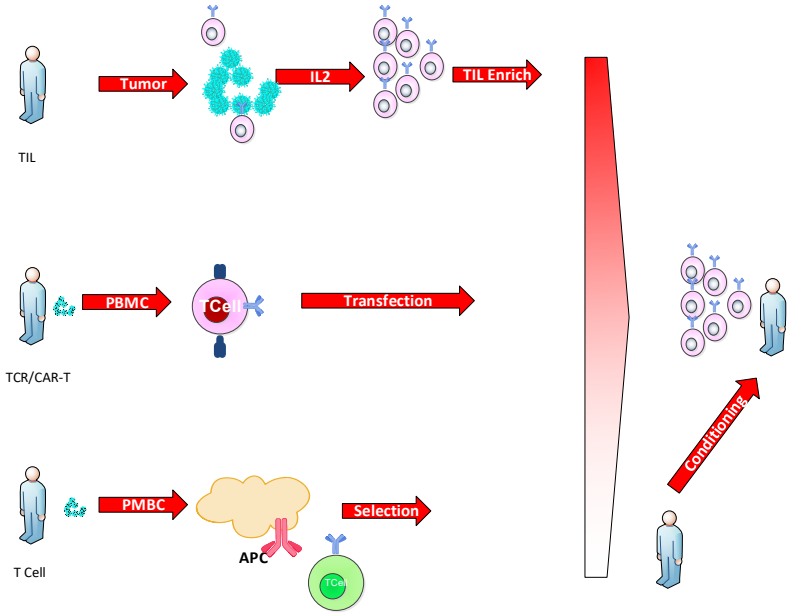


We will then focus on the interrelationship between the cancer cell and the immune cell. For each we ask how they are identified, how they act, and how they may protect themselves. We summarize that below. The battle is between an immune cell and a cancer cell. The cancer cell can be identified but it can also protect itself. The self-protection is inherently part of its ancestry as a descendent of the individuals own cell line. It is a protection against auto-immune diseases. In contrast the immune cell can detect cells that do not belong, and as such can then emit attack mechanisms that destroy the unwanted invader. Immunotherapy is thus a balance between survival and destruction.



1.3 COMPARISONS

We briefly look at a comparison of some of the techniques. From Yee we have the following diagram (as modified):



Yee then notes on the above:

Adoptive Cell Therapy is represented by three general approaches:

1) Enrichment and expansion of tumor-infiltrating lymphocytes (TIL) from a disaggregated tumor biopsy sample

2) Genetic transfer of T Cell Receptor (TCR) recognizing tumor antigen-derived peptide-MHC target or Chimeric Antibody Receptor (CAR) recognizing surface tumor protein

3) Enrichment of endogenous antigen-specific T cells from peripheral blood mononuclear cells by in vitro stimulation followed by cell selection or cloning. PBMCs are a source of both antigen-presenting cells and T cells.

Following enrichment, the population of tumor-reactive T cells undergoes rapid expansion of 1000-5000 fold achieving 10 - 100 billion cells for adoptive transfer. Patients often receive a lymphodepleting conditioning regimen pre-infusion followed by exogenous IL-2. In the case of adoptive TIL therapy, patients receive high-dose near ablative or fully ablative conditioning pre-infusion and a course of high-dose IL-2 post-infusion. … 'young' TIL are generated using a shortened pre-expansion culture phase prior to rapid cell expansion, enabling production of an infusible T cell product within 5-7 weeks from time of tumor collection.

Here Yee included a multiplicity of techniques. Namely Yee sweeps any method extracting, modifying, and re-implanting T cells as ACT. We examine these somewhat and leave them as all separate.

One must recall that T cells are not alone in this fight against cancer cells. The innate immune system has a powerful set of tools which are used as an immediate attack mechanism and if properly triggered may be of adjuvant usage. We have examined the innate system and its

various methods elsewhere. Two strong elements there include the natural killer cells, NK, and the complement chain. Complement has yet to receive a great deal of attention as regards to cancer immunotherapy. Looking at Macor and Tedesco we note:

The contribution of the complement system to the control of tumour growth has been neglected for a long time as the major emphasis has been put mainly on cell-mediated immune response against cancer. With the introduction of monoclonal antibodies in cancer immunotherapy complement has come into play with a great potential as effector system. Complement has a number of advantages over other effector systems in that it is made of molecules that can easily penetrate the tumour tissue and a large majority, if not all, of the components of this system can be supplied locally by many cells at tissue site.

Further advances are being made to increase the anti-tumour efficiency of the complements system using C-fixing antibodies that are modified in the Fc portion to be more active in complement activation. Another strategy currently investigated is essentially based on the use of a combination of two antibodies directed against different molecules or different epitopes of the same molecule expressed on the cell surface in order to increase the number of the binding sites for the antibodies on the tumor cells and the chance for them to activate complement more efficiently.

One of the problems to solve in exploiting complement as an effector system in cancer immunotherapy is to neutralize the inhibitory effect of complement regulatory proteins which are often over-expressed on tumour cells and represent a mechanism of evasion of these cells from complement attack. This situation can be overcome using neutralizing antibodies to target onto tumour cells together with the specific antibodies directed against tumor specific antigens. This is an area of active investigation and the initial data that start to be available from animal models seem to be promising.

Thus we believe that a great deal can be garnered by not only focusing on the adaptive elements but also the innate.

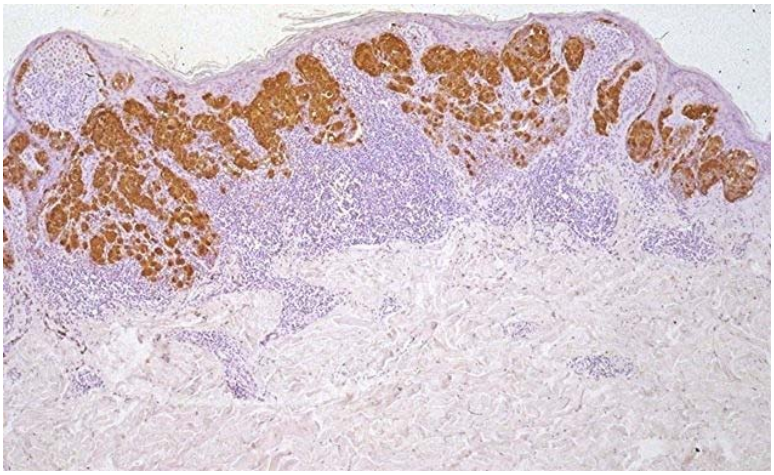
2 TILS

Tumor Infiltrating Lymphocytes ("TIL"s) are T cells that have managed to recognize an aberrant cell and commence attacking it. They often get confused because of the self defence mechanisms inherited by the cancel cells but they accumulate around the new lesion. Pathologists often can see collections of TIL when performing biopsies on many malignancies.

