Winter 2-12-2016

Challenges of Adoptive (T-)cell Transfer Immunotherapy for Cancer

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Biology 464

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February 12, 2016

ABSTRACT

Background and significance: The rebirth of the theory of immunosurveillance in 2001 rejuvenated interest in anticancer immunotherapies. In particular, T-cell-based therapies have garnered substantial interest due to the robustness and tumor antigen-specific cytotoxicity of T-cell anticancer immune responses.

Hypothesis: The efficacy of adoptive cell transfer (ACT) T-cell immunotherapy could significantly improve and gain widespread approval if future innovations in ACT-based approaches account for the pro- and antitumoral properties of non-$CD8^+$ lineages of effector T-cells, evasion of T-cell antitumor immunity, and tumor-induced suppression of antitumor immunity.

Problem analysis: Despite numerous reports of highly successful ACT-based clinical trials, no such therapy is currently approved by the FDA for use in cancer patients. This project explores three limitations of current ACT-based anticancer therapies that may underline their lack of federal approval: inadequate incorporation of the pro- and antitumoral properties of non-$CD8^+$ T-cell lineages as potential immunotherapeutic targets; tumor evasion of T-cell antitumor immunity; and tumor-induced immunosuppression of T-cells.

Broader implications: Substantial patient-incurred economic costs are likely to be associated with ACT-based anticancer therapies if they are approved for clinical use. However, the relatively few side effects associated with anticancer ACT render this approach significantly less abusive than traditional forms of anticancer treatment.

BACKGROUND AND SIGNIFICANCE

One of the most urgent and complicated problems that confronts modern clinical medicine is the treatment of human cancers. Although the incidence rates of most cancers are reported to have begun stabilizing during the past five decades and the rate of cancer deaths appears to be experiencing a slow decline, the numbers of new diagnoses of most cancers continue to increase each year. Even more alarmingly, the types of treatment for all forms of cancer have remained dismally static and ineffective [1,**Fig. 1**]. Indeed, the three most common forms of cancer treatment – surgical excision of tumors, ionizing radiation therapy, and cytotoxic chemotherapies – are so remarkably primitive and impotent, yet ubiquitous and unparalleled in their clinical applications, that they have earned a bleak moniker for themselves: "slash, burn, and poison," respectively. Even in light of significant advancements in the early detection of cancers, these extremely outdated treatment options are, in general, unsuccessful in remedying the cancers of even those persons whose diseases are detected early. The inadequacy of current treatment regimens is evidenced by the fact that cancer claimed an estimated 590,000 lives in the United States in 2015, while only 3 times as many new cases – about 1,660,000 cases – are estimated to have been reported [1]. In other words, for every cancer death, about 3 new cancer diagnoses are being made, yielding a 1:3 ratio of epidemic proportions that highlights the need for novel, more effective treatments. Of equal concern is the abuse that a patient must endure at the hands of a current treatment regimen: it is typical for cancer patients who have been slashed, burned, and/or poisoned to be brought, quite literally, to their knees as the debilitating symptoms of their treatments often equal or even eclipse the physical punishments of the cancer itself [2]. A groundbreaking series of mouse model experiments at the turn of this century, however, finally revealed an encouraging, *effective* target for innovative cancer therapies that offer potent, patient-

friendlier alternatives to the surgeon's unforgiving knife and the chemotherapist's toxic cocktails: the human immune system.

History of cancer immunobiology

The theory of the immune system's potential role in antitumoral immunity is far from new: German physician and scientist Paul Ehrlich postulated as long ago as the early 1900s that the immune system is equipped with the means to protect the body from cancer [3]. At the time, however, the complex biology of the immune system remained to be elucidated, and Ehrlich's experimentally unfounded theory, though enticing, was quickly dismissed. Nearly 50 years later, as immunologists were finally armed with the means to directly study the anatomy and physiology of the immune system, Ehrlich's theory was resurrected by Sir Macfarlane Burnet and Lewis Thomas, this time with a name: the immunosurveillance hypothesis [4]. Burnet and Thomas reasoned that the adaptive arm of the immune system is capable of suppressing tumorigenesis: they suggested that just as the lymphocyte components of the adaptive immune system (AIS) had been shown to survey the body for *exogenous* contagions, those same cells – namely B, T, and natural killer (NK) cells – are responsible for monitoring and eliminating *endogenous* pathologies, including the development of neoplastic lesions. However, after several labs challenged Burnet and Thomas's data, the immunosurveillance hypothesis failed to obtain experimental support once again and remained a forgotten hypothesis until the turn of the 21st century [3].

Finally, in 2001, immunologists Vijay Shankaran, Robert Schreiber, and other colleagues successfully provided compelling, concrete support for the existence of cancer immunosurveillance by demonstrating that immunodeficient mice were at elevated risk of chemically induced and spontaneous tumorigenesis compared to immunocompetent murine

controls [5]. In their initial experiment, Shankaran *et al*. (2001) engineered 15 immunodeficient mice in which they disrupted the activity of recombination-activating gene 2 (RAG2), which is expressed only in lymphocytes and is vital to their function. Their 15 immunocompetent, wild type mice were genetically unaltered. Both groups of mice were exposed to chemical carcinogen methylcholnthrene (MCA) for 160 days, at which point the authors reported that only 2 of the 15 immunocompetent mice, but 9 of 15 immunodeficient mice, had formed MCA-induced tumors [5,**Fig. 2**]. Perhaps more strikingly, the authors performed a second experiment to evaluate the role of immunological tumor suppression of *spontaneous* tumors. After 15-16 months, all immunocompetent and immunodeficient mice (as previously described) were killed; the authors reported that all 11/11 immunocompetent mice were free of cancer (although 2 animals had developed benign tumors), while all 12/12 immunodeficient mice had developed some form of neoplastic disease [5]. Thus, the authors concluded that T-cells, B-cells, and/or NK cells (which had been rendered inoperative by RAG2 disruption) must be essential in the immunological suppression of chemically induced *and* spontaneous tumor development [5]. The ensuing flurry of tumor immunology research spoke for itself: cancer immunosurveillance had finally graduated from "theory" to the realm of experimental verification, marking a monumental – yet poorly understood – addition to the field of tumor biology and rejuvenating interest in immunotherapy as a viable form of cancer treatment.

Since the rebirth and subsequent validation of cancer immunosurveillance, an additional theory, of which immunosurveillance is a key component, has been proposed by Schreiber *et al*. (2011): the cancer immunoediting hypothesis. This hypothesis comprises three phases that characterize the interaction of the immune system with cancer cells, the first of which, termed the elimination phase, is essentially an updated version of Burnet and Thomas's cancer

immunosurveillance during which tumor cells are detected and destroyed by the immune system. The second phase, equilibrium, describes tumor cells that survive elimination but are forced into a sort of dormancy by the AIS, which suppresses additional tumor growth but simultaneously "sculpts" tumor cell immunogenicity, ultimately allowing for the survival of cells that are non-immunogenic and therefore resistant to antitumor immunity. Tumor cells that acquire means by which they may circumvent both elimination and equilibrium enter the third phase of immunoediting – escape – during which they emerge as aggressively malignant tumors that escape immune detection and destruction [3]. One advantage of the immunoediting hypothesis is that it identifies promising targets for cancer therapy. If, for example, one could therapeutically enhance the immunosurveillance capabilities of the AIS, it follows that it may be possible to selectively target tumor cells for destruction; concurrently "shutting off" the means by which cancer cells *escape* the AIS may, in turn, amplify the efficacy of a therapeutically enhanced AIS. However, the AIS is a complex system that comprises many different types of cellular and molecular players, begging the question: what component (or components) of the adaptive immune system could be targeted by cancer immunotherapy to selectively destroy cancer cells? One contender consistently seems to rise above the rest: T-cells.

