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Immunotherapeutic strategies to target prognostic and predictive markers of cancer

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Abstract

Throughout the last century medical advances in cancer treatment in the fields of surgery, radiation therapy and chemotherapy have greatly impacted patients' survival rates. Nevertheless, cancer remains a significant cause of mortality, with an estimated 7.6 million deaths worldwide in 2008, reflecting the inability of existing therapies to effectively cure disease. The emergence of vaccines and their successes in preventing the spread of infectious diseases has demonstrated the unique specificity and therapeutic potential of the immune system. This potential has driven the development of novel cancer immunotherapeutics. This review focuses on the current status of the use of immunologic effectors to target known biomarkers in cancer.

Keywords

adoptive cell therapy; antibody conjugate; cancer vaccine; checkpoint inhibitor; chimeric antigen receptor; dendritic cell vaccine; immunotoxin; monoclonal antibody; tumor-infiltrating lymphocytes

A major challenge in curing cancer is the development of treatments that are truly tumor-specific in nature. Such agents could potentially identify and eliminate malignancies without causing damage to healthy surrounding tissues. Current therapies such as surgery and radiotherapy are largely localized in nature and fail to remove occult metastatic disease that, if present, can lead to eventual recurrence and poor patient survival. Alternatively, chemotherapeutics offer a systemic treatment that can potentially treat metastatic disease; however, these are nonspecific, leading to dose-limiting toxicities that may render some treatments ineffective. The process of cancer immunosurveillance has revealed a role for the immune system in recognizing and eliminating malignant cell growth [1]. This has prompted the development of multiple strategies that use immune effectors to target and eliminate

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disease. These strategies include the passive transfer of immune effectors such as targeted-monoclonal antibodies and T lymphocytes, or the activation of endogenous tumor immunity through the use of immunomodulating monoclonal antibodies and cancer vaccines. In that context, this review will examine the current status of clinical applications of cancer immunotherapy and provide insights into future therapeutic developments.

Antibody-based therapy

The lack of truly tumor-specific targets has been a hindrance on antibody-based therapeutics as no biomarker has yet been found to be expressed solely in cancerous lesions. As such, the predominant antigen targets are hallmarks of the tissue from which they arise, albeit at higher antigen-expression levels. However, this increased expression of certain biomarkers within cancer cell populations still allows for a more focused antibody-based delivery of therapeutic agents, as well as the use of immunestimulatory antibodies as an intervention. Current clinical trials are underway that utilize such antibodies to interfere with the upregulated homeostatic processes found in cancer and its surrounding environment, as well as to deliver payloads, which kill cancer cells directly.

Growth factors

Angiogenic—Since Judah Folkman's paradigm-shifting work on the requirement for newly formed vasculature during tumorigenesis [2], there has been much investigation into the pathways and markers required for such environmental remodeling. VEGF is a signaling protein that promotes the formation of new blood vessels upon engagement with the VEGF receptor. Healthy involvement of VEGF can be found during embryonic development [3], recovery from injury and exercise, or other insults to the vasculature that require new growth [4]. As tumors grow they require increased resources that outpace their initial seeding environment and utilize the VEGF pathway to induce blood vessel renovation and recruitment. Inhibiting this pathway could lead to the slowing of tumor progression. The first approved VEGF inhibitor was the humanized monoclonal antibody bevacizumab (Avastin®, Roche, Switzerland) in 2004 for metastatic colon cancer in combination with traditional chemotherapy and 5-fluorouracil regimens. Bevacizumab blocks the binding site for VEGF-A on the cell surface, thereby inhibiting the tyrosineprotein kinase CSK and its downstream effectors, including Raf-1 and MAP kinase-1 that mediate proliferation, migration, invasion and cell survival [5]. This combination approach, which destroys rapidly dividing cells via chemotherapy while preventing the recruitment of supporting vasculature via VEGF inhibition, increases survival by 4.7 months in patients with metastatic colorectal cancer [6]. Additional indications that have found early promise for bevacizumab therapy include cancers of the lung [7], kidney [8], brain [9] and in macular degeneration [10]. Initial hopes for fighting breast cancer were dashed when the US FDA concluded that no increases in survival rates or quality of life were obtained while significant side effects were observed [11]. Such off-target repercussions stem from the broad scope of the drug target, inhibiting VEGF signaling of healthy tissue in addition to VEGF in malignant lesions. Bowel and nasal perforations [12], hypertension and bleeding [13] top the list of adverse effects associated with targeting of such a widely expressed biomarker.

