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ESSENTIALS The development of a cancer in an immunologically intact host leads to an interaction between the host immune system and the tumour mass. The three phases of tumour/host interactions (Elimination, Equilibrium, and Escape) form the ‘immune editing hypothesis’, which serves as a valuable framework for understanding of the im- mune response to cancer and the approaches by which this might be manipulated for therapeutic benefit. Many immune-oncological strategies have been and are being de- veloped, including (1) cancer vaccines; (2) chimeric antigen receptor T cells; (3) T-cell redirecting engineered antibodies; (4) blocking of the immune checkpoint molecule cytotoxic T lymphocyte antigen- 4; (5) blocking of the immune checkpoint molecule programmed death-1; (6) immune agonist approaches; and (7) immunotherapy combinations. Immunotherapy is emerging as an important treatment modality for many tumour types, including melanoma, lung cancer, kidney cancer, lymphoma, and bladder cancer. By the time you read this chapter it is highly likely that additional monotherapy and com- bination regimens will be approved in multiple tumour types, but an understanding of the basic mechanisms underlying an adaptive antitumour immune response will be valuable in understanding fu- ture agents, as well as their toxicities. Introduction Immunotherapy is emerging as an important treatment modality for many tumour types, including melanoma, lung cancer, kidney cancer, lymphoma, bladder cancer, and several others. This chapter will first provide some general background on the immune system, with the goal of providing a basic scientific framework for under- standing cancer immunity and immunotherapy. It will then provide a very brief description of cytokine therapy, mostly for historical context, following which discussion will move on to cancer vaccines (reviewing one successful and several not so successful approaches) and the use of T cells bearing chimeric antigen receptors (CAR T cells), an exciting technology that is being evaluated in later stage clinical trials. Following that, there will be a brief introduction to a rapidly developing field of immunotherapy involving bi-specific antibodies designed to localize a patient’s endogenous T cells to tumours. Consistent with recent clinical data and interest, discus- sion will then delve more deeply into the notion that the immune response to cancer is attenuated by a series of molecules known as immune checkpoints, reviewing clinical data showing that immune checkpoint blockade can result in meaningful clinical responses in patients with several tumour types. Finally, there will

be a brief account of combination immunotherapy approaches, providing a perspective regarding ongoing and future development. It should be noted that there are a multitude of approaches to cancer immuno- therapy; to adhere to space limitations this account will focus on active immunotherapy approaches that have either achieved regula- tory approval or are the subject of large-scale phase II and III testing. Basics of the immune system relevant to cancer immunity The innate immune system Macrophages and neutrophils For didactic purposes, the immune system is often divided into innate and adaptive arms, both of which play a role in cancer im- munity and immunotherapy. Evolutionarily, the innate immune system is the older of the two; it is present in all vertebrate organ- isms. The innate system recognizes its targets via repeated molecular patterns typically associated with pathogens; these molecules are collectively known as pathogen-associated molecular patterns or PAMPS. Recognition of PAMPs is mediated by a series of receptors known as pattern recognition receptors or PRRs. Although there are many classes of PRRs, many of these are closely related to Toll molecules in drosophila, and are referred to as Toll-like receptors or TLRs. The interaction between PAMPs and their receptors is crit- ical to an organism's recognition of 'danger', and serves to initiate a rapid, but relatively nonspecific immune response. Although PRRs are expressed by many cell types in the immune system, the initial innate immune cell that responds to an invading pathogen is often a tissue-resident macrophage. These cells derive their name from the Greek makros (large) and phagos (to eat); macrophages are large cells which evolved to engulf and destroy pathogens. Recognition 5.4 Cancer immunity and immunotherapy Charles G. Drake

 of PAMPs by tissue-resident macrophages leads to their activa- tion and secretion of chemical messengers known as cytokines and chemokines, which recruit and activate additional immune cells in- volved in controlling a local infection. These secreted cytokines also attract a second cell type of major importance in the innate immune system, the neutrophil (also known as a PolyMorphoNuclear cell, or PMN). PMNs are the most abundant immune cell in the peripheral circulation, comprising approximately 60% of the white cells in the blood. Neutrophils cells have a half-life measured in hours in the peripheral blood, but can survive for days when present in the tissue at a site of infection or inflammation. There, PMNs are the major cellular constituent of pus, and the hallmark of acute inflammation. Like macrophages, neutrophils can synthesize a variety of secretory granules which are released upon PAMP recognition, and which function in the elimination of an invading pathogen. Paradoxically, inflammation driven by the cells of the innate immune system is often subverted to promote tumour cell survival and outgrowth, although a detailed description the mechanisms underlying those effects is beyond the scope of this more clinically oriented chapter. For fuller discussion of the innate immune system, see Chapter 4.1. Cytokines and chemokines Cytokines and chemokines are chemical messengers used by cells in the immune system to communicate with each other, and these molecules are also important in communication with surrounding tissues. There are many such molecules, with a nomenclature that is daunting to nonimmunologists. Nevertheless, they have a crit- ical role in acute and chronic inflammation, in the innate immune response, and in the adaptive immune response to cancer, hence understanding a few prototypical cytokines is critical. In fact, it should be noted that the term cytokine is a very general one, often used to delineate nearly any small molecule with immunological relevance. Since many of these molecules are involved in the mi- gration of cells, the name derives from 'cyto'—cell, and movement 'kinesis'. Typical cytokines include the type I interferons (IFN-α and IFN-β), which are synthesized by epithelial cells under stress, typ- ically in response to viral infection. Immunologically, type I inter-