The past two decades have witnessed multiple publications that support the conclusions of Shankaran *et al*. (2001) regarding the roles of effector T-cells, NK cells, and B cells[1] in the suppression and, paradoxically, the promotion of various cancers (potentially as a consequence the aforementioned sculpting of minimally immunogenic tumor masses and/or suppression of antitumor immunity) [5,6]. Of those different lymphocytes, T-cells in particular appear to be the

[1] The classification of B cells as effector cells is disputable: many immunologists insist that because the antibody *products* of B cells – and not the B cells themselves – directly interact with target cells to effect the immune response, B cells should not be classified as effectors.

primary effectors of cancer immunoediting, during which the immune system both combats and is exploited by cancer. The central role of T-cells in antitumor immunity is evidenced by their increased (and fluctuating) concentrations in the tumor microenvironment (TME) and within the tumor itself throughout the lifetime of the tumor: such localized increases in T-cell populations, even in classical immunity, are signs of immune system priming itself to launch a specific attack against a pathogen (or, in this case, against transformed neoplastic cells) [7-9]. Most importantly, certain lineages of tumor-associated T-cells have been repeatedly shown to selectively lyse tumor cells *via* the same mechanisms by which they lyse bacterial cells and virally-infected host cells [8,10,11]. As such, T-cells are widely considered to be the most attractive targets for anticancer immunotherapy per their apparent roles as the primary effectors of antitumor immunity.

Overview of T-cell-mediated antitumor immune response

An analysis of any type of T-cell anticancer therapy warrants a review of the general mechanism by which a T-cell-mediated adaptive immune response (AIR) is launched, a simplified version of which is offered here. Note that, unless otherwise stated, all discussions of T-cells throughout this project pertain to αβ T-cells – those T-cells whose surface receptor heterodimers comprise one α and one β peptide chain. γδ T cells, which remain poorly studied in general, are discussed briefly below in *1c*.

Central to launching any AIR, including an antitumoral AIR, is an antigenic substance – a molecule that could be anything from a peptide sequence to a sugar – that most often exists on the surface of the pathogenic cell that produced it[2]. Upon detection of a surface-expressed tumor-associated antigen (TAA) by an antigen-presenting cell (APC), the antigen is internalized by the

[2] Antigens may also exist in a free, soluble form.

APC, bound to a major histocompatibility complex (MHC)[3] protein, and finally "presented" on the surface of the APC in a TAA:MHC complex. Naïve T-cells – those that have not yet met their specific antigens – whose T-cell receptors (TCRs) are specific for a single antigenic substance in its MHC-bound form[4] circulate in the blood and lymph. Once a TCR encounters its specific TAA:MHC complex[5], various endocrine and paracrine signaling pathways instruct the T-cell to clonally expand, producing thousands of copies of itself that are each reactive to that same TAA:MHC complex [9,12,**Fig. 3**]. The TCRs of those tumor-specific T-cells will then selectively recognize and react to any cell that expresses its particular TAA:MHC complex. A T-cell's response to TAA:MHC recognition by its TCR depends on the lineage of that particular T-cell. The major T-cell lineages and their pro- and antitumoral functions in tumor immunity are elaborated below in the Problem Analysis section.

Adoptive T-cell transfer (ACT) therapy

Drawing on the tumor-specific lytic capabilities of the T-cell-mediated immune response, Perica *et al*. (2015) enumerate a few of the advantages of using T-cells as effectors of anticancer immunotherapy, citing the specificity and robustness of T-cell antitumor immunity [10]. Perhaps most appealing is the specificity of T-cell AIRs, which selectively destroy only targeted tumor cells, thereby preserving normal, non-tumor cells – something that slashing, burning, and poisoning all fail to do, highlighting the need for the development of novel, tumor cell-specific anticancer immunotherapies.

[3] Human MHC proteins are also referred to as human leukocyte antigen (HLA) proteins.

[4] TCRs are specific to a single MHC protein (which exists in two primary forms, MHC I and MHC II, each of which has hundreds of sub-forms) *and* to the single antigen to which they will react. Any single αβ T-cell will only be activated by its specific antigen that is bound to the single MHC protein to which the TCR is sensitive; that same antigen bound to a different MHC protein, for example, will not elicit an AIR [9].

[5] Several other types of recognition in addition to Ag:MHC recognition are necessary to fully activate a naïve T-cell, a few of which are mentioned below in 2b.

The efficacy of one such T-cell-mediated anticancer immunotherapy – adoptive T-cell transfer (ACT) – has been tested in clinical trials for more than a decade, many of which have reported promising results. At its core, ACT for cancer therapy involves the transfusion of T-cell lymphocytes to enhance antitumor immunity [13-16]. T-cells (primarily $CD8^+$ T cells, which are capable of inducing apoptosis in their target cells, thereby killing them) are harvested from a patient's blood or tumor and genetically modified and/or amplified *in vitro* before being reinfused back into the patient. If T-cells are harvested from a tumor, it is assumed that they are tumor cell-specific; thus, it follows that expanding the populations of those tumor-specific T-cells allows clinicians to foster and unleash a directed attack on tumor cells.

Several modifications of ACT for cancer therapy have been described. One increasingly popular variation involves partial ablation of lymphocytes (including T-cells) *in vivo* prior to reinfusion of the cultured cells in order to temporarily overcome immunotolerance (as is mediated by T_{reg} cells, a unique lineage of T-cells that act to suppress other T-cell effectors of immunity), thus providing a window of time during which reinfused $CD8^+$ T-cells may mount an uninhibited attack on tumor cells. This approach has been shown to significantly improve the efficacy of anticancer ACT [13]. More recently, the remarkable successes of genetically modified T-cell ACT trials have garnered special attention, even catching the eye of the FDA, which noted the "breakthrough status" of these therapies (although no ACT-based anticancer therapy for cancer is currently approved by the FDA) [10,14]. The most common therapeutic modification of T-cells *ex vivo* involves the transfection of tumor-specific chimeric antigen receptors (CARs) – which combine antibody recognition with T-cell activation – and/or tumor-specific T-cell receptors into the surfaces of harvested T-cells, thereby homing in on the cancer cells themselves while sparing normal cells from the T-cells' selective lysis [10,13-15,17].

As stated above, ACT-based anticancer therapies have produced exceedingly promising results in human clinical trials. Hinrichs and Rosenberg (2014) published a review of dozens of such trials in which they analyzed the curative effects of ACT-based therapies that had been reported up to the point of their publication [18]. They conclude that, in general, ACT-based anticancer regimens are associated with a 50-60% overall response in trial patients; further, approximately 30% of patients exhibit complete responses (remission). Perhaps even more importantly, 95% of those patients whose cancers enter remission experience durable, ongoing remissions that persisted for many patients for years after the publishing of their respective trials[6], which speaks to the powerful potential of therapeutically-enhanced antitumor immunity.

As a consequence of such pronounced successes, the numbers of ACT-based clinical trials for cancer continue to increase each year. Additionally, Aranda *et al*. (2015) note the recent creation of startup companies that focus on the development of new approaches to ACT-based anticancer immunotherapy [10]. However, there remains substantial room for progress. For example, the FDA's refusal to approve ACT-based immunotherapy as an accessible form of cancer treatment speaks to the inadequacies of the ACT approach that must not be ignored. This project explores three primary limitations that must be addressed by future innovations in ACT-based immunotherapy in order to garner widespread approval as an effective, advantageous new form of cancer treatment. The next section of this paper describes each of these limitations in turn: inadequate incorporation of non-$CD8^+$ T-cell subpopulations in tumor immunity, tumor evasion of T-cell immunity, and tumor-induced immunosuppression. Overcoming these challenges may significantly improve the efficacy of ACT-based anticancer therapies, perhaps leading to their approval as promising new alternatives to traditional treatment options.

[6] Many of the patients that exhibited such durable responses were reported to have been in remission even at the time of the review's publishing.