EGF—Another growth factor upregulated in some cancers is the EGF receptor (EGFR), which provides signaling for proliferation, inhibition of apoptosis, migration, adhesion and invasion through the MAPK/KRAS pathway [14]. Stanley Cohen and Rita Levi-Montalcini received the Nobel Prize in 1986 for discovering EGF, whose cell surface receptor has since become a popular target for cancer therapy. Cetuximab (Erbitux®, Bristol–Myers Squibb, NY, USA) and panitumumab (Vectibix™, Amgen, CA, USA), chimeric and fully human antibodies, respectively, inhibit EGFR activation via competitive binding to the surface

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receptors, thereby preventing tyrosine kinase activation and its downstream effects [15]. Again, cancer cells are not the only expressers of EGFR and side effects including acne-like rashes, fever, chills, rigor, urticaria, bronchospasm, angioedema, anaphylaxis and photosensitivity occur [16]. Further dampening enthusiasm for these therapies are mutations in genes, such as *KRAS*[17,18] and *BRAF*[19], present in 40% of colon cancers, which render the use of EGFR monoclonal antibody therapy ineffective as the EGFR pathway is constitutively active, requiring no ligand for activation and is, therefore, unaffected by blockade at the receptor level.

Another humanized monoclonal antibody therapy that targets the EGF pathway, is trastuzumab (Herceptin®, Genentech Inc., CA, USA). HER2, the target of trastuzumab, is a transmembrane protein with 100-fold increased expression in 20–30% of breast cancers [20]. The binding of antibody to HER2 prevents dimerization, which normally activates the PI3K and MAPK pathways [21]. Additionally, binding of humanized antibody to HER2 mediates effector cell cytotoxicity [22]. However, even among the patients with overexpression nearly 70% fail to respond to trastuzumab treatment [23], probably due to the loss of the nuclear expression of the CDK inhibitor p27 [24].

Immunologic checkpoint inhibitors

Alternative strategies to limit tumor growth have focused on more active mechanisms of inducing antitumor immune responses. T lymphocytes, major immune effectors responsible for clearing infections, are tightly regulated by the expression of various stimulatory and inhibitory receptors. The inhibitory receptors act as crucial mediators of self-tolerance, preventing autoimmunity as well as tissue damage in infections producing chronic inflammation [25]. These checkpoint inhibitors are commonly dysregulated in cancer, providing an avenue for tumors to escape immune detection by suppressing antitumor T-cell responses. The use of monoclonal antibodies to block engagement of immunologic checkpoint mediators such as CTLA4 and PD-1, have the potential to relieve this immunological break on T cells in order to enhance T-cell activation and promote antitumor immunity. Indeed, the FDA approved the CTLA4-blocking antibody ipilimumab (Yervoy™; Bristol–Myers Squibb) in 2011 for the treatment of late-stage melanoma. When combined with a gp100 peptide-based melanoma vaccine ipilimumab increased survival in patients with late-stage melanoma by 4.4 months compared with patients receiving the vaccine alone [26]. Antibodies targeting the PD-1 inhibitory pathway are currently in earlystage clinical trials and have produced objective regressions in 18% of patients with non-smallcell lung cancer, 28% of patients with melanoma and 27% of patients with renal carcinoma [27].

Antibody conjugates

Rather than targeting immunologic pathways corrupted in cancer, another approach delivers toxic payloads to cancer cells themselves, with monoclonal antibodies as the site-specific conveyor. Preclinical and Phase I/II clinical trials have shown great promise in equipping monoclonal antibodies with cytotoxicity rather than relying on their ability to interfere with survival pathways or recruit immune system effectors, by giving them a lethal payload [28]. Conjugates of antibodies and toxins commonly derived from plants or bacteria (immunotoxins) have evolved since their inception. The coupling of whole antibody to holotoxin gave way to more sophisticated linkages that utilize just the enzymatic chain of the toxin, which is released from the antibody upon endocytosis, avoiding the off-target effect conferred by the binding region of the toxin [29]. The newest generation of immunotoxins employs recombinant DNA technology to genetically fuse the binding domain of an antibody to the enzymatic domain of a toxin [30], while replacing the epitopes that could lead to immunotoxin-specific immune responses and unwanted clearance or neutralization of the therapy [31]. Denileukin diftox (Ontak®, Ligand Pharmaceuticals, CA,

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USA), the only immunotoxin in clinical use, makes use of such technology to deliver the cytotoxic action of diphtheria toxin to T-cell lymphomas, which express the IL-2 receptor. The first interleukin molecule, characterized in 1965 by Gordon and Maclean [32], IL-2 is required for normal proliferation and differentiation of T cells into their effector $CD4^+$ and $CD8^+$ T-cell populations generated during immunological response, in addition to supporting thymic T-cell maturation. In that way, IL-2 is mitogenic for lymphocytes via the Ras/MAPK, JAK/Stat and PI3K/Akt pathways [33]. Specificity still remains elusive as denileukin diftox may also lead to adverse effects such as capillary leak syndrome, fever, chills, fu-like symptoms and other symptoms characteristic of the diphtheria toxin itself [34].