From NCI we have the definition[1]:

Therapeutic tumor infiltrating lymphocytes ("TIL"s) : A preparation of cells, consisting of autologous tumor infiltrating lymphocytes, that are manipulated in vitro and, upon administration in vivo, re-infiltrate the tumor to initiate tumor cell lysis. In vitro, therapeutic tumor-infiltrating lymphocytes (TILs) are isolated from tumor tissue and cultured with lymphokines such as interleukin-2; the therapeutic TILs are then infused into the patient, where, after re-infiltration of the tumor, they may induce lysis of tumor cells and tumor regression. The use of therapeutic TILs is considered a form of adoptive immunotherapy.

From the MMMP[2] we have an example of a melanoma with significant TILs as shown below:



As noted by Eichhoff in the doctoral report on melanoma the author notes on p77:

Melan-A protein, also known as MART1 (Melanoma Antigen Recognized by T-cells), is processed by melanoma cells into a peptide that is presented at their surface, allowing them to be targeted by tumor infiltrating lymphocytes. MLANA mRNA expression in vitro is highest in proliferative phenotype melanoma cells and near background levels in invasive phenotype cells ($p < 0.001$). Microphthalmia-associated transcription factor (Mitf) is critical for the regulation

[1] https://www.cancer.gov/publications/dictionaries/cancer-drug/def/therapeutic-tumor-infiltrating-lymphocytes

[2] http://www.mmmp.org/MMMP/import.mmmp?page=prog_factors.mmmp

of melanocyte development and survival. It is responsible for regulating the expression of many genes including Melan-A and is thought to be central to the regulation of cell phenotype during melanoma progression. MITF expression in vitro is closely related to that of MLANA, with significantly increased expression in proliferative phenotype cells ($p < 0.001$).

We shall see that this observation is quite critical later on in this presentation[3].

[3] See Miller and Mihm, Melanoma, NEJM, July, 2006. *At the stage of the benign nevus, BRAF mutation and activation of the mitogen-activated protein kinase (MAPK) pathway occur. The cytologic atypia in dysplastic nevi reflect lesions within the cyclin-dependent kinase inhibitor 2A (CDKN2A) and phosphatase and tensin homologue (PTEN) pathways. Further progression of melanoma is associated with decreased differentiation and the decreased expression of melanoma markers regulated by microphthalmia-associated transcription factor (MITF). The vertical-growth phase and metastatic melanoma are notable for striking changes in the control of cell adhesion. Changes in the expression of the melanocyte-specific gene melastatin 1 (TRPM1) correlate with metastatic propensity, but the function of this gene remains unknown. Other changes include the loss of E-cadherin and increased expression of N-cadherin, αVβ3 integrin, and matrix metalloproteinase 2* (MMP-2).

3 ACT

Adoptive cell transfer or therapy has been around for well over thirty years. For example, Belldegrun et al noted (1988):

The identification, isolation, and adoptive transfer of selected subsets of immune cells with specific antitumor reactivity into tumor bearing patients to mediate cancer regression in vivo is a prime goal of tumor immunology. Currently, however, there are no available techniques for generating such lymphoid cells with reactivity against specific tumor antigens in the human. Recent experiments have demonstrated that the ***adoptive transfer*** *of lymphokine-activated killer cells plus IL-2 can mediate tumor regression in a variety of animal models (1-4) and human tumors as well.*

This approach, however, requires the transfer of large numbers of sensitized fresh lymphocytes… into tumor bearing humans, along with the systemic administration of relatively high doses of IL-2 …. Many human tumors are infiltrated with chronic inflammatory cells, including lymphocytes. We have recently identified a population of lymphoid cells infiltrating murine tumors that could be expanded in vitro in IL-2 and, when ***adoptively transferred,*** *were capable of totally eliminating 3-day established pulmonary metastases. When compared to LAK cells, these TIL cells were at least 50 times more potent in mediating the therapy of established micrometastases. The simultaneous administration of IL-2 enhanced the in vivo therapeutic effective ness of the adoptive transfer of TIL, although high doses of TIL alone were also effective. The greater therapeutic efficacy of TIL compared to LAK cells in the treatment of established metastases in mice raises the possibility that TIL isolated from human tumors and expanded in vitro in IL-2 may similarly be effective for the treatment of human cancer.*

In effect they idea was that the T cells in and around the tumor were reflecting a response so they must be effective and that all that was needed was an expansion of the cells using IL-2. The idea was based upon some reasonable understanding of the immune system but it was too rudimentary. Fundamentally this approach has not changed, it still is taking T cells, enhancing them, and replacing them back in the patient.

Progress continued as noted in Schoof et which stated(1990):

Collectively, these results suggest that TIL activation by solid-phase antibodies to CD3 prior to adoptive transfer may provide one important link to improved adoptive immunotherapy, since immunologically active cells, in terms of cell growth and cytotoxicity, are available for infusion. In addition, the activation of TIL on anti-CD3- coated surfaces early in the life of a bulk culture may facilitate the selective expansion of tumor-specific cytotoxic T-cell pre cursors at the expense of tumor nonspecific NK cells which have been reported to expand in certain TIL bulk cultures under conditions of high-dose rIL-2).

The use of solid-phase anti- CD3 is useful because it provides a simple and reproducible stimulus for T-cell growth and does not require the procurement and utilization of accessory cells and an often limited supply of autologous tumor which are required for the serial activation of TIL.

The 1990 approach demonstrates a better understanding of some of the CD proteins as targets. Now Norelli et al have noted:

The idea of transferring ex vivo-manipulated cells of the immune system into cancer patients for therapeutic purposes, also known as adoptive cell therapy (ACT), is an idea that dates back to the mid- 1980s, approximately ten years before the debut of targeted therapies, when Steve Rosenberg pioneered the use of lymphokine-activated killer cells in metastatic melanoma. In thirty years, the original concept of ACT has dramatically evolved, now embracing the use of immune effectors as disparate as cytokine-induced killer (CIK) cells, tumor infiltrating lymphocytes or TILs (T cells isolated from tumor sites, expanded and re-infused back into patients]), and, more recently, T cells genetically modified to express clonal T-cell receptors (TCRs) or chimeric antigen receptors (CARs).

Initially conceived by Zelig Eshhar, CARs are monomeric receptors usually designed by fusing the single chain fragment variable (scFv) of a tumor-reactive mAb with a transmembrane domain and one or more signaling molecules containing intracellular immunoreceptor tyrosine-based activation motifs (ITAMs).

Upon CAR modification, originally with viral vectors, but more recently also with non-viral systems, T cells become cytotoxic against tumor cells expressing the antigen recognized by the mAb of origin. The greatest payback of ACT over targeted therapies and mAbs relies on the nature of effector T cells, which are endowed with unparalleled potency, wide biodistribution and long-term persistence, without the need of repetitive administrations. Moreover, at least in the case of CARs, HLA independent recognition ensures intrinsic resistance to the defects in antigen processing and presentation that tumor cells often put in place to evade T-cell recognition.