ferons render epithelial cells (including tumour cells) more sensitive to immune attack, increasing their recognition by cells of the adap- tive immune system, and also by directly promoting programmed cell death. The term ‘chemokine’ refers to a set of cytokines which direct the migration of immune cells along a concentration gradient. A proto- typical example is CXCL8 (also known as interleukin 8 or IL-8), which can be secreted by most epithelial cells in response to inflam- matory signals or stress. In general, CXCL8 is a powerful attractant for neutrophils, but in several tumour types levels of IL-8 are prog- nostic, reflecting the notion that innate inflammation may drive tu- mour progression. Another set of cytokines of note are a series of molecules originally described as based on their role in communi- cation between leukocytes, the interleukins. Interleukins are num- bered in the order of their discovery, so the name for an interleukin is not at all relevant to its functional role. Interleukin-1 (IL-1), for example, is more of an innate cytokine than an interleukin—it is se- creted from stressed epithelial cells and attracts a variety of immune cells. In the systemic circulation, IL-1 is one of the primary medi- ators of fever, and IL-1 has further been postulated to play a role in the innate inflammation that drives tumour progression. There are two important sets of cytokines associated with polar- ization of an adaptive (T-cell mediated) immune response. These are known as TH1 and TH2 cytokines, and are a group of molecules se- creted by CD4 (helper) T cells in response to various stimuli. The pat- tern of cytokines is associated with well-defined patterns of immune response; TH2 responses are associated with chronic inflammation and are thought to be tumour-promoting, whereas TH1 cytokines are important in clearing viral infections and tumours. Functionally, CD4 T-cell skewing occurs when naïve CD4 helper cells are acti- vated. In an environment rich in the cytokine IL-12, CD4 T cells differentiate into TH1 CD4 T cells, and in turn secrete interleukin-2 (IL-2), tumour necrosis factor alpha (TNFα), and interferon gamma (IFN-γ). These TH1 cytokines activate CD8 (killer) T cells (discussed next), and are especially important in an antitumour immune re- sponse. Alternatively, when naïve CD4 T cells encounter their spe- cific antigen in the context of interleukin-4 (IL-4), they differentiate towards a phenotype associated with chronic inflammation and antibody production, and in turn secrete additional interleukin-4 (IL-4), as well as IL-5, IL-10, and IL-13. For fuller discussion of cytokines, see Chapter 3.3. The adaptive immune system The key cells in the adaptive immune system are CD4 (helper) T cells, CD8 (killer) T cells, and B cells. These cells are brought into an immune response only after activation of the innate immune system. The key aspects of the adaptive immune response are its exquisite specificity and its ability to ‘remember’ prior antigen encounter— responding more robustly when that antigen is encountered again in the future. Dendritic cells Information is transmitted from the innate immune system to the adaptive immune system via a unique cell type known as the den- dritic cell, which serves as the bridge between an innate and an adap- tive immune response. Dendritic cells (DC) get their name from their fine cytoplasmic projections; microscopically they resemble nerve cells. Functionally, dendritic cells are distributed throughout the peripheral tissues, as exemplified by Langerhans cells in the skin. DC spend most of their lives at rest, continually sampling their microenvironment, taking in fluid and protein antigens through the process of pinocytosis. In the absence of an activating or ‘danger’ mediated by the interaction between PAMPs and PRRs, DC remain in a quiescent state. From the perspective of a DC, a danger signal can also come in the form of cytokines like TNFα secreted from in- nate immune cells like macrophages, or through direct contact with bacterial products recognized by pattern receptors (TLRs) on the DC. DC activation initiates a coordinated cascade of events: (1) ac- tivated DC cease taking in antigens; their new role will be to present the antigens they have already taken up to T cells. (2) Their dendrites are retracted and the cells develop a more compact morphology. (3) DC upregulate cell surface molecules important for

presenting the antigens they have taken up to T cells. Molecules important in DC communication to T cells include major histocompatibility molecules (MHCs) which bind 9–12 amino acid peptides in their grooves for interacting with specific receptors on T cells (TCR), as well as several molecules which evolved to stimulate T cells; these are costimulatory molecules such as B7-1, B7-2, and others. (4) So, activated DC must solve a localization problem: T cells reside in the