These classic plant toxin payloads, serving as proof of principle, have provided support to deliver almost any desirable payload. Brentuximab vedotin (Adcetris®, Seattle Genetics, WA, USA), a monoclonal antibody targeted against CD30 that is used to deliver monomethyl auristatin E, a synthetic antineoplastic agent, was approved by the FDA in 2011 for the treatment of anaplastic largecell lymphoma and Hodgkin lymphoma [35]. Additional conjugates currently in late phase trials include trastuzumab conjugated to emtansine (T-DM1) [36], a derivative of a plant toxin that inhibits microtubule assembly [37] and inotuzumab ozogamicin, a CD22-targeted antibody that delivers the calicheamicin antibiotic from bacteria [38].

Even further in the future, but well within realization, are engineered payloads such as liposomes or polymeric micelles. Liposomes are lipid bilayers that given the proper linkage to an antibody, could serve to deliver doxorubicin or other drugs encapsulated within [35]. Polymeric micelles, or amphiphillic block copolymers, are yet another way to create a nanoscale shell in which drugs can be encapsulated or additionally covalently attached to the surface, providing further options for attachment and delivery [39]. Both strategies of nanoscale material engineering theoretically serve to cloak the payload from the systemic environment until it can reach the target of interest where it can then release its contents, thereby reducing the off-target effects that direct conjugates suffer. The advances in payload technology are the next step required to move the field forward.

Antibody-based therapies: summary

Antibodies as carriers of toxins or acting as inhibitors themselves have been around for some time, but have not yet hit their stride. Altering conjugation techniques as well as varying payloads offers great hope in tailoring the effector moiety to the natural function and conditions of each specific receptor. At the forefront of such manipulation is recombinant DNA technology and engineering payload strategies providing precision adjustments beyond the advantages offered by naked monoclonal antibodies. Seemingly, all that remains is the correct target–payload combinations with sufficient affinity and specificity for this field's potential to be fully realized.

Vaccines

Translation of prognostic and predictive markers of disease into vaccine targets has seen the greatest adoption in cancer immunotherapy. Much of the enthusiasm for employing vaccines in cancer results from the unique ability of the immune system to produce ‘memory’. Immunologic memory is maintained by a small population of memory cells that respond with enhanced speed and magnitude upon re-encountering an infectious disease, providing life-long protection against the pathogen. Generation of immune memory could protect against cancer recurrence for years after immunization.

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Our immune system evolved during a time when human life expectancy was insufficient to make cancer a primary cause of death. The most common forms of cancer (lung, breast, prostate and colorectal) typically occur in individuals >50 years of age. Thus, infectious diseases, but not cancer, have shaped the evolution of our immune system and cancer immunotherapy aims to manipulate a system suited for fighting pathogens to one capable of eradicating cancer. While challenging, progress has been made in several areas employing biomarkers as cancer vaccine targets. The most commonly used biomarkers in cancer are serum proteins shed by the growing malignancy; these are used to detect and monitor the progression of various cancers including colorectal, prostate, gynecologic and other epithelial cancers. Ideal target antigens for cancer vaccines are disease specific, universally associated with the disease and sufficiently immunogenic to induce robust immune responses that are capable of eliminating cancer cells without affecting normal tissues [40]. While no antigen fits this ideal scenario, several biomarkers have been studied as vaccine targets and translated to cancer patients with some success.

Colorectal cancer

CEA (or CEACAM5) is a membrane glycoprotein that was discovered in 1965 [41]. At the time it was hypothesized that CEA was expressed during fetal development and in cancers, but not in adult tissues. However, it is now known to be expressed by various human tissues. CEA is one member of the large CEACAM family of proteins [42]. The small hydrophobic C-terminus of CEA is modified to create a glycosylphosphatidylinositollinked membrane anchor. This anchor is readily cleaved by phosphoinositol-specific phospholipases C and D, releasing a soluble CEA molecule. Importantly, the liberated CEA can be detected in sera of colorectal cancer patients and is elevated in the serum of about half of these patients. However, because of very high false-positive and false-negative rates, serum CEA measurement does not have utility as a routine screening diagnostic in the general population. By contrast, since the 1970s, serial CEA measurements have been used in colorectal cancer patients presumed to be cured surgically to detect tumor recurrence [42]. Relatively modest elevations in serum CEA levels are predictive of disease recurrence up to 1 year before the onset of clinical symptoms [43].