More recently Wang et al note (2014):

Cancer immunotherapy holds several key advantages over traditional therapies: high specificity, little or no side effects for active immunization, although adverse effects may occur in adoptive cell transfer (ACT) and good safety profile. The key point of immunotherapy is to use the patient's own immune system to control and destroy cancer cells. Cancer immunotherapy approaches include active immunization, reversal of immunosuppression, nonspecific immune stimulation and ACT.

To date, ACT has been demonstrated to be the most effective immunotherapy method for cancer treatment and has achieved very promising results in cancer clinical trials. The first exciting clinical trial of ACT used tumor-infiltrating lymphocytes (TILs).

In the TIL-based ACT approach, TILs are isolated from the tumor tissues of cancer patients, expanded in vitro using a high concentration of IL-2 (6000 U/ml), and then infused back into the patient. The feasibility of the TIL-based ACT approach was first demonstrated in melanoma, with a current objective response rate of 49–72% when lymphodepleting preparative regimen is performed prior to TIL infusion.

Successful TIL-based immunotherapy has promoted the rapid development of ACT. In addition to TIL-based immunotherapy, genetically modified cancer-specific T cells, such as T-cell receptor (TCR)- and chimeric antigen receptor (CAR)- transduced T cells, are being developed to augment ACT-mediated immunotherapeutic responses against various types of cancer and have already shown encouraging therapeutic effects in clinical trials

From June we have:

Adoptive T cell therapy of rodent malignancies was first reported in 1955, and there are no forms of FDA-approved T cell therapy for cancer available after more than 60 years of research into adoptive immunity for tumors. However, there is increasing optimism that the scientific barriers preventing clinically effective adoptive immunotherapy have been addressed. Evidence in support of the cancer stem cell hypothesis and the idea that these cells present a substantial barrier to complete tumor elimination using cytotoxic chemotherapy raises the hope that it might be possible to target these cells using adoptive T cell therapy. Given the success of allogeneic cellular therapy for chemotherapy-resistant hematologic malignancies, it is of interest to learn whether T cells can target cancer stem cells, as is suggested by the durability of responses following allogeneic T cell therapies. Advances in the understanding of T cell biology and the tumor microenvironment have provided multiple novel adoptive transfer strategies that are now poised for translation into clinical trials.

As Rosenberg and Restifo recently noted (as modified) the history of the ATC process:

1960s: Very little was known about the function of T lymphocytes until the 1960s, when it was shown that lymphocytes were the mediators of allograft rejection in experimental animals. Attempts to use T cells to treat transplanted murine tumors were limited by the inability to expand and manipulate T cells in culture.

Thus, ACT used transfer of syngeneic lymphocytes from rodents heavily immunized against the tumor, and modest growth inhibition of small established tumors was observed. In early preclinical studies, the importance of host inhibitory factors was suggested by findings that lymphodepletion using either chemotherapy or radiation before cell transfer enhanced the ability of transferred lymphocytes to treat established tumors.

1976: The ability to use ACT was facilitated by the description of T cell growth factor [interleukin-2 (IL-2)] in 1976, which provided a means to grow T lymphocytes ex vivo, often without loss of effector functions.

1982: The direct administration of high doses of IL-2 could inhibit tumor growth in mice, and studies in 1982 demonstrated that the intravenous injection of immune lymphocytes expanded in IL-2 could effectively treat bulky subcutaneous FBL3 lymphomas.

In addition, administration of IL-2 after cell transfer could enhance the therapeutic potential of these adoptively transferred lymphocytes.

1985: The demonstration in 1985 that IL-2 administration could result in complete durable tumor regressions in some patients with metastatic melanoma (9) provided a stimulus to identify

the specific T cells and their cognate antigens involved in this cancer immunotherapy. Lymphocytes infiltrating into the stroma of growing, transplantable tumors were shown to represent a concentrated source of lymphocytes capable of recognizing tumor in vitro, and studies in murine tumor models demonstrated that the adoptive transfer of these syngeneic tumor-infiltrating lymphocytes (TILs) expanded in IL-2 could mediate regression of established lung and liver tumors.

1986: In vitro studies in 1986 showed that human TILs obtained from resected melanomas contained cells capable of specific recognition of autologous tumors (11),

1988: and these studies led in 1988 to the first demonstration that ACT using autologous TILs could mediate objective regression of cancer in patients with metastatic melanoma (12). Populations of TILs that grow from tumors are generally mixtures of CD8+ and CD4+ T cells with few if any major contaminating cells in mature cultures. The ability of pure populations of T lymphocytes to mediate cancer regression in patients provided the first direct evidence that T cells played a vital role in human cancer immunotherapy.

2002: A critical improvement in the application of ACT to the treatment of human cancer was reported in 2002, when it was shown that lymphodepletion using a nonmyeloablative chemotherapy regimen administered immediately before TIL transfer could lead to increased cancer regression, as well as the persistent oligoclonal repopulation of the host with the transferred antitumor lymphocytes. In some patients, the administered antitumor cells represented up to 80% of the CD8+ T cells in the circulation months after the infusion.

2006: it was shown for the first time in humans in 2006 that administration of normal circulating lymphocytes transduced with a retrovirus encoding a TCR that recognized the MART-1 melanoma-melanocyte antigen could mediate tumor regression

As Koury et al note with a simple description of ACT:

The adoptive cell transfer (ACT) technology takes advantage on the reliance of immune cells in surrounding the tumor environment, stimulating cells ex vivo, and manipulating the immune environment for the introduction of effector cells.

ACT typically consists of three parts:

1) *lymphodepletion,*

2) *cell administration, and*

3) *therapy with high doses of IL-2.*

Lympho-depletion using chemotherapy or radiation has proven to enhance the antitumor effects of transferred lymphocytes. It was also shown that IL-2 was crucial for the expansion of the transferred lymphocytes ex vivo, as well as for the regression in metastatic melanoma when directly administered. Tumor-Infiltrating Lymphocytes (TILs).

One form of transferred lymphocytes is tumor-infiltrating lymphocytes (TILs) which were discovered to be mononuclear lymphocytes that had a propensity to surround and invade tumors. These TILs were first discovered in resected melanomas and were found to contain a mixture of both CD4 and CD8 T cells.

The general procedure for autologous TIL therapy is stated as follows:

(1) the resected melanoma is digested into fragments;

(2) each fragment is grown in IL-2 and the lymphocytes proliferate destroying the tumor;

(3) after a pure population of lymphocytes exists, these lymphocytes are expanded; and

(4) after expansion up to 1011 cells, lymphocytes are infused into the patient.