5.4 Cancer immunity and immunotherapy 473 secondary lymphoid structures (i.e. in the lymph nodes), whereas DC are situated in the tissues. So, DC must migrate into the lymph- atic system, and enter into the lymph nodes through afferent lymph- atic vessels. This is accomplished via chemotaxis; fully activated DC follow a gradient of secondary lymphoid chemokine into the lymph nodes. Once in the lymph nodes, DC interact with (and activate) specific CD4, CD8, and B lymphocytes, completing the transfer of information from the innate immune system to the adaptive. T cells T cells include both helper (CD4 T) and killer (CD8) subsets. As outlined earlier, CD4 T cells in a lymph node are generally acti- vated when an antigen-presenting DC presents the specific peptide antigen (usually 11 AA long) recognized by a T cells receptor in the context of a Class II MHC. These activated CD4 (helper) cells are capable of either helping CD8 T cells to become fully activated in terms of lytic function, or of helping B cells to secrete antibodies. As just described, CD4 T-cell responses fall into several basic categories, including a TH1 response which serves to fully activate CD8 (killer cells) and a TH2 response, which helps B cells to mature into antibody secreting plasma cells. An additional CD4 T-cell sub- type of interest in cancer immunity is the regulatory T cell (Treg). These cells suppress adaptive immune responses, and appear to play an important role in preventing a successful adaptive anticancer re- sponse. The origin of Treg is complex; a population of ‘natural’ Treg arises de novo in the course of T-cell development in the thymus, while a second population may be ‘induced’ in the periphery when naïve CD4 T cells recognize their antigen in a microenvironment that is poor in proinflammatory signals and rich in transforming growth factor beta (TGFβ). The relative contributions of these two types of Treg to the progression of cancers in humans is unclear, however, recent laboratory data point to a critical role for natural, thymus-derived Treg. In terms of cancer immunity, the most critical adaptive immune cell is the CD8 T cell. These cells recognize their specific antigen in the form of peptides 9 AA long presented in the context of class I MHC, which is present on almost all cell types, and which is upregulated in the context of inflammation as well as on virally infected cells. When a specific CD8 T cell recognizes its target, it se- cretes molecules which result in destruction of that target cell. This killing process is exquisitely specific; in the autoimmune disease type 1 diabetes, CD8 T cells can lyse β cells in the pancreas while leaving immediately adjacent α cells completely intact. Similar specificity is a hoped-for outcome in cancer immunotherapy, the goal of which is to kill tumour cells while leaving adjacent normal tissue cells intact. The mechanism of killing is robust; CD8 T cells employ multiple molecular mechanisms to induce their target cells to commit suicide (i.e. to undergo programmed cell death or apoptosis). Finally, CD8 T cells are serial killers, able to destroy specific targets in a sequen- tial manner. As discussed next, the major goal of cancer vaccines is to activate antigen-specific CD8 T cells, and to thus eliminate an evolving tumour. For a fuller discussion of the adaptive immune system, see Chapter 4.3. The immune editing hypothesis Before proceeding with a discussion as to how the immune system may be activated to treat cancer, it is important to briefly consider the immune system’s response as tumours arise within the context of an immunologically intact host. Indeed, with the exception of some virally mediated tumours that arise in immunocompromised individuals, human cancers generally develop in immunologically intact hosts. As tumorigenesis proceeds from low-grade/localized disease to

metastatic disease, an interaction between the host im- mune system and the tumour mass occurs. This process has been well-characterized in several elegant animal models, and can be con- ceptually divided into three stages: 1. Elimination—in first stage of the process, early tumours may be recognized by the immune system in a productive manner, leading to elimination of small, clinically undetectable masses. Elimination is most likely driven by a coordinated effort between the innate (macrophages and dendritic cells) and the adaptive immune systems. 2. Equilibrium—as tumours evolve, they acquire genetic and epi- genetic alterations that render an antitumour immune response less efficacious. So, in the next phase of tumour/immune system interaction, tumours are able to exist in equilibrium with the host immune response, with progression inhibited by an ongoing im- mune response, but in a stage in which tumours can no longer be successfully eliminated without intervention. Equilibrium likely persists for a significant period of time, and some tumours may remain in the equilibrium stage for the life of the host. 3. Escape—eventually, clinically relevant tumours proceed to es- cape the host immune response. The immunological and mo- lecular mechanisms involved in the escape phase are not fully understood, but may include downregulation of tumour antigens against which a host response is directed, downregulation of MHC molecules, as well as the induction or expansion of regula- tory T cells (Treg) that inhibit an immune response. Together, the three phases of tumour/host interactions (Elimina tion, Equilibrium, and Escape) form the ‘immune editing hypoth- esis’ (see Fig. 5.4.1), which serves as a valuable framework by which to understand the immune response to cancer. Indeed, subversion of a productive host antitumour response is now designated as one of the hallmarks of cancer. Cytokine therapy for cancer One way to reverse tumour escape might be to exogenously pro- vide cytokines that augment T-cell function, hopefully driving T-cell proliferation and effector (lytic) function. Clinically, this has gen- erally been attempted by the systemic administration of the cyto- kine known as interleukin-2 (IL-2), which was originally described as ‘T-cell growth factor’ as it proved critical for expanding and maintaining human T-cell growth in vitro. In both melanoma and kidney cancer, intravenous (IV) administration of IL-2 is associated with objective response in some patients, although that response rate is lower than that currently achieved via immune checkpoint blockade (discussed next). The attractive feature of IL-2 administra- tion is that some of the induced tumour regressions are complete, and some are persistent for decades. Thus, although the objective response rate to systemic IL-2 is low, the persistence of some of those responses drives ongoing interest in the approach. Limiting its application is the fact that systemic IL-2 is associated with severe