CEA is generally thought to be restricted to secretory and absorptive epithelia including that of the colon, stomach, tongue, esophagus, cervix, sweat glands and prostate, although it can be detected in other tissues including the bladder, spleen, lymph nodes and blood [44, 45]. A variety of vaccine strategies targeting CEA have been employed in patients, such as dendritic cell vaccines, DNA vaccines and viral vector vaccines. Administration of dendritic cells loaded with CEA epitope peptides resulted in cellular and humoral responses in some patients [46]. In other studies, plasmid DNA or recombinant viral vectors failed to induce clinical responses, although CEA-specific immune responses were detected [47–50]. However, combining viral vector approaches to create prime–boost regimens generated cellular responses and disease stabilization in some advanced colorectal cancer patients [51]. A significant advancement in CEA vaccines was achieved by the incorporation of a triad of costimulatory molecules, including B7–1, ICAM-1 and LFA-3 (TRICOM), within the viral vector to increase CEA-specific immune responses and clinical responses [52,53]. Moreover, a prime–boost regimen combining different CEA–TRICOM-expressing viral vectors induced CEA-specific T-cell responses and prolonged disease stabilization in a majority of patients [54].

While CEA has been utilized as a colorectal cancer biomarker for four decades, GUCY2C is an emerging prognostic biomarker in colorectal cancer [55]. GUCY2C is a membrane-spanning receptor of the guanylyl cyclase family [56]. It is the receptor for the paracrine hormones guanylin and uroguanylin, and the bacterial diarrhea genic heat-stable enterotoxin, ST. Following ligand engagement, GUCY2C converts GTP to the second messenger cyclic

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GMP that activates numerous down stream signaling pathways that regulate intestinal physiology and pathophysiology [57]. Importantly, GUCY2C has been detected in >500 normal intestinal samples, but not in >1000 nonintestinal tissue samples [55, 58 – 61]. Moreover, GUCY2C expression is maintained during colon carcinogenesis and GUCY2C protein and mRNA have been detected in >95% of primary and metastatic human colorectal cancer specimens [55,58–66]. These observations suggest that GUCY2C may be a useful biomarker of metastatic colorectal cancer, a hypothesis that was confirmed in a large, multicenter prospective clinical trial examining >2500 specimens from 257 patients [67].

In the context of enterocyte-selective expression and universal expression in metastatic colorectal cancer, a recombinant adenoviral vector possessing GUCY2C (Ad5-GUCY2C) was generated as a candidate vaccine for colorectal cancer [68]. Immunization of mice with a mouse GUCY2C-expressing vaccine induced GUCY2C-specifc $CD8^+$ T-cell responses and extended the survival of mice with colorectal cancer metastases in lung and liver. Importantly, this occurred in the absence of autoimmunity, suggesting that Ad5-GUCY2C is both safe and effective. Currently, Ad5-GUCY2C is poised for clinical evaluation in patients during a Phase I clinical trial in early-stage colorectal cancer patients scheduled to begin in 2013.

Prostate cancer

There are two prominent biomarkers expressed by prostate cancer, PSA and PAP. PSA is a serine protease from the kallikrein family of proteases. Importantly, only prostate epithelial cells express PSA and secreted PSA can be measured in the sera of prostate cancer patients. In addition to digital rectal exams, serum PSA measurement is useful as a prostate cancer diagnostic and in monitoring prostate cancer progression. PAP, discovered in 1938, was the first biomarker with utility in diagnosing cancer [69]. PAP was widely used in the diagnosis, prognosis and monitoring of prostate cancer from the 1950s to the 1980s [70]. However, upon the discovery of PSA and the comparison of PSA and PAP testing, PAP fell out of favor primarily due to its considerably lower sensitivity than PSA [71]. However, both PSA and PAP have been utilized in prostate cancer vaccines. Similar to CEA, the viral vector delivery of PSA and TRICOM (PROSTVAC®-VF [Bavarian Nordic, Denmark]–TRICOM) has been employed to treat prostate cancer patients. In a recent Phase II clinical trial [72], 82 patients received PROSTVAC-VF while a control cohort of 40 patients received control vectors. Overall survival 3 years after vaccination was better in the PROSTVAC-VF group (30%) than controls (17%), increasing median survival by 8.5 months. Importantly, the vaccine was also well tolerated, while reducing the death rate to 44%. TRICOM-based vaccines have had the greatest success among viral vector vaccine approaches in cancer, although none are currently FDA-approved. To date, TRICOM-based vaccines in prostate and colorectal cancer remain experimental, but ongoing efforts could lead to their approval and adoption in patients.