Adoptive T cell transfer of TILs produces a 50% cancer response rate and 20% complete response rate in metastatic melanoma, and since the responses are very durable, the 20% complete response rate translates into a 20% cure rate. Before the recent development of checkpoint modulators (anti-PD-1), which shows a comparable level of response, TILs had been the only agent approved by the US FDA for patients with metastatic melanoma.

Overall ACT is a simple process compared to many of the current alternatives. It has a modicum level of success and is of generally a lower cost. However its efficacy is not that great and it lacks specificity.

4 ALTERNATIVES

It is worth comparing ACT approaches with CAR-T cells. On a simple level, ACT as used by Rosenberg takes T cells from a malignant lesion and grows them and reinserts them. The ACT approach is an attempt to magnify the natural process. On the other hand the CAR-T approach is one where a surface marker on a lesion is identified and T cells are "made" with receptors to link to that ligand.

There are a multiplicity of therapeutic alternatives. We use Abbas et al as an excellent source for these.

4.1 CHECKPOINT INHIBITORS

We start with the checkpoint inhibitors. As Abbas et al note:

Tumors evade antitumor T cell responses by engaging inhibitory molecules that normally function to prevent autoimmunity or regulate immune responses to microbes. There is strong experimental and clinical evidence that T cell responses to some tumors are inhibited by the involvement of CTLA-4 (cytotoxic T lymphocyte–associated protein 4) or PD-1 (programmed cell death protein-1), two of the best-defined inhibitory pathways in T cells.

Studies of mouse tumor models and human cancers have shown that both PD-1 and CTLA-4 are often upregulated on tumor infiltrating T cells, consistent with their role in inhibiting tumor-specific T cell function. In fact, tumor infiltrating T cells often have a dysfunctional (exhausted) phenotype that was first described in the context of chronic viral infections. This dysfunctional state is characterized by impaired effector functions and increased expression of CTLA-4, PD-1, and other inhibitory molecules. A possible reason for why tumors exploit CTLA-4 to regulate antitumor responses is that tumor antigens are presented by APCs in the absence of strong innate immunity and thus with low levels of B7 costimulators. These low levels may be enough to engage the high-affinity receptor CTLA-4.

The PD-1 pathway may be engaged in tumor-specific T cells because PD-L1 (PD-ligand 1), a B7 family protein that is a ligand for PD-1, is expressed on many human tumors, sometimes because of PDL1 gene amplification. PD-L1 on APCs may also be involved in inhibiting the activation of tumor-specific T cells.

Thus targeting these inhibitors using monoclonal antibodies, Mab, then block the blocking functions and allow the T cells which are targeting the cancer cells to do their job. This technique has exploded as a new means to do cancer therapeutics. It was first used with a CTL4A blockage on melanoma cells and is now proliferating with PD-1 and PD-L1 blocking as well. The issue is that these may not be the sole checkpoint sites. The interesting thing about cancers is that in finding and fighting off one approach then reveals another.

4.2 ADOPTIVE CELL IMMUNOTHERAPY

The comments in Abbas et al on ACT or as they call it adoptive cell immunotherapy parallels what Rosenberg and others have noted. Specifically they note:

Adoptive cellular immunotherapy is the transfer of cultured immune cells that have antitumor reactivity into a tumor-bearing host. The immune cells are derived from a cancer patient's blood or solid tumor, and then are treated in various ways in vitro to expand their numbers and enhance their antitumor activity, before reinfusion back into the patient.
Chimeric Antigen Receptor T Cell Therapy.

T cells specific for tumor antigens can be harvested from a patient's tumor tissue or blood, expanded and activated in vitro, and reinfused into cancer patients. This general approach has been used in various trials for many years, but has had limited success, probably because the cells that are isolated from patients contain a low frequency of potent tumor-specific T cells. With the advent of the technologies discussed earlier to identify the neoantigens that drive tumor-specific T cell responses in individual patients, there is renewed excitement about adoptive therapy with T cells specific for these antigens.

The approach will involve harvesting T cells from the blood or tumors of patients, stimulating the cells with the antigen in vitro to increase the numbers and functional activity of cells specific for the tumor neoantigens, and then transferring the activated T cells back into the patient. There have already been some successes with small trials using this approach in melanoma patients.

The reference to the melanoma patients in the above is a reflection of the work of Rosenberg. It is worth reflecting here on Rosenberg's work. In his earlier book (1992) he recounts that in 1968 while at the Brigham as a surgical resident he met a man who had terminal gastrointestinal cancer, but then after a massive infection he had the cancer disappear. That insight led him to MCI and his work on immunotherapy. The interesting thing is that at that time we knew little if anything about the immune system and furthermore the tools to work with it were absent. Yet fifty years later, Rosenberg continues and the techniques and tool proliferate.

4.3 PASSIVE IMMUNOTHERAPY WITH ANTIBODIES

The classic approach is using antibodies to embolden the immune system. As Abbas et al note:

Passive antibody therapy involves the transfer of tumor-specific antibodies into patients, which is a rapid and theoretically very specific approach (often called, with some optimism, "magic bullets") but does not lead to long-lived immunity. Paul Ehrlich wrote about the potential to treat tumors with antibodies over a century ago. Some monoclonal antibodies have been in use to treat cancers for over 20 years, and many more are now approved or in advanced development. Although the checkpoint blockade reagents discussed earlier are monoclonal antibodies, most of them do not bind to tumor cells, and their mode of action, which is to block inhibitors of T cell activation, is fundamentally different from the mechanisms of the antibodies discussed here.

Some antitumor antibodies bind to cell surface molecules on tumor cells and engage host effector mechanisms that kill the tumor cells. These mechanisms include NK cell-mediated cytotoxicity, complement-mediated lysis, and complement- or Fc receptor–mediated phagocytosis by macrophages. Several antitumor antibodies that are now approved for the treatment of certain cancers work in this way. For example, as mentioned earlier, anti-CD20 is used for treating B cell lymphomas, and it works by depleting all CD20-expressing cells, including B cells and B cell–derived lymphoma cells, mainly by antibody-dependent cellular cytotoxicity and perhaps also by complement activation.

Other monoclonal antibodies used in cancer therapy bind to growth factor receptors on cancer cells and interfere with the signaling required for tumor growth and survival. Anti-Her2/Neu is an approved monoclonal antibody used to treat breast cancers that overexpress the cell surface growth factor signaling molecule Her2/Neu. An antibody that binds and blocks the function of the epidermal growth factor receptor (EGFR) is approved for the treatment of metastatic colorectal cancers and head and neck cancers. Another antibody in clinical use for several cancers blocks not a tumor cell molecule but a growth factor, VEGF, that stimulates the angiogenesis that is required to maintain tumor growth.

Overall this approach has seen limited success. It is a bit akin to the strategy of "flinging the spaghetti on the wall and seeing what sticks".