 toxicity, including hypotension, fluid retention, and even a small risk for death. Based on those features, systemic IL-2 administration is currently limited, mostly to academic centres, and with the number of patients treated declining each year. Cancer vaccines Basic biology A ‘cancer vaccine’ is intended to either initiate or expand an adaptive immune response against a patient’s tumour. In general, a cancer vac- cine includes components known as pathogen-associated molecular patterns, which, as described here earlier, activate resting dendritic cells and programme them to migrate to a local lymph node. So, a vaccine always includes some component or components intended to activate dendritic cells, but the precise substance(s) employed vary widely between different vaccines. Another common term for these activating components is ‘adjuvant’, since they ‘add’ immuno- genicity to the protein or peptide components of the vaccine. The other important component of a vaccine is a target protein or pro- teins that are expected to be overexpressed in a tumour relative to normal tissue. To date, the majority of cancer vaccines have targeted so-called ‘shared’ antigens that are relatively

overexpressed in a tu- mour, but several new approaches focus on antigens derived from tumour-specific mutations that alter the coding regions of proteins (one term for these antigens is ‘mutation-associated neoantigens’ or MANA). In response to vaccination, activated DC migrate to the draining lymph node, where they display fragments of proteins in the form of small peptides (see Fig. 5.4.2). Cellular recognition of these small peptide fragments (antigens) is complex; as just discussed, these peptides are not presented alone, but instead are bound within a genetically diverse set of host molecules collectively encoded by a set of genes within the major histocompatibility complex (MHC). Specific receptors on CD4 and CD8 T cells recognize a structure composed of both MHC molecules and a specific peptide. Simple recognition of a peptide/MHC surface (a good fit) is necessary but not sufficient for full T-cell activation—T cells must also receive additional costimulatory signals provided by mature DCs to pro- liferate and acquire effector function. Two particularly important costimulatory molecules are B7-1 (CD80) and B7-2 (CD86), which bind to CD28 on the T cell to induce full T-cell activation. Specific recognition of peptide/MHC plus costimulation leads to full T-cell activation. In the case of CD8 T cells, this leads to the ac- quisition of their key effector function: the ability to lyse target cells expressing the same MHC/peptide complex that activated them (i.e. their target antigen). Once fully activated, CD8 T cells emigrate from the lymph node, and roam widely through the host in search of their targets. For CD4 T cells, the desired outcome of vaccination is the generation of a TH1 response associated with the secretion of IFN-γ and TNFα, which helps CD8 T cells to achieve their full potential. Dendritic-cell-based vaccines One rationale for DC-based vaccines is the observation that cancer patients often have DC that are dysfunctional in either number or in phenotype. Thus, the principal of DC-based vaccines is to generate new DC ex-vivo, load them with a cancer antigen, activate them, and then reinfuse them into patients. From a practical perspective, DC can be generated by maturing peripheral blood monocytes in the presence of GM-CSF. Although several experimental DC vaccines are currently being evaluated, for the purposes of discussion we will focus here on Sipuleucel-T, a vaccine directed against prostate cancer (a) (b) (c) Elimination CD8+ CD8+ CD4+ CD4+ Genetic instability/tumour heterogeneity Immune selection Equilibrium Escape CD8+ CD4+ D4+ CD8+ NK NKT NK Fig. 5.4.1 The immune editing hypothesis. In this model, small tumours are eliminated (a) by the immune system before they are detectable. This can occur through natural killer (NK) cells, which recognize the tumour losing major histocompatibility molecule (MHC) class I, or natural killer-activating ligands being expressed on the tumour (due to cellular stress). It can also occur when CD8 T cells recognize tumour antigens, which can represent either new, mutated proteins, or overexpression of a tissue-specific ligand to which tolerance is not complete. Some tumours are not eliminated, but instead progress to a state in which they coexist with the immune system in an ongoing equilibrium. Equilibrium (b) is a balance between immune pressure and a tumour’s ability to progress. Clinically apparent tumours have almost certainly evolved to escape (c) the immune system. Multiple immune escape mechanisms have been described, although the precise mechanisms responsible are not completely understood. Adapted from Dunn GP, et al. (2004). The immunobiology of cancer immunosurveillance and immunoediting. Immunity, 21(2), 137–48.