As of yet, the only FDA-approved cancer vaccine targets PAP for the treatment of advanced prostate cancer [73]. The vaccine, sipuleucel-T (Provenge®) manufactured by Dendreon (WA, USA), consists of enriched monocytes collected from the prostate cancer patient and exposed *ex vivo* to a fusion protein of PAP and granulocyte-macrophage colony-stimulating factor. Granulocyte-macrophage colony-stimulating factor aids in PA P delivery to monocytes and induces their maturation to optimize T-cell activation. These cells are then administered to the patient where they migrate to lymphoid organs to activate PAP-specific T cells. In the Phase III IMPACT trial of 512 patients with metastatic castration-resistant prostate cancer, treatment with the vaccine improved median overall survival by 4.1 months compared with the placebo and reduced the risk of death by 22% [74]. While those results are modest, sipuleucel-T has established a model for successful translation of cancer vaccines to patients and it sets the stage for FDA approval of future cancer vaccines.

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Vaccines: summary

Several successful Phase II and III clinical trials, including those described above, suggest that the cancer vaccine community has obtained a sufficient understanding of the mechanisms regulating vaccine efficacy and optimal vaccine design strategies to begin giving patients the long-awaited benefit these approaches have promised. However, beyond cancer, vaccines may have utility in treating other chronic diseases of noninfectious origin. These include Alzheimer's disease, heart disease, Type 1 diabetes and others. The immunologic nature of the mechanisms underlying these diseases is becoming clear and immunologic approaches are being developed for their treatment.

Adoptive cell therapy

The ability of cancer vaccines to induce tumor-specific T-cell responses has prompted the development of adoptive cell therapy (ACT). ACT involves the *ex vivo* expansion of autologous tumor-specific T lymphocytes and the subsequent reintroduction of these cells into patients to promote antitumor therapy [75]. ACT is particularly advantageous over existing immunotherapies as it permits introduction of large numbers of tumor-specific immune cells into patients. Furthermore, lymphodepletive conditioning regimens, including various chemotherapeutics and low-dose total-body irradiation administered prior to T-cell transfer eliminates immunosuppressive tumor microenvironments, and increases production of the homeostatic cytokines IL-7 and IL-15 that facilitate additional expansion of transferred T cells *in vivo*[76,77]. In addition, advances in genetic engineering have permitted the introduction of antigen-targeted receptors of desired specificity as well as genes that enhance T-cell potency [78,79] and tumor trafficking [80, 81]. Antigen-specific receptors may be natural T-cell receptors (TCRs) or a class of engineered single-chain molecules known as chimeric antigen receptors (CARs) [82]. CARs provide T cells with antibody specificity by fusing an extracellular antigen-binding domain derived from a monoclonal antibody, to intracellular domains derived from T-cell activation receptors such as CD3ζ, CD28 and 4-1BB [83]. CARs provide the advantage of targeting any native cell-surface antigen to which an antibody can be generated in a MHC-independent manner, allowing broad application of ACT irrespective of MHC haplotype. ACT has demonstrated successes in eradicating large well-established melanomas and leukemias and a multitude of prognostic disease markers are currently under investigation as ACT targets for a wide range of cancers [84, 85].

Melanoma

Initial studies with ACT utilized tumorinfiltrating lymphocytes (TILs) from surgically resected tumors in patients with metastatic melanoma. T cells in the TIL population can be expanded *in vitro* via coculture with patient tumors in the presence of the T-cell growth factor IL-2 [86]. Transfer of these T-cell products back into patients results in objective response rates in 72% of patients and complete regressions in 40% of patients when combined with lymphodepleting regimens [85]. Analysis of TILs in melanoma has revealed the presence of T cells specific for melanoma differentiation antigens such as MART-1 and gp100 [87]. MART-1 and gp100 are expressed by 89 and 76% of melanomas, respectively, and are found in normal healthy melanocytes in the body [88]. TCRs specific for these antigens have been isolated from TILs as well as generated in transgenic mice that express human *HLA-A2 MHC* genes. T cells engineered to express MART-1- and gp100-specifc TCRs produce objective responses in 30 and 19% of patients, respectively [89]. However, these T cells also recognize and destroy normal melanocytes. Indeed, 55% of patients receiving MART-1-specifc T cells, and 25% of patients receiving gp100-specific T cells developed anterior uveitis requiring the administration of steroid eye drops [89]. Interestingly, patients treated with expanded TIL products experienced anterior uveitis in