4.4 BISPECIFIC T CELL ENGAGERS (BITE)

The Bispecific T cell approach has seen limited use. As Huehls et al note:

Bispecific T cell engagers are a new class of immunotherapeutic molecules intended for the treatment of cancer. These molecules, termed BiTEs, enhance the patient's immune response to tumors by retargeting T cells to tumor cells. BiTEs are constructed of two single chain variable fragments (scFv) connected in tandem by a flexible linker. One scFv binds to a T cell-specific molecule, usually CD3, while the second scFv binds to a tumor-associated antigen. This structure and specificity allows a BiTE to physically link a T cell to a tumor cell, ultimately stimulating T cell activation, tumor killing and cytokine production. BiTEs have been developed that target several tumor-associated antigens for a variety of both hematological and solid tumors. Several BiTEs are currently in clinical trials for their therapeutic efficacy and safety. This review examines the salient structural and functional features of BiTEs as well as the current state of their clinical and preclinical development….

The concept of using T cell retargeting for cancer therapy stretches back to the 1970s. Unlike macrophages, dendritic cells, and other accessory cells, T cells are present in copious numbers, expand rapidly upon activation, give robust and durable cytotoxic responses, and have the potential to generate immunologic memory. Furthermore, T cells have been found to attack tumors from the outside as well as infiltrating into the tumor. These features make T cells optimal therapeutic effectors for cancer. T cell redirection does suffer one significant challenge, which is the requirement of a second stimulatory signal to achieve full T cell activation and prevent anergy. Multiple bispecific formats have been developed to meet or circumvent this requirement.

Then Abbas et al also have noted:

Bispecific T cell engagers (BiTEs) facilitate the targeting of host T cells of any specificity to attack tumor cells. These reagents are recombinant antibodies engineered to express two different antigen binding sites, one specific for a tumor antigen and the second specific for a T cell surface molecule, usually CD3. In many of these antibodies, each antigen binding site is composed of a single chain variable fragment containing Ig heavy and light chain variable domains, similar to the CARs described earlier. The presumed mechanism of action of BiTEs, based on in vitro studies, is the formation of immune synapses between the tumor cells and the T cells and the activation of the T cells by CD3 crosslinking. A CD19-specific BiTE is approved for treatment of acute lymphocytic leukemia. BiTEs specific for many other tumor antigens have been developed, including CD20, EpCAM, Her2/neu, EGFR, CEA, folate receptor, and CD33, and are at various stages of preclinical and clinical trials.
As Ross et al note:

For targets that are homogenously expressed, such as CD19 on cells of the B lymphocyte lineage, immunotherapies can be highly effective. Targeting CD19 with blinatumomab, a CD19/CD3 bispecific antibody construct (BiTE®), or with chimeric antigen receptor T cells (CAR-T) has shown great promise for treating certain CD19-positive hematological malignancies.

In contrast, solid tumors with heterogeneous expression of the tumor-associated antigen (TAA) may present a challenge for targeted therapies. To prevent escape of TAA negative cancer cells, immunotherapies with a local bystander effect would be beneficial. As a model to investigate BiTE®-mediated bystander killing in the solid tumor setting, we used epidermal growth factor receptor (EGFR) as a target. We measured lysis of EGFR-negative populations in vitro and in vivo when co-cultured with EGFR-positive cells, human T cells and an EGFR/CD3 BiTE® antibody construct. Bystander EGFR-negative cells were efficiently lysed by BiTE®-activated T cells only when proximal to EGFR-positive cells.

Our mechanistic analysis suggests that cytokines released by BiTE®-activated T-cells induced upregulation of ICAM-1 and FAS on EGFR-negative bystander cells, contributing to T cell induced bystander cell lysis.

Namely the BITE approach is to create using an Ab a molecule which is CD3 on one end and say CD19 on the other and use this to cover a target and then to attract a T cell. In some ways this is akin to CAR-T where we place the receptor to the target on a T cell, here we use a T cell and attach the target to a known receptor on a T cell.

4.5 IMMUNOTOXINS

Various therapeutics have been developed along the line of anti-toxins. As Abbas et al note:

Immunotoxins, or conjugated monoclonal antibodies, are antibodies specific for tumor antigens that are linked to a chemotherapy drug or to a radioisotope. The rationale for these agents is they will allow high local concentrations of the cytotoxic drug or isotope to be delivered to the tumor cells, because of the antibody specificity. Approved drug-conjugated antibodies specific for HER2/neu and CD30 are approved for treatment of breast cancer and Hodgkin's lymphoma, respectively. Many more conjugated antibodies have been developed but failed in clinical trials because of significant systemic toxicity due to the nonspecific accumulation of the toxic component in various tissues.

Specificity of Antibody[4]	*Drug Name*	*Form of Antibody Used*	*Clinical Use*
HER2/Neu (EGFR)	*Trastuzumab*	*Humanized*	*Breast cancer*
CD19	*Blinatumomab*	*CD19-/CD3-bispecific antibody*	*Acute lymphoblastic leukemia*
CD20	*Rituximab* *Ofatumumab*	*Chimeric* *Human*	*B cell lymphomas and leukemias* *Chronic lymphocytic leukemia*
CD20	*90Y-Ibritumomab tiuxetan*	*Radioisotope conjugated mouse*	*Low grade or transformed B cell non-Hodgkin's lymphoma*
CD30	*Brentuximab vedotin*	*Drug-conjugated chimeric*	*Hodgkin's or systemic anaplastic large cell lymphoma*
CD33	*Gemtuzumab ozogamicin*	*Humanized*	*Acute myelogenous leukemia*
CD52	*Alemtuzumab*	*Humanized*	*CLL, CTCL, and T-cell lymphoma*
CTLA-4	*Ipilimumab*	*Human*	*Metastatic melanoma*
PD-1/PD-L1	*Nivolumab* *Pembrolizumab*	*Humanized* *Humanized*	*Metastatic melanoma; lung cancer*
EGFR	*Cetuximab* *Panitumumab* *Nimotuzumab*	*Chimeric* *Human* *Humanized*	*Colorectal, breast, and lung cancer; other tumors* *Colorectal cancer* *Head and neck cancer*
VEGFA	*Bevacizumab*	*Humanized*	*Colorectal and lung cancer*
CD254 (RANK Ligand)	*Denosumab*	*Human*	*Solid tumor bony metastases*

4.6 CYTOKINES

Cytokines are the products of immune cells that are used to activate other immune cells. Identifying and using cytokines is now several decades old. It can be a hit and miss process and

[4] CLL, Chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; EGFR, epidermal growth factor receptor; VEGEFA, vascular endothelial growth factor A

it may also have the problem of creating an immune storm which can be debilitating to the patient. As Abbas et al note:

Cancer patients can be treated with cytokines that stimulate the proliferation and differentiation of T lymphocytes and NK cells. These cytokines can enhance the activation of dendritic cells and tumor-specific T cells, particularly CD8+ CTLs. Many cytokines also have the potential to induce nonspecific inflammatory responses, which by themselves may have antitumor activity.