 which has been approved by several regulatory agencies. To generate Sipuleucel-T, patients first undergo a leukapheresis procedure to obtain peripheral blood leukocytes, which are then shipped (un- frozen) to a processing facility. There, peripheral blood monocytes (immature DC) are enriched from the total product via density gradient centrifugation. These immature DC are then incubated with the targeted immunogen, a fusion

protein coupling GM-CSF to prostatic acid phosphatase (PAP). This fusion protein approach is unique to the Sipuleucel-T DC vaccine; usually monocytes are first differentiated in the presence of GM-CSF and then loaded with peptide. The GM-CSF fragment of the fusion protein serves to mature/activate the monocytes, while simultaneously facilitating DC loading with peptide. The target antigen of Sipuleucel-T is PAP, an antigen shared by more than 95% of all prostate cancer metastases. Once DC have been incubated with the fusion protein, the product is then shipped to the local clinic site, where it is admin- istered intravenously. After infusion, functional DC are thought to activate PAP-specific CD4+ and CD8+ T cells in treated patients. In terms of therapeutic cancer vaccines, this agent has progressed the furthest: three phase III studies were completed and FDA approval was granted in April of 2010, making Sipuleucel-T the first antigen- specific immunotherapy approved for cancer treatment. It should also be noted that this approach is theoretically adaptable to other tumour types by changing the nature of the immunogen (i.e. by swapping out the antigen coupled to GM-CSF). Peptide-based cancer vaccines In lung cancer, several vaccine efforts have focused on MAGE-A3—a cancer testis antigen which is expressed at significant levels nearly exclusively in the testes; in that location the antigen is not accessible by circulating T cells because MHC molecules are not expressed in the testes. So, central and peripheral immune tolerance to MAGE- A3 (and other cancer testis antigens) is usually not present. MAGE- A3 expression has been shown to increase with tumour stage, and MAGE-A3 is expressed in approximately one-third of lung tumours. To therapeutically vaccinate against MAGE-A3, a series of adjuvants were refined over time, starting with AS02, an oil/water emulsion that includes two stimulatory molecules; the first is monophosphoryl lipid A (MPL), a PAMP which activates resting DCs through Toll-like receptor 4 (TLR4). The second component of AS02, QS21, enhances antigen uptake by DCs. Early phase II studies involving a MAGE- A3 vaccine compromised of AS02 and the MAGE-A3 peptide that binds the MHC class I molecule HLA-A2 showed that the vaccine was well-tolerated, but that overall survival of lung cancer patients Intradermal vaccine: protein or peptide and adjuvant Dendritic cell (DC) Adjuvant activates DC Antigen uptake by immature DC Class I MHC Class II MHC Specific CD4+ T cell Activated CD8+ T cell CD8 T cell Fig. 5.4.2 Cancer vaccines. Cancer vaccines provide a target antigen and/or antigens in the context of a proinflammatory substance known as an immune adjuvant. Antigens are taken up by dendritic cells (DC) and the adjuvant serves to activate the DC and promote DC maturation and trafficking to the lymph node, where activated DCs present antigens to T cells in the context of MHC molecules. If a specific T cell recognizes its target antigen, that T cell is in turn activated, resulting in the acquisition of effector function and migration from the lymph node into the circulation. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Clinical Oncology (Drake CG, et al., 2013, Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer, Nature Reviews Clinical Oncology, 11, 24–37), copyright © 2013.

 treated with the vaccine was not significantly improved as compared to placebo. Eventually, a phase III trial was launched to investigate non-small cell lung cancer (NSCLC), this 2000+ patient trial was the largest interventional trial ever completed in lung cancer. In this study, MAGRIT, the adjuvant, was slightly modified to include CpG 7909, a synthetic 24-mer oligonucleotide aimed at activating DC by targeting the Toll-like receptor, TLR9. The primary end point of MAGRIT was disease-free survival, and 2270 patients with completely resected, MAGE-A3 expressing tumours were randomly as- signed to either vaccine or placebo. In keeping with current clinical practice, patients were permitted to receive adjuvant chemotherapy before randomization. Unfortunately, despite being the largest inter- ventional trial