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only 6.5% of cases potentially indicating that antigen specificities other than melanoma differentiation antigens are mediating this antitumor effect [89]. Indeed, T cells specific for mutated antigens have also been identified in TIL products [89].

Cancer–testis antigens

Cancer–testis (CT) antigens are a class of antigens that are upregulated in various cancers whose normal expression is limited outside the immunoprivileged testes [90]. CT antigens are particularly attractive targets for this reason because, unlike cell-differentiation antigens, CT-specific immune responses do not pose the risk of developing autoimmune toxicities against normal healthy tissues. One CT antigen, NY-ESO-1, is expressed by 10–50% of melanomas, 80% of synovial cell sarcomas and to a lesser extent in various other tumors including cancers of the bladder, esophagus, liver and lung [91]. T cells engineered to express NY-ESO-1-specifc TCRs produced objective responses in five out of 11 patients with melanoma as well as four out of six patients with synovial cell sarcoma [92]. Importantly, objective responses in these patients occurred in the absence of autoimmune toxicities [92]. Other CT antigens used as targets for ACT include MAGE-A3 and SSX-2 and are currently in early stages of clinical development [93,94]. MAGE-A3 is of particular interest for its broad expression pattern in melanomas as well as in tumors of epithelial and leukemic origins [94]. T cells expressing MAGE-A3-specifc TCRs produce antigen-specific killing and IFN-γ cytokine production when cocultured with melanoma and non-small-cell lung cancer cell lines *in vitro*[94].

Leukemia & lymphoma

The use of antibody-based CARs for ACT has allowed for a more universal approach in targeting tumor-antigens since one CAR can potentially be used across all patients for a particular antigen due to their independence from MHC restriction. The majority of CARs target cell surface differentiation antigens and as such pose the risk of causing autoimmune toxicities against normal antigen-expressing tissues. To date, cancers of leukemic origin have been the most widely targeted in CAR ACT, with CD19 being the most frequent target. CD19 is a marker of mature B cells and its expression is maintained in B-cell leukemias. CD19-specifc CARs were first shown to be effective in promoting a partial remission in a patient with follicular lymphoma with a concomitant reduction in normal B cells [95]. A following report demonstrated six out of eight patients treated with CD19-specifc CARs experienced objective responses, one of which experienced a complete response as measured by the RECIST criteria [96]. Half of the treated patients experienced reductions in normal B cells [96]. In a separate study, three out of three patients with chronic lymphocytic leukemia treated with CD19-specific CARs experienced a reduction in tumor burden of at least 1 kg, two of which were complete responders [84]. Each patient also experienced B-cell aplasia and it was estimated that each introduced CAR-expressing T cell contributed to the elimination of 1000 leukemic cells [84]

Translation to other cancers

The success of ACT in the treatment of melanoma and B-cell leukemias has galvanized efforts to treat cancers of various histological origins. Expanding from initial success in cancer vaccines, CEA has been an active target for ACT. T cells engineered to express a CEA-specific TCR have been introduced into patient T cells for the treatment of metastatic colorectal cancer [97]. All three patients experienced a transient reduction in serum protein levels of CEA, one had a reduction in tumor burden of 17% and another experienced a 49% reduction; however, in each case the disease eventually progressed [97]. In addition, all three patients experienced severe transient colitis that resolved after 2–3 weeks [97]. Her2 is another frequent target for cancer immunotherapies. It is over-expressed in approximately 30% of breast cancers and can also be found in approximately 10% of gastrointestinal

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cancers of epithelial origin [98]. A patient with Her2-expressing colorectal cancer was treated with T cells expressing Her2-specific CARs [99]. The patient experienced respiratory distress within 15 min of infusion and eventually died of cardiac arrest 5 days after treatment [99]. Death was attributed to a cytokine storm resulting from T-cell recognition of low levels of Her2 on normal lung epithelial cells [99]. CAR T cells have also been used to treat renal cell carcinoma. Here the targeting of carbonic anhydrase-9, which is expressed on >95% of clear-cell renal cell carcinomas [100], resulted in acute liver toxicities attributed to low levels of expression of carbonic anhydrase-9 on bile duct epithelial cells [101]. Results from these different antigen targets demonstrate that while transferred T cells can have potent antigen-specific reactions, it is important to select antigen targets that produce minimal or 'acceptable' toxicities, such as the case with CD19. Another antigen target that has had success in the clinic is disialoganglioside, an antigen expressed by nearly 100% of neuroblastomas [102]. Disialoganglioside-specific CAR-expressing T cells induced the complete remission of tumors in three out of 11 patients with active neuroblastoma [103]. This occurred in the absence of toxicities, again demonstrating the importance of antigen selection when employing ACT in the clinic [103].