PROBLEM ANALYSIS

One of the most glaring shortcomings of ACT-based immunotherapy for cancer is its failure to address each of the effector subpopulations of T-cells. Although cytotoxic $CD8^+$ T-cells are firmly established as potent killers of cancer cells, other lineages of T-cells, many of which augment or even *suppress* the cytotoxic capabilities of $CD8^+$ T-cells, have also been shown to participate in tumor immunity. Ignoring those other lineages limits the efficacy of ACT-based immunotherapy. Additionally, evasion and suppression of tumor immunity, which are relatively common characteristics of cancers (especially late-stage malignancies), can render any current form of ACT immunotherapy entirely ineffective. Even with the development of genetically-enhanced variations of ACT that attempt to overcome tumor evasion and suppression of immunity, resistance to ACT-based immunotherapy trials has been consistently reported.

Underlying each of these problems – the heterogeneous (and sometimes counterproductive) effects of certain T-cell subpopulations, tumor evasion of immunity, and tumor suppression of immunity – is, first and foremost, the absence of critical information. What *are* the roles of the other, non-$CD8^+$ lineages of T-cells in tumor immunity? *How* do tumor cells evade or suppress antitumor immune responses? This section responds to those questions with an analysis of the progress that has been made since the turn of this century in deciphering T-cell-mediated tumor immunity and how it is sidestepped and even sabotaged by cancer cells.

1. What are the roles of the major T-cell lineages in tumor immunity?

Current ACT-based approaches target only CD8+ T-cells (discussed below), which severely limits their therapeutic potential due to the presence of other T-cell lineages in the tumor environment which may augment or inhibit the antitumoral toxicity $CD8^+$ T-cells. Here, six T-

cell lineages and their roles in tumor immunity are organized into three categories: antitumoral, protumoral, and ambiguous.

1a. Antitumoral T-cells: $CD8^+$ cytotoxic T_c cells and Type-1 $CD4^+$ T_H1 cells

The aggressors of the T-cell family, T-cells that express the protein CD8 on their surface are responsible for the direct cytolysis of target cells. In general, as discussed above, elevated densities of $CD8^+$ T_c cells at sites of neoplastic lesions are thought to recognize TAA on tumor cells and selectively lyse those cells *via* the same cytotoxic mechanisms by which they destroy exogenous pathogens [8,10,11]. Additionally, $CD8^+$ T_c cells are known to secrete antitumoral cytokines, the most potent of which – interferon-γ (IFN-γ) and interleukin-2 (IL-2) – are elaborated below in the context of T_H1 cells [9]. As such, high densities of $CD8^+$ T_c cells in the tumor and the TME are strongly associated with a positive prognosis, which includes overall survival and/or longer disease-free survival after surgical resection of the primary tumor, for almost all types of cancer cells [3,6,19,**Fig. 4**]. Indeed, that association is so pronounced that Galon *et al*. introduced the "immunoscore" in 2012 as a means by which cancer survival prognoses may be quantified – with remarkable accuracy – in terms of relative tumoral densities of *anti*tumoral $CD8^+$ T_c cells and (often) *pro*tumoral T_{reg} cells (the latter of which which are developed below in *1c*) [20].

Effective cancer immunotherapy is now believed to target the activation of $CD8^+$ T_c cells [21]. However, it is becoming increasingly apparent that $CD4^+$ T-cells, which express CD4 instead of CD8 on their surfaces, are critical in the activation and regulation of said $CD8^+$ T_c cells, although they themselves are not effectors of direct cytolysis [9]. $CD4^+$ T_H1 cells have long been known to govern the development and cytotoxicity of $CD8^+$ T_c cells during immune responses to exogenous pathogens. After nearly two decades of questioning the role(s) of T_H1

cells in tumor immunity, it is now understood that T_H1 cells, like $CD8^+$ T_c cells, are prominent at sites of cancer lesions and are strongly associated with positive prognoses for most cancers [5,19,21,22,**Fig. 4**]. The mechanisms by which T_H1 cells induce the differentiation and augment the cytotoxicity of $CD8^+$ T_c cells are mediated by the cytokines that they secrete, the most well-documented of which include IFN-γ and IL-2, each of which is also secreted by $CD8^+$ T_c cells [8,9]. Also noteworthy, T_H1 cells have been shown to polarize another type of immune cell, tumor-associated macrophages (TAMs), to the M1 phenotype; M1 TAMs, as opposed to the protumoral M2 type, are known to suppress the growth and metastasis of tumors [13,23,24]. Multiple studies have reported a significant decrease in the M1/M2 (antitumoral/protumoral) ratio that worsens as patients' cancers progress; moreover, those same studies report a strong association of an increased M1/M2 ratio with improved survivability [24,25]. Thus, T_H1 cells indirectly enhance antitumor immunity *via* mediation of $CD8^+$ T_c cell and M1 macrophage responses.

In addition to directly initiating the development of tumor-specific $CD8^+$ T_c cells, the IFN-γ that is secreted by T_H1 and $CD8^+$ T_c cells is also known to bolster a tumor-specific AIR by increasing the immunogenicity of cancer cells by upregulating surface expression of MHC molecules, which increases the likelihood of Ag:MHC presentation to $CD8^+$ T_c cells, thereby encouraging tumor cell lysis[7] [8,19,26]. In order to take advantage of IFN-γ-induced immunogenicity of target cells, T_H1 and $CD8^+$ T_c cells also secrete IL-2, which stimulates the clonal expansion and survival of activated T-cells, ultimately resulting in a robust population of tumor-specific T-cells [27].

[7] Other, *pro*tumoral effects of IFN-γ have been reported; however, these effects are not well-supported in the primary literature.

The future development of effective ACT immunotherapies for cancer must not neglect to incorporate antitumoral T_H1 cells. Unfortunately, however, the antitumoral properties of T_H1 cells are almost always eclipsed by the efforts of a second subpopulation of $CD4^+$ T-cells, T_H2 cells, concentrations of which are similarly increased at sites of neoplastic lesions.

1b. Protumoral T-cells: Type-2 $CD4^+$ T_H2 cells

Unlike $CD8^+$ T_c cells and T_H1 cells, which are strongly associated with positive prognoses in almost all primary literature, T_H2 cells are increasingly becoming associated with *negative* prognoses for many types of cancers [9,19,21,**Fig. 4**]. This can be explained by the fact that T_H2 cells often express themselves as the protumoral antipodes of T_H1 cells; indeed, the T_H1/T_H2 inverse relationship is so well-documented – in tumor immunity and otherwise[8] – that it is often referred to as the T_H1/T_H2 paradigm [9,22]. In cancer patients, the T_H1/T_H2 relationship manifests as a prognostic tool: an increased T_H1/T_H2 ratio in the tumor microenvironment and within the tumor itself is often associated with a more positive prognosis [22]. On the other hand, decreased ratios of intratumoral and circulating T_H1/T_H2 cells have been associated with poor prognoses, highlighting the largely protumoral characteristics of T_H2 cells [22]. T_H2 cells mediate their protumoral effects through their immunosuppressive cytokines: IL-10 and transforming growth factor-β (TGF-β). In contrast to the M1-polarizing cytokines secreted by T_H1 cells, IL-10 and TGF-β polarize TAMs to the M2 (tumor-promoting) type, again speaking to the protumoral effects of this lineage [9]. Studies have reported that up to 70% of tumor-associated macrophages are of the M2 (protumoral) type, suggesting that populations of T_H2 cells are preferentially drawn to tumor cells, where they indirectly support the development of

[8] The effector $CD4^+$ T-cell response can be polarized to favor either a T_H1 cell-mediated/inflammatory response, which is induced by elevated levels of IFN-γ, or a T_H2 humoral/antibody-mediated response, which is induced by elevated levels of IL-4. Significant differences distinguish each type of response, as is evidenced by the very different cytokine secretions of each type, and the T_H1/T_H2 paradigm has implications in many disease states [9].

the tumor *via* M2 polarization [28]. Finally, IL-10 and TGF-β are also known to inhibit a host of other antitumor immunity parameters, including antigen presentation to antitumoral T-cells, proliferation of tumor-specific T-cells, and the production of T_H1 antitumoral cytokines [13,22,23,29].