ever conducted for NSCLC, MAGRIT was a negative trial, and further development of this approach has not proceeded. Peptide-based vaccines have also been evaluated in fairly large trials in kidney cancer (RCC). One noteworthy approach focused on targeting a series multiple antigens in the context of a less complex adjuvant. To select relevant antigens, kidney tumours from a series of 32 patients expressing the common class I MHC molecule HLA- A2 were isolated, and the cell surface peptides bound to class I MHC molecules were eluted and analysed. This work led to the identification of a set of nine tumour-associated peptides (TUMAPs), which were tested in a vaccine using granulocyte-macrophage colony stimulating factor (GM-CSF) as an adjuvant. GM-CSF is a strong inducer of DC migration, but is probably not as potent as the PAMPs discussed earlier in terms of DC activation. In two early phase trials, the vaccine IMA901 was shown to be safe, well-tolerated, and to in- duce T-cell responses when the vaccine was given with a low dose of the chemotherapy agent cyclophosphamide to deplete regulatory T cells (Treg). Notably though, no objective responses were reported in the early trials. A randomized phase III trial was initiated in which IMA901 was added to first-line tyrosine kinase inhibition (sunitinib) in patients with metastatic RCC. Enrolment was completed in 2012 (330 patients total), and results were recently reported in abstract form. Like the MAGRIT trial in NSCLC, this randomized phase III trial was negative, with no benefit on overall or progression-free survival. Peptide-based vaccines have also been evaluated in large, ran- domized controlled trials in patients with glioblastoma multiforme. In this disease, approximately 30% of patients express a common mutant form of the epithelial growth factor receptor known as epi- dermal growth factor receptor (EGFR) variant III (EGFRvIII). In glioblastoma multiforme, data suggest that mutated EGFR may drive malignant cell proliferation, differentiation, and survival, so EGFRvIII represents both a tumour-specific antigen as well as a po- tential driver mutation. To target EGFRvIII a peptide-based vaccine was developed, the vaccine couples a mutant EGFRvIII peptide to the non-self-protein keyhole limpet hemocyanin which serves as an adjuvant. A phase III randomized double-blind trial comparing vac- cination to placebo in 745 newly diagnosed EGFRvIII-positive glio- blastoma multiforme patients was recently discontinued based on the recommendation by the Data Safety and Monitoring Committee that the trial was unlikely to meet its overall survival (primary) end point. Taken together, these phase III experiences (and several others) suggest that peptide-based vaccines are unlikely to have a significant impact in cancer as a monotherapy. It is worth noting, however, that multiple preclinical models suggest that cancer vaccines may prove synergistic with immune checkpoint blockade, and several early phase trials are currently exploring such combinations. Cell-based vaccines Perhaps an ideal source of antigens for cancer vaccination would come from a patient’s individual tumour cells, such vaccines could theoretically include multiple mutation-associated neoantigens, as well as a panoply of overexpressed shared antigens. However, harvesting viable tumour cells and converting them to an individu- alized vaccine has proved challenging, so several approaches have focused on using immortal allogeneic tumour cells engineered to increase immunogenicity. One noteworthy approach transduced tumour cells with GM-CSF, which (as already described) attracts dendritic cells to the vaccine site; this approach is known as GVAX. Phase III trials of GVAX were launched in prostate cancer, and as was the case for the vaccines listed here were negative in terms of their primary overall survival end points. A similar approach was developed for pancreatic cancer; here cells were modified based on the observation that in humans, a large fraction of pre-existing immunity is directed against a sugar moiety (α-gal) present in all mammalian species other than humans. So, tumour cells were modified to express α-gal, when injected intradermally the alpha-gal expressing vaccine induces a hyperacute immune response, me- diated by pre-existing antibodies and characterized by the rapid, immunogenic destruction of the vaccine cells. Although

early trials were promising, a recently reported phase III trial in 700 patients with fully resected pancreatic cancer was negative for its primary overall survival end point. Viral vector-based vaccines As compared to the other approaches discussed earlier, viral vectors have several advantages for cancer immunotherapy: they are rela- tively straightforward to generate, they are able to carry significant amounts of genetic material, and there is a great deal of clinical ex- perience with some of these vectors. In particular, the poxvirus vec- tors are well-understood since vaccinia was used in the worldwide campaign that led to the eradication of smallpox. Mechanistically, poxvirus vectors likely infect epithelial cells, a proportion of which will undergo cell death. Cellular debris, including encoded antigens, are then taken up by immature DC, which, when appropriately acti- vated, will present those encoded antigens to CD4+ and CD8+ T cells in the draining lymph node. Direct infection of antigen-presenting cells like the Langerhans cells in the skin is another possible mech- anism by which poxviral vector may prime an antitumour immune response. This approach was extensively studied in prostate cancer, where prostate-specific antigen (PSA)-targeted vaccinia-based im- munotherapy has been refined over subsequent iterations. Current clinical vectors now include costimulatory molecules as well as a modified version of the PSA protein designed to better fit into the MHC class I-binding groove of the most common MHC molecule. A major limitation of poxvirus-based vaccines is their tendency to induce a neutralizing antibody response, making prime-boost re- gimens ineffective because the antibody response to viral proteins dominates over the desired response to encoded antigen(s). To overcome such limitations, a heterologous prime-boost strategy involving a vaccinia virus prime followed by a fowlpox virus boost was developed. At the current time, this agent (ProstVac VF) is the subject of a large, international randomized phase III trial, which,