Enhancing ACT efficacy

Genetic engineering approaches have also facilitated the introduction of genes to increase T-cell potency. Modification of T cells to express the chemokine receptors CCR4 or CCR2 have shown increased trafficking to tumors that produce their respective ligands. Transduction of these chemokine receptors into CAR-expressing T cells has shown increased trafficking and antitumor activity in preclinical mouse models targeting lymphomas, neuroblastomas and mesotheliomas [80,81,104]. T cells can also be engineered to express cytokines such as IL-12. IL-12 is a potent inflammatory cytokine that has the potential to restructure immunosuppressive tumor microenvironments that limit antitumor immunity; however, it is highly toxic when delivered systemically [105]. The introduction of an inducible form of IL-12 into T cells, which is only produced after the T cell comes into contact with its cognate antigen, ensures production of IL-12 at local tumor sites, limiting toxic side effects from systemic administration. CAR T cells expressing IL-12 have been demonstrated to drastically increase antitumor activity compared with T cells with CAR alone [106]. Alternatively, genes can be introduced that directly counteract tumor-induced immunosuppression of T cells. TGF-β is a cytokine produced by many tumors that is able to directly inhibit antigen-specific cellular immune responses. Introducing a dominant negative form of the TGF-β type II receptor into T cells, renders them resistant to TGF-β-mediated suppression and enhances antitumor efficacy in preclinical mouse models [107,108]. Furthermore, introduction of chimeric costimulatory receptors that fuse the extracellular domains of T-cell immune checkpoint inhibitors CTLA4 or PD-1 to the intracellular domain of the activating costimulatory receptor CD28 are able to redirect tumor-induced inhibitory signals to promote T-cell activation [109,110].

ACT: summary

ACT has demonstrated success in the treatment of melanoma, B-cell leukemias and neuroblastoma. Success in the treatment of other cancers has been limited, in part due to on-target, off-tumor toxicities. There are a variety of AC T antigen targets in development, including those for additional targets in leukemia [111,112], prostate cancer [113], mesothelioma [114] and ovarian cancer [115]. The success of these antigen targets will depend on the ability of these cells to induce antitumor activity in the absence of life-threatening toxicities. Proper dose-escalation strategies should be executed in initial clinical trials to ensure patient safety [116]. Alternatively the introduction of suicide genes into transferred T cells, such as herpes simplex virus thymidine kinase or inducible caspase 9, may impart an additional layer of safety by allowing the elimination of transferred T cells if

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toxicities occur [117]. Despite issues with antigen-specific toxicities, the development of novel antigen targets with confirmed safety, or the development of mechanisms to preserve antitumor efficacy while limiting toxicity, will probably provide different avenues for ACT success.