The effects of T_H2 cells in tumor immunity have not yet been generalized in the majority of primary literature due to the accumulation of reports only recently that concrete their protumoral properties. In spite of this, the potential of T_H2 cells to suppress therapeutic $CD8^+$ T_c immunity should not be ignored by immunotherapies for cancer and techniques should be developed to specifically interrupt their immunosuppressive effects in order to maximize the efficacy of ACT-based anticancer regimens.

1c. Ambiguous T-cells: Type-17 $CD4^+$ T_H17, $CD4^+$ $Foxp3^+$ regulatory T_{reg}, and γδ T-cells

T_H17 cells – named after IL-17, their primary cytokine product – are perhaps the least-studied T-cells in tumor immunity and even in classical immmunity; thus, relatively little is known about their function(s) in suppressing and/or promoting tumor development and growth [9]. As a result, it is too early to decisively categorize T_H17 cells as either pro- or antitumoral for any one type of cancer [19,22,**Fig. 4**].

Although it would seem that T_H17 cells are not candidates for T-cell immunotherapy due to their confusing role(s) in tumor immunity, one particularly important product of the past decade of studying T_H17 cells in the context of tumor immunity has been the establishment of a T_H17/T_{reg} paradigm, which appears to be a promising target for cancer immunotherapy [22,34]. This idea is discussed below in the context of T_{reg} cells.

Concentrations of regulatory $CD4^+$ $Foxp3^+$ T (T_{reg}) cells, like each of the above T-cell lineages, are significantly elevated in the peripheral blood and at the tumor sites of patients with

many types of cancer, strongly indicating that they are involved in tumor immunity [30]. Distinguishable by their expression of the Foxp3 protein, which acts as their master regulator (the transcription factor that controls differentiation into the T_{reg} cell lineage), T_{reg} cells are also set apart from the other subpopulations of T-cells per their main function: the regulation and shutdown of the immune system once a pathogen has been cleared from the body [9]. Given the destructive capacity of the immune system, properly-functioning immunosuppressive T_{reg} cells are critical in preventing excessive tissue damage to the host. In tumor immunity, however, T_{reg} cells are progressively being associated with negative prognoses due to their ability to suppress the very immune system that is equipped with the means to destroy cancer cells [7,19,31,**Fig. 4**]. Their primary immunosuppressive secretions – TGF-β and IL-10 – are discussed above in the context of T_H2 cell-mediated immunity [9]. Interestingly, T_{reg} cells have even been shown to reprogram the TME towards an angiogenic phenotype, thus contributing to tumor growth and even metastasis [32,33]. T_{reg}-induced suppression of tumor immunity is extensively explored below in *3a*.

Recent studies have challenged the pervasive association of T_{reg} cells with negative prognoses [34,35]. In a meta-analysis of gastrointestinal cancers – hepatocellular carcinoma (HCC), colorectal cancer (CRC), and gastric cancer (GC) – Huang *et al*. (2014) demonstrated that, as was expected, overall survival rates 1, 3, and 5 years following tumor resection were significantly lower in high T_{reg} infiltration patients with HCC and GC [34]. Surprisingly, though, CRC patients with high levels of infiltrating T_{reg} cells were shown to have significantly *higher* survival rates 1, 3, and 5 years following tumor resection, as compared with patients with low levels of infiltrating T_{reg} cells [34]. Their results suggest that T_{reg} cells may exhibit deleterious effects in certain cancers (including HCC and GC), but not in others (including CRC). Note,

however, that these results should be interpreted carefully as the authors' analyses of the prognostic value of infiltrating T_{reg} cells did not account for the effects of other lineages of T-cells (such as protumoral T_H2 cells) that could have contributed to the apparent results of the study. In sum, the prognostic value of T_{reg} cells remains somewhat controversial for certain cancers; thus, future research should focus on discerning which types of cancers might benefit from infusions of T_{reg} cells, à la ACT, thereby individualizing antitumoral immunotherapies to best fit a patient's particular disease [31].

As mentioned above, a recent breakthrough in understanding tumor immunity was the proposal of the T_H17/T_{reg} paradigm, which has largely replaced the previously described ubiquitous T_H1/T_H2 relationship in its prognostic value for many cancers. Perhaps most strikingly, recent studies have revealed strong associations between the development of lung cancer – both small cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC) – and cytokine imbalance as a result of a skewed T_H17/T_{reg} ratio [22,34]. Thus, the T_H17/T_{reg} paradigm should be considered a valuable target for ACT immunotherapy, especially for patients with SCLC or NSCLC, which, together, comprise the deadliest forms of cancer in the United States [1].

One final subpopulation of T-cells, γδ T-cells, warrants discussion due to its growing appeal as a potential target for ACT-based immunotherapy for cancer. γδ T-cells differ significantly from their αβ counterparts both phenotypically and mechanistically: phenotypically, they rarely express either $CD4^+$ or $CD8^+$ surface proteins, and their TCRs comprise one δ and one γ chain instead of one α and one β chain; mechanistically, most γδ T-cells do not require Ag:MHC presentation in order to be activated, and they are known to play a prominent role in the recognition of lipid antigens, possibly in response to heat shock proteins [9,36-38].

Additionally, γδ T-cells comprise only a small subset, about 5%, of circulating lymphocytes, leaving their precise role(s) in immunity – including tumor immunity – largely unexplored [37]. However, far more antitumoral than protumoral effects of γδ T-cells have been described in recent studies [9,36-38]. Additionally, therapeutic γδ T-cells have been shown to exhibit significant antitumoral effects in ACT-based clinical trials, which is attributed to their ability to recognize isopentenyl pyrophosphate, a phosphoantigen that is upregulated on the surface of many tumor cells as a byproduct of mutation-induced dysregulation of the mevalonate pathway [36-38]. The immunotherapeutic potential of γδ T-cells is becoming increasingly clear and is presently the subject of numerous clinical trials as a novel effector of ACT for cancer [37,39]. The advantages of γδ T-cell-based therapy will be considered below in the context of tumor evasion of immunity.

1d. Conclusions and potential solutions

Future approaches to ACT-based anticancer immunotherapy must not neglect to address the effector functions of the above lineages of T-cells due to their potential for both desirable and undesirable interactions with $CD8^+$ T_c cells. Indeed, Chodon *et al*. (2015) conclude that future trials incorporating $CD8^+$ and $CD4^+$ lineages are expected to elicit synergistic responses that demonstrate considerable improvement in the durability and efficacy of ACT-based therapies [14]. However, additional research is required to fully and concretely elucidate the different role(s) of those $CD4^+$ lineages in human cancers, particularly the roles of T_H17 and T_{reg} cells.

2. How do tumor cells evade immune detection and destruction?

Despite the human immune system having evolved to combat tumors, as is evidenced by the antitumoral T-cell lineages explored above, the majority of clinically significant cancers acquire means of evading tumor immunity. Such tumor escape mechanisms are the product of genetic

mutations that plague cancer cells due to their marked genetic instability, which is well-established as one of the cornerstones of tumorigenesis and tumor survivability in the face of various cancer therapies [4,7,40]. The hypermutability that occurs as a direct result of genomic instability means that cancer cells are at increased risk of bearing mutations in the various genes whose products participate in tumor immunity. Malignant genomic instability is aggravated by the laws of natural selection, which elicit pressure on tumor cells in a sort of accelerated survival of the fittest[9], in which only those cells that possess genetic (and epigenetic) traits that allow them to escape immune destruction are selected for survival [9,39]. Future approaches to ACT-based immunotherapy for cancer must address tumor evasion of immunity to ensure that a therapeutically enhanced immune response is not futile.