5.4 Cancer immunity and immunotherapy 477 if positive, could prove potentially pivotal. In this trial, men with later stage prostate cancer (metastatic, castration-resistant dis- ease) were randomized to either ProstVac VF alone, the combin- ation of ProstVac VF and GM-CSF, or to placebo. The trial's primary end point is overall survival, and although accrual has been com- pleted, survival data were not available at the time this chapter was completed. Chimeric antigen receptor (CAR) T cells Introduction and mechanism of action Monoclonal antibodies are high-affinity molecules which can be reliably generated against cell surface proteins. Indeed, tumour- directed monoclonal antibodies have shown efficacy in several tu- mour types including anti-Her-2 in breast cancer (trastuzumab) and anti-CD20 (rituximab) in B-cell malignancies. These agents func- tion through several mechanisms, including antibody-dependent cellular toxicity and by blocking pro-survival and pro-proliferation signals. Despite their high affinity and exquisite specificity, mono- clonal antibodies lack intrinsic lytic function, instead relying on the host immune system for this facet of their function. The T-cell re- ceptor, by contrast, is of fairly low (micromolar) affinity, but exists in nature on the cell surface of T cells, incredibly efficient engines of cellular destruction which employ multiple nonoverlapping mechanisms to drive facilitate lysis of their specific targets. A clever approach to cancer immunotherapy involves the fusion of these two biological mechanisms via the generation of chimeric molecules which fuse the portions of an antibody involved in specific recogni- tion of a target antigen (the variable heavy (VH) and variable light (VH) chains) to the transmembrane and intracellular domains of the T-cell receptor (zeta) chain. This short antigen recognition re- gion (a single-chain fragment variable or ScFv) provides for a high specificity of recognition, while the remainder of the construct func- tions to fully activate the associated T cells, driving proliferation, effector function, and persistence. Together, the fusion of the ScFv with the T-cell apparatus is known as a chimeric antigen receptor (CAR), and T cells

modified to express CAR are known as CAR T cells (see Fig. 5.4.3). CAR T-cell technology has evolved over time; first-generation CAR included only native portions of the T-cell receptor, and suf- fered from poor persistence and a relative lack of clinical activity. Second-generation CAR incorporated a costimulatory signalling domain, which was often CD28 as discussed earlier. Although second-generation constructs have been the subject of much clinical investigation, third-generation CAR have also been evaluated: these include a second costimulatory signalling domain to provide even greater persistence and activation. The generation of CAR T cells for therapy is somewhat involved; patients first undergo apheresis to provide adequate numbers of peripheral blood mononuclear cells for subsequent modification. These cells are cryopreserved, Lipid bilayer Endodomain (stimulation) Ectodomain (antigen recognition) Linker Light (or heavy) chain Heavy (or light) chain Hinge region Transmembrane domain Derived from CD8 or lgG4 Derived from an scFv of known specificity Derived from transmembrane domain of CD8 or CD28 Costimulatory molecule(s) Stimulatory molecule CD3 ?chain or FcRy chain None, one, or more of: CD27, CD28, ICOS, 4-1BB, OX40 Fig. 5.4.3 Chimeric antigen receptor (CAR) T cells. These combine the high-affinity antigen recognition capacity of an antibody with the cell-based killing machinery of a T cell. They are constructed by linking the heavy and light chain of an antibody with a known specificity via a short linker. This fragment, the single-chain variable fragment (ScFv), provides antigen recognition. The ScFv is linked to the transmembrane region of the CD8 molecule and then to the stimulatory region of the CD3 zeta chain. First-generation CARs have a single stimulatory region, while later generation CARs include additional stimulatory regions from 41BB, CD28, or others. CAR T cells are manufactured using a patient's T cells obtained by pheresis, and the construct may be inserted via electroporation or viral transfection. Reprinted with permission from Gill S and June CH (2015). Going viral: chimeric antigen receptor T-cell therapy for hematological malignancies. Immunol Rev, 263(1), 68–89, © 2014 John Wiley & Sons A/S.

 and later thawed and modified to generate the biological product. To modify a patient's T cells to express appropriate CAR, several approaches have been tested: these include lentiviral transfection, retroviral transfection, and a system involving the Sleeping Beauty transposon system. Each of these has relative advantages and dis-advantages, a discussion of which is beyond the scope of the cur- rent chapter. Following transduction, T cells are expanded in vitro by engaging the T-cell receptor via CD28/CD3 costimulation; this results in an approximate 1000-fold expansion. The final product, which contains both CD8 and CD4 T cells is infused intravenously, usually in split doses ranging from 1.5 × 106 cells/kg to approximately 3 × 107 cells/kg. Clinical considerations The complexity of this treatment approach would be of little interest if not for the significant activity observed in several studies. By far the largest number of CAR T-cell studies have been involved pa- tients with B-cell acute lymphoblastic leukaemia (ALL) for which the target antigen is CD19. For patients refractory to prior treat- ments, 6-month event-free survivals of approximately 70% have been reported, with 6-month overall survival rates in the 80% range. After infusion, the CAR T cells undergo additional expansion and proliferation, with cell numbers peaking at approximately 2 weeks post infusion. Fascinatingly, CAR T cells may persist long term in some patients, with persistence partially determined by the par- ticular construct under evaluation. Thus, CD28 containing CAR T cells appear to persist for 2–4 months, whereas second-generation CAR T cells incorporating the 41BB signalling domain have been detected more than 2 years post infusion. Despite the dramatic responses noted in some treated patients, significant hurdles will need to be overcome before CAR T cells become a standard of care therapy for CD19 expressing malignan- cies. First among these is