Future perspective

The FDA approvals of Bristol–Myers Squibb's ipilimumab and Dendreon's sipuleucel-T have set the stage for future cancer immunotherapeutics [118]. Unlike previous cancer targets, these are the first FDA-approved drugs that promote active modulation of the endogenous immune system in order to promote antitumor immunity. The recent partnership between the University of Pennsylvania (PA, USA) and Novartis (UK) to create the Center for Advanced Cellular Therapies (University of Pennsylvania), focused on the development of CAR-based ACTs, ensures that ACTs will be aggressively pursued in the near future [201]. Despite a promising outlook, significant hurdles remain for the development of these therapies. One major limiting factor of immunotherapy is the lack of truly tumor-specific antigens, particularly for epithelial cancers. As evidenced with many ACT treatments, the targeting of tumor-associated self-antigens results in on-target, off-tumor toxicities. While these toxicities may be alleviated with suicide genes, the future of immune therapy will rely on the identification of novel targets that do not pose such risks. Another potential hurdle for immune therapies is the high cost of drug manufacturing. Unlike chemical entities used in chemotherapeutics, immunotherapeutics are derived from biological materials and are therefore intricately more complex and exponentially more expensive to produce. The personalized nature of cellular therapeutics seen in sipuleucel-T and ACTs, which require a custom-made product for each individual patient, will further increase production costs. Continued success of these therapies is likely to spur active investigation into methods for optimizing cellular production strategies in order to maximize therapeutic potential in a cost-effective manner [119]. Considering the current production limitations, greater clinical efficacy demonstrating increases in survival over existing treatments will be necessary to justify these therapies. Another significant limitation of immune therapies is the presence of intricate immunosuppressive microenvironments within large, well-established tumors that counteract antitumor immunity [120]. Tumors corrupt the endogenous immune system and promote the differentiation of myeloid-derived suppressor cells and regulatory T cells, both of which are capable of inhibiting immune responses by the production of anti-inflammatory cytokines [120]. One major advantage of ACT has been the global elimination of immune cells prior to T-cell transfer with chemical or radioactive conditioning regimens, which effectively eliminate these immunosuppressive cells [77]. However, it is absolutely essential for the endogenous immune system to remain intact when utilizing immune-modulating antibodies and cancer vaccines. As such, the future of these therapies is likely to require combination approaches that act to relieve immunosuppression while preserving immune effectors in order to maximize therapeutic potential. In essence, immunological checkpoint inhibitors such as ipilimumab provide one potential mechanism to circumvent tumor-initiated tolerance. Additional use of non-myeloablative chemotherapy or local radiation therapy have been demonstrated to promote ‘immunogenic cell death’ of tumors, which may temporarily reverse tumor immunosuppression and promote synergistic effects when combined with checkpoint inhibitors or cancer vaccines [121,122]. The success of antibody-based therapeutics, cancer vaccines and ACTs in clinical development provides substantial evidence that cancer immunotherapy is emerging from its infancy and will soon become a mainstay in cancer therapeutics.

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Executive summary

Immune therapy

- The ability of the immune system to recognize and eliminate targets with high specificity make it a promising tool in cancer therapy.

Monoclonal antibody therapy

- Monoclonal antibodies can be used to block tumor angiogenesis by targeting VEGF-mediated growth of blood vessels.
- Monoclonal antibodies can be used to directly block EGF signaling.
- Immune checkpoint inhibitors, which target T-cell inhibitory molecules such as CTLA4 and PD-1, have the potential to relieve T-cell tolerance and promote T-cell-mediated destruction of tumors.
- Immunological factors can be conjugated to toxic payloads including toxins and chemotherapeutics, which can mediate selective elimination of targeted tumors.

Vaccines

- Cancer vaccines induce active immune effector responses from the patient's endogenous immune system.
- Viral-based vaccine vectors encoding the tumor antigens CEA and PSA, in combination with TRICOM costimulatory molecules, have promoted antigen-specific immune responses.
- Cellular vaccines, such as the US FDA-approved sipuleucel-T, have increased survival in patients with advanced prostate cancer.

Adoptive cell therapy

- Adoptive cell therapy (ACT) expands large numbers of tumor-specific autologous patient lymphocytes ex vivo that are intended for passive transfer into patients.
- Lymphodepleting conditioning regimens with low-dose chemotherapy or total-body irradiation prior to T-cell transfer assist ACT by removing endogenous immunosuppressive cells in the tumor microenvironment and by facilitating the increased expression of the homeostatic cytokines IL-7 and IL-15 that promote engraftment and expansion of transferred cells.
- Expanded tumor-infiltrating lymphocytes have successfully promoted dramatic regressions of tumors in patients with melanoma.
- Genetic engineering approaches permit the generation of T-cell products with T-cell receptors or antibody-based chimeric antigen receptors of desired specificity.
- Potential for on-target, off-tumor toxicities demonstrates the need to be selective in tumor-antigen targets, employing only those with low expression outside the tumor, such as cancer-testis antigens, or found in expendable tissues, such as CD19 expression in B cells.

Future perspective

- FDA approval of the immune therapies ipilimumab and sipuleucel-T has provided promise for the future of cancer immunotherapeutics.

- Limited efficacy of immune-modulating antibody and cancer vaccine monotherapies will probably lead to the use of combination therapies that may also utilize radiotherapy and chemotherapy for the development of synergistic immune activation.
- In order to translate ACT treatments into a broader range of patients, novel antigen targets, particularly for solid tumor malignancies, will need to be identified in the future.
- Streamlined manufacturing processes will need to be developed in the future in order to make immunotherapies a cost-effective option for the treatment of advanced cancer.

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