Three of the most common of those evasive/non-immunogenic traits are discussed in this section: loss of surface-expressed tumor-associated antigens (TAA); loss of MHC proteins, antigen processing machinery, and/or costimulatory molecules that are necessary for antigen recognition and subsequent immune effector cell activation; and loss of function of the IFN-γ receptor signaling pathway by which the immunogenicity of cells is augmented.

2a. Loss of surface tumor-associated antigens

Decreased expression of several known TAAs has been associated with disease progression of many types of cancer, with advanced tumors often expressing fewer immunogenic TAAs than lower-grade tumors. Loss of TAA is thought to contribute to resistance to anticancer immunotherapies because such therapies inevitably destroy only those cancer cells that *do* express a target TAA, while sparing – and allowing for the survival and proliferation of – cancer cells that have lost, or never expressed, that particular TAA [39].

[9] "Fittest" here refers to the cancer cells' immunogenicity; those cells that elicit strong immune responses will not be selected for survival.

Although the mechanisms that regulate the selective loss of certain TAAs are poorly understood (aside from the fact that TAA loss is often the result of genomic hypermutability), one phenomenon that may offer some answers is the theory of immunodominance [39,41]. Originally observed in certain viral infections that express multiple viral antigens, immunodominance as it relates to tumor immunity describes situations in which immune responses are controlled toward the first TAA that is encountered. It has been suggested that tumor immune responses are activated by only one or a select few TAA, which consequently become the dominant, preferentially immunogenic TAA, while other TAA are ignored by the immune system and become immunorecessive (non-immunogenic). Tumors cells that do not express that particular immunodominant TAA (say, for example, antigen "D"), but express other, immunorecessive TAA (antigen "R"), are spared from that particular D-specific immune response and are allowed to survive and proliferate. All of the D-expressing tumor cells, on the other hand, will be selectively destroyed by the D-specific immune response, and the R-expressing cells that survive will produce progeny that inherit the expression of the intact antigen R gene, but not the intact antigen D gene. This results in a non-immunogenic tumor whose constitutive cells have undergone widespread loss of antigen D due to immune pressure, effectively neutering any future D-specific immunotherapies [39,41]. Due to the potential links between immunodominance and drug resistance, future approaches to ACT-based anticancer regimens must combine the infusion of several populations of activated T-cells, each of which has been modified to respond to different tumor antigens. This may, in theory, prevent or at least stall the development of resistance to such therapies while more effectively destroying the entire tumor mass.

On the other hand, it is also worth noting that the genomic instability and hypermutability that characterize cancer cells may on occasion work against a tumor cell by *increasing* expression of surface TAA. Multiple recent studies have shown tumor mutational burden to improve a patient's response to immunotherapy, suggesting that hypermutable cells may occasionally sustain mutations that result in the expression of antigenic substances that are recognized by the immune system [7,42,43]. This phenomenon highlights the need for individualized anticancer regimens that account for the unique genomic landscape of a patient's particular disease.

2b. Loss of MHC proteins, antigen processing, and co-stimulatory molecules

Loss of human MHC class I proteins (which present antigens of intracellular origin to immune cells) has been documented in many cancers, including melanoma, colorectal carcinoma, prostate adenocarcinoma, SCLC, NSCLC, breast carcinoma, and renal cell carcinoma [3,8,9,36,39]. Although little unequivocal evidence has accumulated to show that complete or partial loss of expression of MHC class I proteins contributes *directly* to tumor escape, because mutative loss of MHC proteins leads to loss of TAA presentation, it is hypothesized that loss of MHC protein expression therefore causes an overall failure of TAA to stimulate an immune response [39]. Thus, it is presumed that cancer cells that have sustained mutations in MHC genes and/or in the genes that code for the machinery that is responsible for the intracellular processing of TAA prior to its presentation on MHC proteins are evolutionarily favored as they are effectively able to escape immune detection [9,39].

Finally, many advanced tumors lose expression of co-stimulatory molecules that are necessary for the complete activation of T-cells upon recognition of Ag:MHC by a T-cell's TCR [9,17,39]. In addition to the specific recognition of the Ag:MHC complex by a TCR that was

discussed above, certain co-stimulatory molecules (such as B7, which is recognized by CD28 on the T-cell) are required to alert the T-cell to a pathogenic, non-self antigen; recognition of tumor antigens by a T-cell *without* co-stimulation induces T-cell anergy, a precautionary measure by which the host's immune system attempts to protect itself from potentially self-reactive T-cells [9,17,39,44].

Many tumor cells that lose expression of MHC proteins or functionality of antigen processing machinery may fail to escape γδ T-cell-induced apoptosis because, as discussed above in *1c*, γδ T-cells do not require MHC presentation of antigen to mount an immune response [37,39]. This makes γδ T-cells a particularly attractive target for the development of ACT-based immunotherapies that can destroy even cancer cells that have evolved to evade detection by other T-cell lineages. However, effective ACT immunotherapy must not rely on γδ T-cells alone; such an approach would ignore the potent antitumoral properties of $CD8^+$ T_c and $CD4^+$ T_H1 cells. Thus, future innovations in ACT immunotherapy for cancer should focus on developing techniques to engineer T-cells *ex vivo* that do not require Ag:MHC presentation *or* activation by B7 and other co-stimulatory molecules. Of note, recent trials of ACT-based immunotherapy using T-cells that have been modified to express a chimeric antigen receptor (CAR) (a complex that comprises an immunoglobulin-like antigen receptor and co-stimulatory molecules, essentially eliminating the need for activation by co-stimulation) have reported significant success [14,15]. Clinical trials involving CAR-modified T-cell anticancer treatments have been particularly effective in the treatment of lymphoblastic leukemias due to the attractiveness of CD19 – which is predominantly expressed on normal and transformed B-cells – as a therapeutic target antigen for CARs; these clinical trials have reported positive responses in 60-90% of patients [16]. As a result of the remarkable efficacy of these trial therapies, CAR-

modified T-cell therapies are being developed for solid tumors as well. These approaches are relatively new and few reports of their effecacy are yet available, although Pule *et al*. (2008) and Louis *et al*. (2011) have reported early signs of success in the treatment of neuroblastoma [45,46].

2c. Loss of function of the IFN-γ receptor signaling pathway

As mentioned above in *1a*, IFN-γ is one of the major effector molecules of antitumor immunity per its ability to upregulate the surface expression of MHC molecules on which TAA is presented to $CD8^+$ T_c cells [9]. However, an emerging characteristic of many advanced, non-immunogenic cancer cells is the loss of function of one or more components of the IFN-γ receptor signaling pathway, including the partial or total loss of the IFN-γ receptor itself [4,9,26,39]. The loss of function of at least one component of the IFN-γ signaling pathway was recently shown to be present in at least one-third of melanoma and lung adenocarcinoma cell lines [26]. Additionally, it has been shown that mice that do not exhibit a functional IFN-γ signaling pathway are at much higher risk of carcinogen-induced cancers, including sarcomas, lymphoma, and epithelial tumors, suggesting a strong association between loss of IFN-γ signaling and survivability [39].

It must be noted that the IFN-γ signaling pathway alone does not appear to be an advantageous target for cancer immunotherapy due to the results of recent studies that have shed light on a potential "dark side" of IFN-γ. These studies concluded that IFN-γ may exhibit striking protumoral effects, potentially by inducing apoptosis of certain immune cells in some human cancers [26]. Indeed, multiple trials of IFN-γ-based immunotherapies during the 1990s reported significant deleterious side effects associated with the therapies; the patients of one trial fared so poorly compared to untreated patients that the trial was terminated early [47,48]. Therefore,

while it is clear that IFN-γ signaling usually augments the antitumoral properties of the immune system, it is unlikely that intravenous administration of IFN-γ will be approved for the treatment of human cancers due to the potential for unwanted side effects.