The largest clinical experience is with high-dose IL-2 given intravenously, which has been effective in inducing measurable tumor regression in about 10% of patients with advanced melanoma and renal cell carcinoma and is currently an approved therapy for these cancers. The use of high-dose IL-2 is, however, limited because it stimulates the production of toxic amounts of proinflammatory cytokines such as TNF and IFN-γ, which act on vascular endothelial and other cells and lead to a serious vascular leak syndrome.

IFN-α is approved for treatment of several cancers, including malignant melanoma, certain lymphomas and leukemias, and AIDS-related Kaposi sarcoma. The mechanisms of the antineoplastic effects of IFN-α probably include inhibition of tumor cell proliferation, increased cytotoxic activity of NK cells, and increased class I MHC expression on tumor cells, which makes them more susceptible to killing by CTLs.

Other cytokines, such as TNF and IFN-γ, are effective antitumor agents in animal models, but their use in patients is limited by their toxic side effects. Hematopoietic growth factors, including GM-CSF and G-CSF, are used in cancer treatment protocols to shorten periods of neutropenia and thrombocytopenia after chemotherapy or autologous bone marrow transplantation. Nonspecific Inflammatory Stimuli

Immune responses to tumors may be stimulated by the local administration of inflammatory substances or by systemic treatment with agents that function as polyclonal activators of lymphocytes.

One of the oldest examples of tumor immunotherapy was practiced by the 19th century physician William Coley, who treated his cancer patients with mixtures of dead bacteria, so called "Coley's toxin." This approach may have been intermittently successful due to the induction of strong innate responses causing acute inflammation that killed tumor cells. Nonspecific immune stimulation of patients with tumors by injection of inflammatory substances such as killed bacillus Calmette-Guérin (BCG) at the sites of tumor growth has been used for many years. The BCG mycobacteria activate macrophages and thereby promote macrophage-mediated killing of the tumor cells. In addition, the bacteria function as adjuvants and may stimulate T cell responses to tumor antigens.

4.7 STEM CELLS

Stem cells have become a staple to dealing with hematological cancers. As Abbas et al have noted:

In leukemia patients, administration of T cells and NK cells together with hematopoietic stem cells from an allogeneic donor can contribute to eradication of the tumor. The T cell–mediated graft-versus-leukemia effect is directed at molecules present on the recipient's hematopoietic cells, including the leukemia cells, that are recognized as foreign by the administered T cells. Donor NK cells respond to the tumor cells because tumors may express low levels of class I MHC molecules or they express class I MHC alleles not recognized by the donor NK cells. Recall that recognition of self-class I MHC normally inhibits the activation of NK cells. The challenge in use of this treatment to improve clinical outcome is to minimize the dangerous graft-versus-host disease that may be mediated by the same donor T cells.

4.8 CAR-T CELLS

CART cells seems to be the most attractive of all of the current approaches. We have been remarking on them for the past several years and they seem to have the ability to have specificity for a tumor and the ability to leverage the immune system. As Abbas et al note:

Adoptive therapy using T cells expressing chimeric antigen receptors (CARs) has proven successful in some hematologic malignancies, and this approach is in trials for other tumors. CARs are genetically engineered receptors with tumor antigen–specific binding sites encoded by recombinant immunoglobulin (Ig) variable genes and cytoplasmic tails containing signaling domains of both the TCR and costimulatory receptors.

The reason for using an Ig with a binding site specific for the tumor antigen as the recognition receptor, even though it has to function in T cells, is that this avoids the problem of the MHC restriction of TCRs, so the same CAR construct can be used in any patient. The Ig binding site is attached to a genetically engineered cytoplasmic tail that contains signaling domains that normally serve critical roles in T cell activation.

Several variations of signaling constructs have been used so far in CARs developed at different centers, but all contain the TCR ζ chain ITAM motifs and the cytoplasmic singling motifs of costimulatory receptors such as CD28 or 4-1BB (a TNF receptor family member). Expression of these signaling domains confers on the tumor-specific Ig receptor the ability to activate T cells.

In current protocols, a patent's peripheral blood T cells are isolated, stimulated with anti-CD3 and/or anti-CD28 antibodies to expand all the T cells, and transfected with CAR-encoding retroviral or lentiviral vectors. The expanded CAR-expressing T cells are then injected back into the patient. The transferred T cells undergo further robust proliferation in the patient, in response to tumor antigen recognition by the CAR. The specificities of the TCRs on these T cells (which are still present) becomes irrelevant to the goal of killing tumor cells, since all the transfected cells can be activated by the tumor antigen that binds to the antigen binding site encoded by the CAR gene.

Tumor killing is achieved by both direct cytotoxic and cytokine-mediated mechanisms. Patients with B cell malignancies, including chronic lymphocytic leukemia and acute lymphoblastic leukemia, have been very effectively treated with CAR-expressing T cells specific for CD19, a pan–B cell marker also expressed on the tumor cells. Normal B cells as well as tumor B cells are

killed, but patients can be supplemented with pooled immunoglobulin to make up for the lack of B cells. Because long-lived antibody-producing plasma cells, found in adult bone marrow and mucosal tissues, do not express CD19 and are not killed, they continue to provide antibody-mediated immunity in adult patients treated with CD19-specific CAR-T cells.

Memory CAR-T cells may persist in the treated patients for at least many months, so that surveillance against tumor recurrence is maintained. CAR therapy is being used in several medical centers around the world to treat B cell malignancies that are refractory to other treatments, and several facilities have been created that can produce large numbers of CAR-T cells for each patient in a short time.

Abbas et al note some of the deficiencies of CAR-T therapy. Specifically that they may not eliminate all the tumors. This can result from a multiplicity of factors. CAR-T are designed to target a mass of tumor cells. As such it may not target the cell of origin or cancer stem cell. That cell may escape and allow the cancer to regrow. We have seen effects that may be reflective of this on the other side, namely a random depletion of a stem cell and the collapse of residual immunity. Abbas et al note:

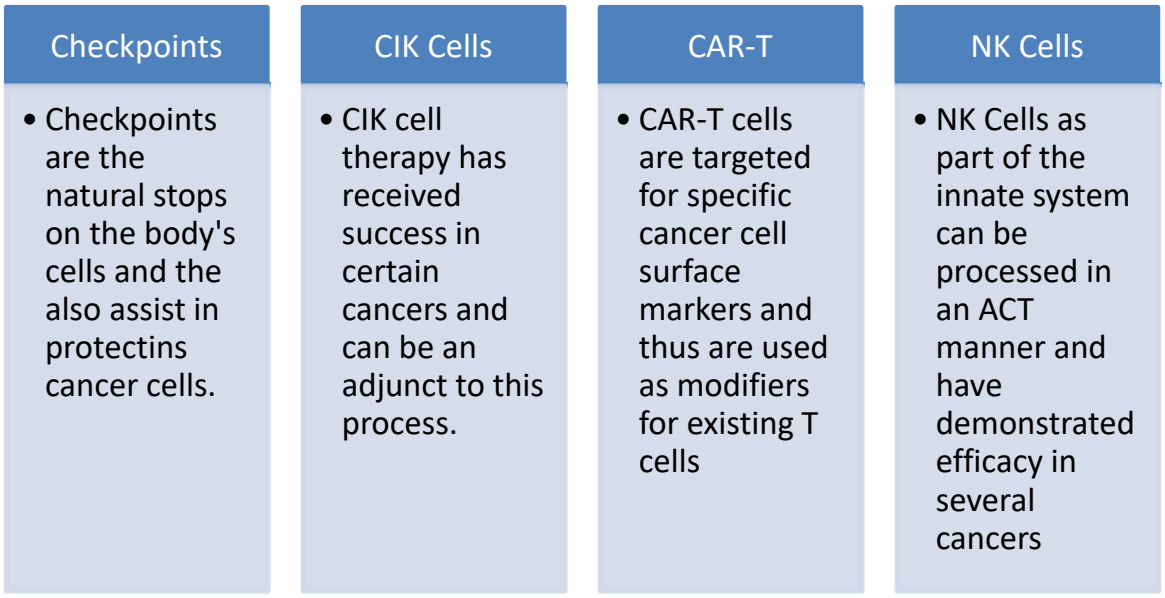
If the tumor is not completely eradicated, surviving cells may lose the antigen being targeted by the CAR and the tumor may recur. This is another example of the clonal evolution of cancers. One way of minimizing this problem is to introduce two CARs, specific for two tumor antigens, into T cells and transfer these cells into patients. Trials using this approach are ongoing.

In some patients, transferred CAR-T cells appear to become unresponsive over time, and initially controlled tumors have recurred. The CAR-T cells in these patients express markers of dysfunction (so-called exhaustion, see Chapter 11), including high levels of PD-1. This observation has led to exploratory studies using genome editing methods to eliminate the PD-1 gene in CAR-T cells before transfer. To avoid the risk of autoimmunity induced by the PD-1–negative T cells, one idea is to also eliminate endogenous TCRs from the CAR-T cells. This will create T cells that have only the introduced tumor-specific antigen receptor with its signaling domains, and will also lack an important checkpoint mechanism.

We shall comment on CAR-T a bit more. There is one argument worth noting here. Namely is CAR-T another adoptive T cell therapy, just a different way of handling the T cells. Rosenberg seems to admit that and we introduced this earlier in a fashion which also admits this.

5 OBSERVATIONS

ACT can be interpreted in a broad manner. We now examine several areas of collateral interest. They are summarized in the following graphic where we have presented 4 of the eight described above.



5.1 CHECKPOINT INTERACTIONS

Cancer cells are derivative of the body's own cells and as such reflect an ability to stop the immune system from destroying them. These surface markers called checkpoints can tell an attacking immune cell not to do so because this cell is part of the whole, even is expressing clear signs of aberrancy. The creation of monoclonal antibodies, Mabs, have yielded tools that work on may cancers and allow for the attack which otherwise would have been halted.

As Liu et al have noted:

Targeted therapies for cancer with small molecules and monoclonal antibodies (MoAb) have led to significant improvement in the long-term survival of multiple malignancies. The discovery of programmed death- 1 (PD-1) and the ligand 1 (PD-L1) has opened the door to the modern era of cancer immunotherapy. It is well known now that many tumor cells are able to upregulate the expression of PD-L1 which leads to anergy of cytotoxic T cells upon PD-1 binding to the ligand. Blocking the PD-1 pathway using monoclonal antibodies against PD-1 or PDL1 can therefore revamp the immune response against tumor cells.

The development of MoAbs against PD-1 and PD-L1 has led to the fast and fundamental paradigm shift in cancer therapy. The anti-PD drugs are the new form of tumor-site immune

modulation therapy through resetting immune reservoir in the tumor microenvironment. This is fundamentally different from the conventional chemotherapy and radiation that mainly target cancer cells themselves.

PD-L1 expression on the tumor cells and immune cells have become biomarkers that can assist clinical decisions in the choice of treatment strategies. Biomarker assays for PD-L1 are playing bigger roles and are being routinely done nowadays. However, PD-L1 assays can be highly variable, which makes it a clinical challenge to employ the results. In this review, we summarized latest clinical development of PD antibodies and immunohistochemistry (IHC) assays for PD-L1 biomarker expression in clinical practice.

5.2 CIK CELLS

CIK or cytokine induced killer cells, have seen use in multiple areas. I have reported on their use in the case of MDS, myelodysplastic syndrome, patients resulting is what the attending physicians have labelled as a cure. As Jiang et al have noted:

The number of immune cells, especially dendritic cells and cytotoxic tumor infiltrating lymphocytes (TIL), particularly Th1 cells, CD8 T cells, and NK cells is associated with increased survival of cancer patients. Such antitumor cellular immune responses can be greatly enhanced by adoptive transfer of activated type 1 lymphocytes. Recently, adoptive cell therapy based on infusion of ex vivo expanded TILs has achieved substantial clinical success.

Cytokine-induced killer (CIK) cells are a heterogeneous population of effector CD8 T cells with diverse TCR specificities, possessing non-MHC-restricted cytolytic activities against tumor cells.

Preclinical studies of CIK cells in murine tumor models demonstrate significant antitumor effects against a number of hematopoietic and solid tumors. Clinical studies have confirmed benefit and safety of CIK cell-based therapy for patients with comparable malignancies. Enhancing the potency and specificity of CIK therapy via immunological and genetic engineering approaches and identifying robust biomarkers of response will significantly improve this therapy. The presence of cytotoxic tumor infiltrating lymphocytes (TIL) within tumor is associated with increased survival of cancer patients. Both antitumor adaptive and innate cellular immunity are important for resistance of tumor growth and eventual elimination of cancer.

Theoretically, antitumor cellular immune responses can be greatly enhanced by adoptive transfer of lymphocytes, a term encompassing a strategy in which autologous T or NK cells are acquired from a cancer patient and then activated and expanded ex vivo prior to reinfusion.

Adoptive cell therapy of cancer, first demonstrated in mice more than 50 year ago, has gained momentum in recent years due to impressive clinical experiences with melanoma patients.

This approach is based on ex vivo expansion of large numbers of TILs and selection of tumor-specific T cell lines. The major effectors of TIL cells are phenotypically CD3+CD8+ T cells and their anti-tumor functions are MHC restricted [5]. In contrast to tumor antigen-specific

immunotherapy, there is potential utility of non-antigen specific cell-based therapy. Many patients with cancer are ineligible for TIL-based therapy because their TILs do not expand sufficiently or because their tumors have lost expression of antigens or MHC molecules or have extremely low numbers of TILs.