2d. Conclusions and potential solutions

Few, if any, variations of ACT-based immunotherapy to resolve the phenomenon of immunodominance have been explicitly described. However, as mentioned above, it may be worthwhile to develop ACT-based regimens that respond to a variety of tumor-associated antigens, potentially eliminating the possibility of evolutionarily/therapeutically selecting for the survival of select cancer cells that do not express a single particular TAA and ensuring *complete* lysis of a tumor mass. In addition to CAR-engineered T-cells that do not require co-stimulation, the efficacy of γδ T-cells is being tested in patients whose cancers have mutated to lose Ag:MHC presentation [14,37,39]. Together, these approaches are promising improvements to ACT-based anticancer therapies. Indeed, Aranda *et al.* (2015) note optimistically that CAR-modified T-cell immunotherapies are so effective that they are likely to be the first forms of ACT-based anticancer therapies to be approved by the FDA for use in human cancers [10]. However, the problematic loss of function of IFN-γ signaling remains unaddressed in current approaches.

3. How do cancer cells induce and maintain suppression of tumor immunity?

In addition to *evading* detection and destruction at the hands of tumor immunity, the majority of cancer cells are also able to *suppress* the immune system. These two phenomena – immune evasion and immunosuppression – are not differentiated in the majority of the current literature; however, each strategy involves significantly different mechanisms and outcomes (though some overlapping pathways certainly do exist). Furthermore, the proposed "solutions" to tumor

evasion and tumor immunosuppression differ significantly; therefore, for the purposes of this paper, evasion and suppression are treated as separate concerns.

This section explores the two most prominent immunosuppressive strategies employed that are by cancer cells: exploitation of T_{reg} cells to over-suppress the very immune system of which they are key components; and tumoral secretions of immunosuppressive molecules. Finally, an emerging hypothesis of indirect immunosuppression by TME-associated hypoxia is briefly acknowledged.

3a. Exploitation of T_{reg} cell-induced immunosuppression

T_{reg}-induced suppression of antitumor immunity exemplifies the paradoxical role(s) of the immune system in both the suppression *and* the promotion of tumors. As was summarized above in *1c*, T_{reg} cells are the primary effectors of immunosuppression in normally-functioning systems and it is increasingly apparent that cancer cells are often equipped with the means to hijack the "normal" immunosuppressive capabilities of T_{reg} cells to induce *aberrant*, deleterious immunesuppression [22,29-32,35,40,49-53]. Thus, one mechanism by which cancer cells may suppress tumor immunity involves recruiting significant concentrations of T_{reg} cells to the site of lesions; indeed, T_{reg} cells have been found to account for as much as 60% of all tumor-infiltrating $CD4^+$ T-cells in some cancers, suggesting that they must be preferentially drawn to cancer cells [49]. At least three explanations for such drastic concentrations of intratumoral T_{reg} cells have been described: first, chemokines (molecules that mediate chemotaxis – the movement of cells along chemical "paths") released by tumor cells may cause preferential migration of T_{reg} cells to the site of the tumor; second, cocktails of cytokines released by tumor cells may facilitate uncontrolled proliferation of T_{reg} cells; third, tumor cells may release certain cytokines that drive the conversion of conventional, non-regulatory $CD4^+$ T_H cells into $CD4^+$ $Foxp3^+$ T_{reg} cells

[22,30,32,50,51. In addition to increased numbers of T_{reg} cells at sites of tumor growth, enhanced *potency* of those intratumoral T_{reg} cells (as compared to circulating T_{reg} cells) has also been reported, though the mechanisms that may account for such a phenomenon remain wholly unclear [30]. But how do these hyper-suppressive T_{reg} cells work in favor of the tumor cells that recruit them? The answer is multifold.

The primary means by which T_{reg} cells "turn off" the immune system is through their immunosuppressive secretions, including TGF-β, IL-10, and prostaglandin E_2 (PGE_2) [3,9,35,39]. The immunosuppressive effects of TGF-β and IL-10 include: hindering the differentiation of the antitumoral T_H1 cell lineage; suppressing the cytotoxicity of $CD8^+$ T_c cells (potentially by decreasing production of granzyme B, a protease released by $CD8^+$ T_c cells that induces apoptosis of target cells); and decreasing antigen presentation by target cells (including cancer cells) [3,22,23,39,49,50,52]. PGE_2, on the other hand, upregulates the secretion of IL-10, selectively suppresses antitumoral effector T_H1 cells, and promotes the protumoral functions of T_H2, T_H17, and T_{reg} cell lineages [54]. However, the protumoral immunosuppressive capabilities of T_{reg} cells extend beyond their secretory molecules. For example, T_{reg} cells have been shown to consume and sequester IL-2, a cytokine that is critical for maintaining the functions of other T-cell lineages that are involved in tumor immunity [3,49]. Finally, most T_{reg} cells express surface proteins CD39, an enzyme that is responsible for the conversion of ATP and ADP to cAMP, and CD73, which catalyzes cAMP to adenosine, a powerful immunosuppressive molecule that disrupts the normal functions of T-cells and other effectors of immunity [30,35,49].

3b. Tumoral secretions of immunosuppressive molecules

In addition to capitalizing on the immunosuppressive features of T_{reg} cells, cancer cells themselves are also known to secrete multiple immunosuppressive agents, many of which

overlap with the secretions of T_{reg} cells. For example, tumor cells have also been shown to secrete TGF-β, IL-10, and PGE_2, thereby augmenting the immunosuppressive activities of recruited T_{reg} cells [9,21,39,40,51]. (Note that TGF-β is the primary cytokine that induces differentiation of naïve T-cells into mature T_{reg} cells, thus accounting for one of the ways by which T_{reg} cells are thought to preferentially accumulate at sites of tumor growth) [9,51]. Additionally, and somewhat controversially, it has been suggested that certain high-grade tumor cells express and may even secrete apoptosis stimulating fragment ligand (FasL), which induces apoptosis upon binding to its receptor (FAS) on target cells. This "tumor counter-attack" hypothesis suggests that $FasL^+$ tumor cells are capable of suppressing tumor immunity by inducing apoptosis in FAS^+ lymphocytes (including FAS^+ T-cells) [10,39,55]. However, the quality of many of the studies that proposed the tumor counter-attack hypothesis have been questioned, and additional research has found little or no evidence of significant FasL expression by tumor cells [41]. One hypothetical approach to manage FasL-mediated immunosuppression is to manipulate T-cells *in vitro* to knockout expression of the FAS receptor. At this time, no trials incorporating such FAS-modified T-cells have been reported.

3c. Indirect immunosuppression by TME-associated hypoxia

Finally, recent studies involving T-cell metabolism and the TME have introduced the intriguing theory of TME-associated hypoxia-induced immunosuppression [23,56]. Building onto the well-established observation that tumor growth tends to result in a hypoxic TME as malignant cells rapidly outgrow their angiogenic blood supply, it has been suggested that tumor-associated hypoxia may create a hostile TME in which T-cells and other lymphocytes cannot thrive due to their metabolic needs, leading to an impaired immune response. Indeed, Egebald *et al*. (2008) reported that the migration of T-cells into tumor tissues is dependent on normoxic conditions,

suggesting that an inevitable consequence of tumor cells' hyperproliferative biology may indirectly suppress the antitumoral immune system [22,57].

Although relatively few reports of TMA-assocaited hypoxia-induced immunosuppression have been published, the expression of certain hypoxia-related molecules, such as HIF-1α (hypoxia-inducible factor), has been tentatively linked to T-cell suppression in the TME. Carraro *et al*. (2007) reported that effector T-cells that had been exposed to hypoxic conditions increased the expression of HIF-1α – a transcriptional regulator – which ultimately resulted in apoptosis of the T-cells [58]. However, the results of other studies demonstrated precisely the opposite, reporting that increased expression of HIF-1α in T-cells was associated with prevention of apoptosis [59]. Due to this confusion, focused studies to examine the possibility of TME-associated hypoxia-induced immunesuppression *via* HIF-1α-induced apoptosis are warranted to determine the potential role of TMA-associated hypoxia in the suppression (or the improvement) of ACT-based T-cell infusions [23,56].

3d. Conclusions and potential solutions

Given the various means by which tumor cells attract and subsequently exploit T_{reg} cells to suppress tumor immunity, it is clear that future innovations in ACT-based immunotherapies should focus not only on maximizing concentrations of $CD8^+$ T_c cells, but also on *minimizing* concentrations of T_{reg} cells, depending on a patient's particular cancer. Otherwise, any attempts to therapeutically enhance a $CD8^+$ T_c antitumor immune response may be ineffective. Mockler *et al*. (2015) report that therapeutic suppression of T_{reg} cells alone has not produced significant clinical results; on the other hand, Chodon *et al*. (2015) note that $CD8^+$ T_c-based ACT trials have reported "spectacular" results, although the responses are short-lived due to effector suppression [14,56]. Together, these reports highlight the need to *combine* T_{reg} suppression with $CD8^+$ T_c-

based ACT; however, no such approaches (other than the previously described nonspecific ablation of lymphocytes prior to reinfusion of enhanced T-cells) have been reported.

BROADER IMPLICATIONS

In conclusion, although ACT-based immunotherapy for cancer has demonstrated encouraging results in clinical trials for human cancers, the efficacy of this novel form of anticancer therapy will inevitably plateau in most patients if researchers and clinicians overlook the effects of non-$CD8^+$ T-cell lineages and the challenges of tumor evasion and tumor suppression of T-cell antitumor immunity. Future innovations in ACT-based approaches must address these challenges in order to ensure maximum efficacy of a therapeutically enhanced adaptive immune response to cancer.

Aside from the three problems described above, it is important that any discussion of an anticancer therapy does not fail to address its true goal: the safe and successful treatment of *persons*. A premium should be placed on translational medicine that considers what a patient is to endure at the hands of any therapy that appears to be effective in the lab. What does ACT-based anticancer immunotherapy mean for the patients that receive it? Is it cost-effective? Is it safe? Although these therapies appear to increase survival rates, what is their effect on the *quality* of a patient's life? That is, will ACT-based approaches render patients incapacitated and unable to work or play, as do many chemotherapeutic regimens? Will patients be able to leave the hospital at all? These questions, although not biological in nature, should not be discounted in any project such as this one. As such, this final section considers the broader, person-centered implications of T-cell-based ACT anticancer therapies.

Economic cost

At this time, it is difficult to estimate the costs of ACT-based anticancer immunotherapies due to the fact that all current trials are conducted at academic medical centers; thus, all costs are reimbursed and the recipients of these experimental therapies do not incur charges. However, Perica *et al*. (2015) note that the costs of ACT-based approaches may be comparable to hematopoietic stem cell transplantation (HSCT) fees due to the technical similarities of the two processes. HCST fees range from $36,000 to $88,000 for autologous transplants, and $96,000 to $204,000 for allogenic transplants [13]. Unfortunately, this means that a cancer patient requiring multiple ACT-based-transfusions could quickly amass medical debt that climbs well into the hundreds of thousands of dollars. Such staggering costs are associated with the labor-intensive, technological demands of these therapies. Each ACT-based regimen must be individualized to specifically counteract each patient's respective disease by engineering T-cells to respond to the TAAs that are expressed by a particular patient's tumor cells. Thus, universalization of an antitumoral T-cell response that may be administered to more than one cancer patient does not appear to be within reach [13]. However, it is clear that if translational ACT-based regimens are to be commonplace in the future, emphasis should be placed on reducing their extreme costs in order to maximize accessibility. Emerging strategies to address this issue include the cryopreservation of expanded populations of engineered T-cells so that patients need not undergo extraction of T-cells prior to each course of treatment [14].

Finally, patients may incur economic costs that are extraneous to the ACT procedure itself. The majority of trial patients who receive ACT-based regimens also receive lymphodepleting full-body irradiation and/or chemotherapy, both of which have been reported to drastically improve therapeutic response by obliterating suppressive lymphocytes prior to

reinfusion of modified T-cells. Unfortunately, such procedures require prolonged hospitalization due to the devastating side effects that are generally associated with irradiation and chemotherapy [13,14]. Thus, it is assumed that patients are generally unable to work, thereby incurring additional financial burden.

Side effects and other non-economic considerations

Although relatively few side-effects have been reported in cancer patients undergoing ACT treatments, it is important that patients and clinicians alike be aware of the potential adverse effects that may occur during the course of treatment. The primary side effect that has been reported is cytokine release syndrome, which is characterized by fever, hypotension, and even respiratory insufficiency. This syndrome occurs as the result of extremely elevated levels of T-cell cytokine secretions post-reinfusion. Management of these symptoms includes cytokine receptor antagonists and intensive, supportive hospital care [14]. Additionally, despite efforts to engineer T-cells *ex vivo* to selectively respond to and lyse tumor cells, certain TAAs may be expressed on normal cell populations from which the malignant cells were born, resulting in non-tumor tissue damage; however, such autoimmune reactions are rare and most ACT-trials are reported to be well-tolerated and safe [10,16]. Finally, as mentioned above, many patients experience chemotherapy and irradiation-related symptoms during lymphodepletion prior to T-cell re-infusion. However, ACT-based lymphodepletion is only performed *once* in the majority of patients, rendering it far less abusive overall than traditional repetitive chemotherapy and radiation therapy. As such, the aforementioned therapeutic and economic challenges of current ACT-based anticancer approaches should be given considerable attention by researchers in the hope of making patient-friendly treatment options widely available to patients in the future.

FIGURES

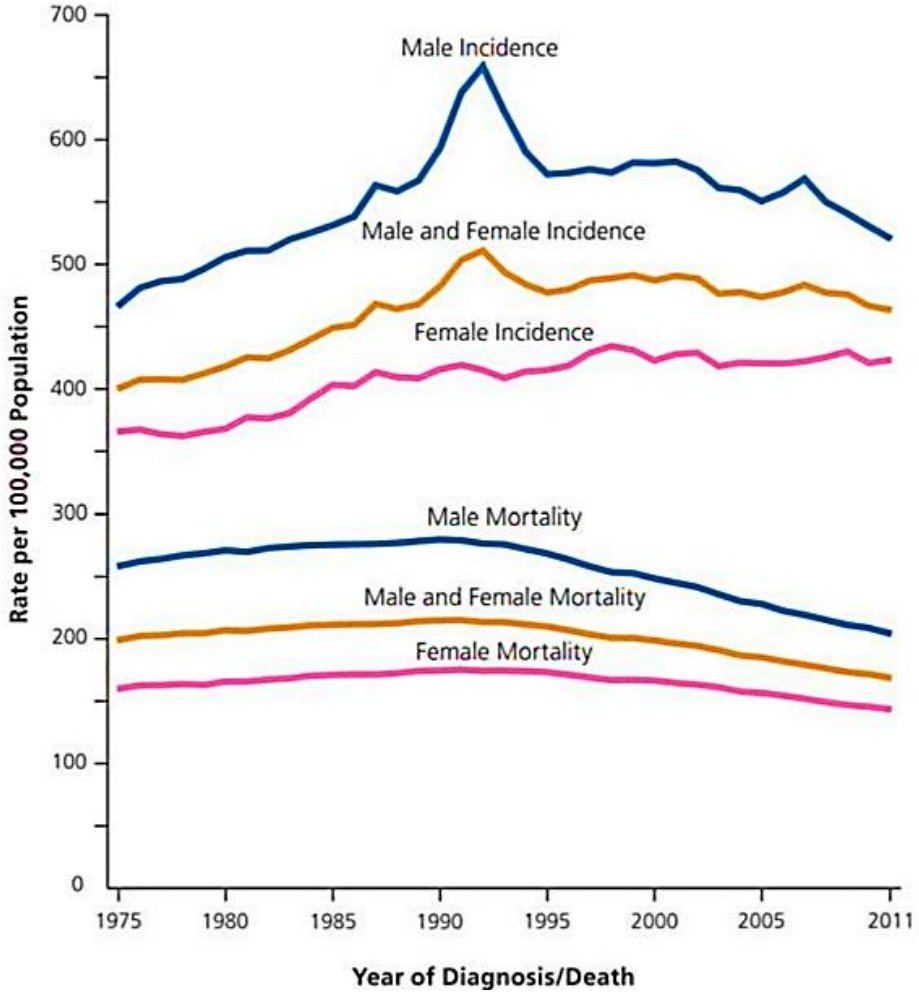

Figure 1. Graph of gender-specific (blue and pink) and overall (orange) rates of cancer incidence and mortality in the United States from 1975-2011. Approximately one cancer death occurs per every three new diagnoses [1].