Cytokine-induced killer (CIK) cells are a heterogeneous population of effector CD8 T cells with diverse TCR specificities, possessing non-MHC-restricted cytolytic activities against tumor cells. Therefore, CIK cells can lyse tumor cells in a non- MHC-restricted manner and can serve as an alternative cellular immunotherapy.

The CIK approach is to some degree more akin to ACT but that it tries to use a multiplicity of immune cells. We believe that using CIKs with perhaps better targeting and checkpoint inhibitors may have significant advantages in many malignancies.

5.3 ACT vs CAR T

ACT can be viewed in broad terms. I believe it is fair to say that the Rosenberg approach is the classic one of removing TILs and then multiply them and strengthen them ex vivo and then place them back in the patient, without any added modifications. CAR-T cell therapy looks at the cancer cell itself and seeks a unique surface marker, such as CD19, and then designs and builds a T cell to attack just that marker. As Ott et al note:

Adoptive T cell therapy, CAR-T cell therapy Adoptively transferred T cells generated from tumor TILs, T cells bearing engineered, tumor specific T cell receptors, and chimeric antigen receptor (CAR) T cells all have shown remarkable anti-tumor activity in select solid and hematological malignancies. CAR T cells and T cells with engineered tumor specific TCRs may have the ability to induce an inflamed tumor microenvironment and therefore to be promising partnering strategies with PD-1/PD-L1 blockade.

CAR-T are effective and clearly more than a passing fad. Yet they are costly to prepare and may miss the critical cancer cells. ACT is a broad brush approach and hopes that the mix of cells may effectively hit the target. However the problem is always the stem cell or cell of origin problem. This is a substantial issue to be faced.

5.4 NK Cells: An Option

Natural Killer cells are considered part of the innate immune system. This classification seems to be based upon their sense of immediacy in responding and the simplicity of their response mechanism. However NK cells are very powerful tools in attacking malignancies as well.

As Pahl and Cerwenka have recently noted:

Natural Killer (NK) cells are classically considered innate immune effector cells involved in the first line of defense against infected and malignant cells. More recently, NK cells have emerged to acquire properties of adaptive immunity in response to certain viral infections such as

expansion of specific NK cell subsets and long-lasting virus-specific responses to secondary challenges.

NK cells distinguish healthy cells from abnormal cells by measuring the net input of activating and inhibitory signals perceived from target cells through NK cell surface receptors. Acquisition of activating ligands in combination with reduced expression of MHC class I molecules on virus-infected and cancer cells activates NK cell cytotoxicity and release of immunostimulatory cytokines like IFN-.

In the cancer microenvironment however, NK cells become functionally impaired by inhibitory factors produced by immunosuppressive immune cells and cancer cells. Here we review recent progress on the role of NK cells in cancer immunity. We describe regulatory factors of the tumor microenvironment on NK cell function which determine cancer cell destruction or escape from immune recognition. Finally, recent strategies that focus on exploiting NK cell anti-cancer responses for immunotherapeutic approaches are outlined.

One of the concerns regarding immunotherapy is that the panoply of options may at times be shadowed by a single strand of success and thus leaving behind a set of tools of great power. The authors continue in their discussion focusing on the use of NK cells as the entity in adoptive transfer:

Adoptive transfer of NK cells: To potentiate NK cell activity, the application of IL-2 in patients has remained challenging because high doses of IL-2 can result in serious adverse effects and expand regulatory T cells.

As an alternative, NK cells can be (re-)activated ex vivo and used for adoptive cell transfer therapy.

In the case of T cells, adoptive transfer using autologous tumor-reactive T cells (e.g. anti-MART-1) and chimeric antigen receptor (CAR) T cells (e.g. anti-CD19-CD3+-CD28) achieved significant clinical responses in some patients with advanced melanoma or B cell malignancies. These T cells, however, fail to control epitope-negative variants and have the potential for long-time adverse effects on epitope-positive non-malignant cells.

Similar to CAR T cells, genetically-engineered CAR NK cells are currently explored to more specifically direct NK cell cytotoxicity toward cancer cells. Analogous to therapeutic antibodies, this approach enables the killing of cancer cells which are otherwise poorly susceptible to NK cell recognition in addition to 'natural' cytotoxicity against epitope-negative cells.

Adoptive transfer of ex vivo cytokine-activated autologous or haploidentical NK cells resulted in favorable responses in a subset of pediatric and adult patients with hematological malignancies without causing graft-versus-host disease in the recipients.

This discussion expands the set of calls used in an adoptive transfer mode. Perhaps there can be alternative beyond these as well. My thoughts would include the complement system and its ability to isolate and neutralize aberrant intruders.

5.5 MULTIFACETED APPROACH

As much as I find the term "precision medicine" inaccurate, for we really mean accuracy not precision but I suspect this is a politically chosen term, the above approaches represent a collection of tools we now have at our command in treating cancers. In addition we also have pathway modifies such as kinase inhibitors whose use in such cancers as CML truly opened the door to treatment based upon detailed knowledge. One suspects that ultimately cancer treatment will be an integrated usage of all of these therapeutic techniques and not just one at a time. If we have learned anything from the treatment of Hodgkins Lymphoma it is that single threaded treatments are rarely effective and that an integrated approach is essential.

5.6 COMPARISONS

We can now make some overall comparisons as shown below:

Approach	Method	Advantages	Disadvantages
Adoptive Cell Transfer	Collect T cells surrounding tumor and enhance growth then reinsert.	Requires less processing and may be less costly.	May not be specific enough and may cause immune responses that are not beneficial Misses cancer stem cell
CIK	Collect multiple immune cells and enhance and then reinsert	Requires less processing and may be less costly.	May not be specific enough and may cause immune responses that are not beneficial Misses cancer stem cell
CAR-T Cell	Obtain T cell and make specific receptor/ligand to match tumor and insert via viral entity	Highly specific for tumor markers Has the ability to persist	Requires identifying a tumor marker Very costly May have immune storm effects Misses cancer stem cell
BITE	Make ligand with one end targeted for T cell and the other for tumor cell.	Focuses on simple molecules Targeted at specific cancer cell May not be as costly	Requires identifying a tumor marker May have immune storm effects Misses cancer stem cell
Cytokines	Use existing immune cell enhancers	Generally inexpensive May work for limited time	Can cause poor immune response Not specific, broad brush approach
Immunotoxins	Use toxins as one would in immunizations targeted for tumor cell	Generally inexpensive May work for limited time	Can cause poor immune response Not specific, broad brush approach
NK Cells	Use the NK cells instead of T cells as above	NK cells are immediate response vectors Possible targeting of stem cells	Yet to be determined

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