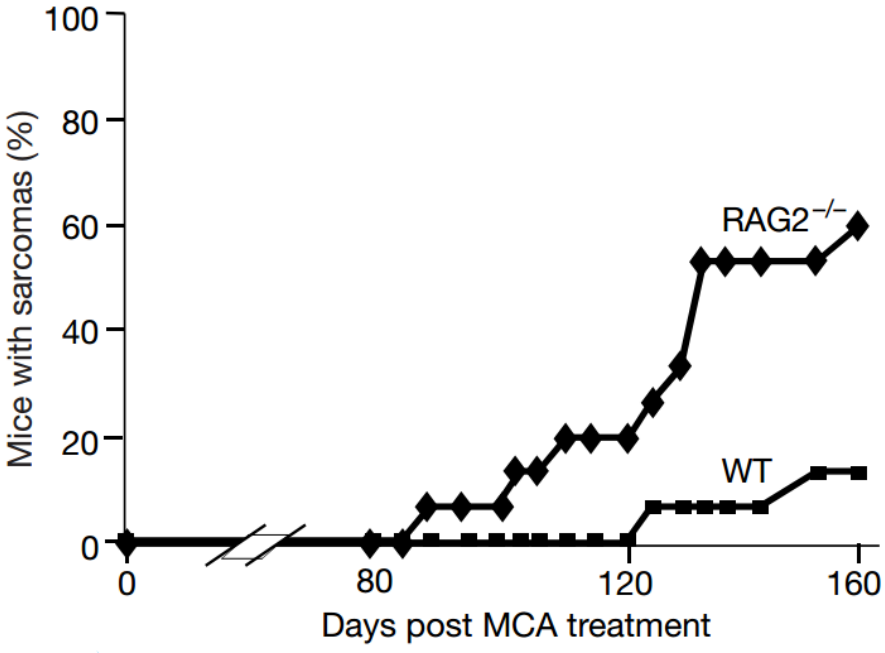

Figure 2. Results of an experiment by Shankaran *et al*. (2001) in which immunocompetent (WT) and immunodeficient ($RAG2^{-/-}$) mice were evaluated for development of malignant sarcomas after exposure to chemical carcinogen methylcholanthrene (MCA) for 160 days. Immunodeficient mice were significantly more prone (~60%) to the development of sarcomas than were immunocompetent mice (~15%), confirming the role of the immune system in the suppression of cancers [5].

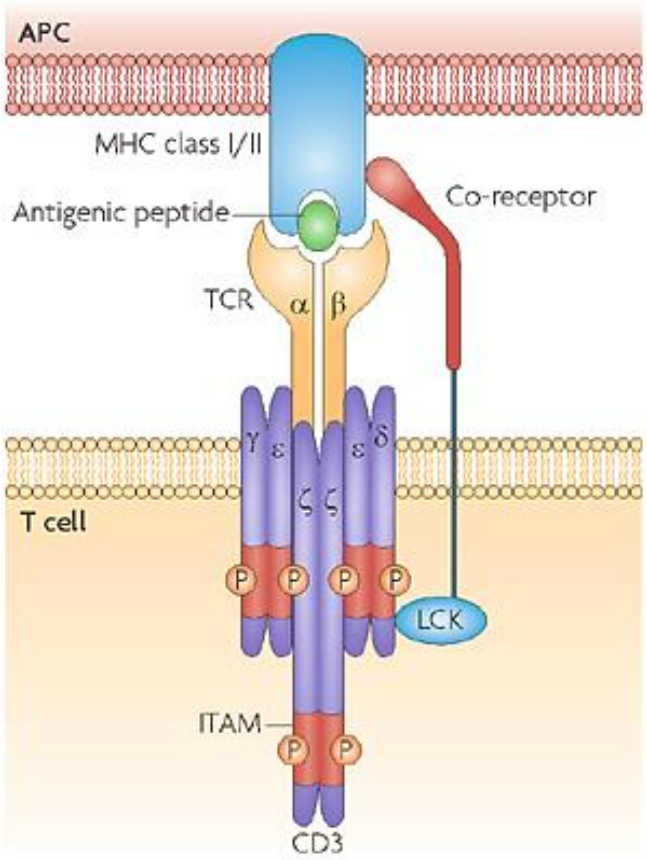

Figure 3. Schematic of a typical T-cell receptor (TCR; shown in yellow/orange in middle) specifically interacting with an Ag:MHC complex (shown at top; antigen is green, MHC is blue). Purple domains of TCR denote structural and intracellular signaling components. "Co-receptor" refers to the CD8 or CD4 protein on the T-cell surface that specifically recognizes the type of MHC proteins [12].

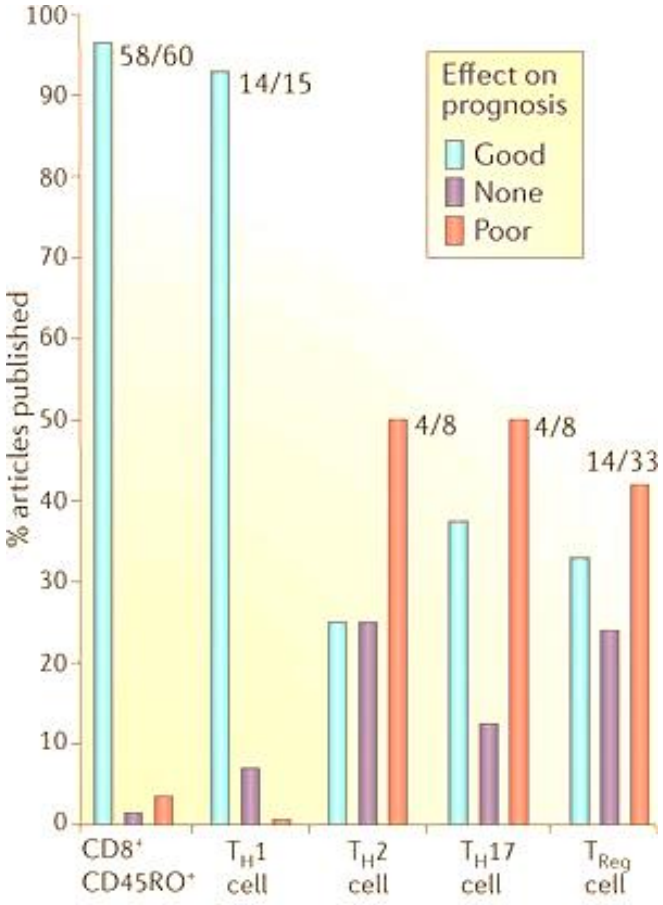

Figure 4. An analysis by Fridman *et al*. (2012) of 124 published articles regarding the prognostic impacts of 5 distinct T-cell lineages: $CD8^+$ T_c ($CD8^+$ $CD45RO^+$), T_H1, T_H2, T_H17, and T_{reg}. Bars indicate respective numbers of articles that reported good (green), poor (orange), and inconclusive (purple) prognostic associations for each lineage of T-cell [19].

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