the complexity of manufacture, as is evi- dent by the aforementioned description. Second among these are the toxicities involved with this approach. To provide ‘space’ for CAR T cells to expand, patients typically undergo treatment with a lymphoablative conditioning regimen, which in and of itself car- ries significant toxicity. Three other toxicities are of note: cytokine release syndrome (CRS), encephalopathy, and B-cell aplasia. CRS typically occurs as the infused T cells expand, and is somewhat variable in severity. In milder cases, CRS may be manifest only by laboratory abnormalities, but in more severe cases patients experi- ence tachycardia, hypotension, and other symptoms necessitating treatment in an intensive care unit. CRS is mediated by several cytokines, including IFN-γ, IL-10, and IL-6. The latter of these is of particular importance, since CRS can be significantly mitigated by blockade of IL-6 signalling using the monoclonal antibody tocilizumab. Encephalopathy is often associated with CRS, but is considered to be a separate toxicity and occurs in up to 50% of treated patients. The aetiology of CAR T associated encephalop- athy is unclear at the present time. Since CAR T cells have generally targeted CD19, which is present on the majority of mature B cells, B-cell aplasia is an expected treatment-related adverse event. Like the other adverse events associated with CAR T cell therapy, B-cell aplasia is variable among patients and can persist for several years. Treatment with intravenous immunoglobulin can mitigate the inci- dence of opportunistic infections. CAR T cell therapy for nonhaematological malignancies is less well established, and has been complicated by the observation that surprisingly low-level expression of the target antigen on normal cells is sufficient to enable CAR T cell-driven destruction, resulting in organ dysfunction and even death. Future CAR constructs may include an ‘off switch’ to potentially decrease toxicity, and well as more complicated recognition constructs requiring the coexpression of multiple tumour associated antigens for CAR T cell activation. At the current time, several large phase II trials of CAR T cells are underway; and the United States Food and Drug Administration has granted breakthrough status to this modality. T-cell redirecting engineered antibodies Introduction and mechanism of action The immune checkpoint blocking antibodies discussed next, and most other antibodies in the clinic are of the IgG class; these anti- bodies are bivalent. For naturally occurring IgG antibodies, both antigen-binding fragments (Fab) are identical, and hence recognize the same target antigen, usually with high affinity. Modern genetic engineering technology allows the generation of antibodies in which the two Fab recognize distinct targets; such antibodies are termed bispecific. While several bispecific antibodies are in various stages of clinical development, one particular variant of this technology has led to an effective and FDA-approved agent for acute lymphoblastic leukaemia. Certain of these reagents are known as bispecific T-cell engagers (BITE®) and consist of two distinct antigen-binding single- chain variable fragments, coupled by a short 5 amino acid linker (see Fig. 5.4.4). One of these, ScFv, is directed at a cell surface target on the tu- mours, and in the case of ALL the target antigen chosen was CD19. As previously described here, CD19 has also been targeted by CAR T cell technology. The second ScFv of this agent targets CD3, a cell sur- face molecule expressed on both CD8 and CD4 T cells. Functionally, this drug (blinatumomab) functions by physically enforcing a T- cell/ tumour interaction, similar to that which naturally occurs when a T cell recognizes and localizes to a cell expressing its target antigen. In addition to physically localizing T cells to tumour cells, it should be noted that cross-linking CD3 on either CD4 or CD8 T cells serves to activate them so that they exert their effector function. In vitro studies confirmed that blinatumomab can activate both CD4 and CD8 T cells, and that these activated cells can lyse CD19 expressing target cells. What is unique about blinatumomab’s mechanism of ac- tion is that the specificity of the engaged T cell is irrelevant (i.e. the T cell does not need to be tumour-specific to kill the colocalized tumour cell). Clinical considerations Full-length human IgG molecules have a half-life in the 1–3

week range, but truncating them into small fragments like bispecific engagers dramatically reduces their persistence. The half-life of blinatumomab is less than 2 hours. It was no surprise, then, that early trails involving 2 or 4 hour intravenous infusions showed no evidence of activity. When the drug was given by continuous intra- venous (CIV) infusion, a significant overall response rate of approxi- mately 70% was noted in a phase I study of patients with relapsed

5.4 Cancer immunity and immunotherapy 479 and/or refractory non-Hodgkin lymphoma. This led to further de- velopment in several haematological malignancies, most notably ALL. A single-armed phase II trial treated approximately 200 ALL patients with a dose-escalating CIV regimen and showed a rate of complete remission of approximately 40%, leading to accelerated ap- proval as well as to further studies in several other CD19-expressing malignancies. In general, CIV treatment with blinatumomab is reasonably well-tolerated, with approximately 10% of patients discontinuing treatment due to treatment-related toxicity. Some of these toxicities are reminiscent of those previously noted with CD19- directed CAR T-cell therapy; and include neurological toxicities and B-cell aplasia. Unlike CAR T cells which persist for months to years, the short half-life of blinatumomab is perhaps an advan- tage here, as discontinuing the CIV infusion results in a reason- ably rapid clearance of the agent. Also, management of central nervous system toxicities with corticosteroids appeared not to limit the efficacy of this reagent, which might not be the case for CAR T cells. Currently, the major barrier to widespread adoption of blinatumomab is the requirement for continuous infusion; al- ternative regimens are being explored in ongoing studies. Of fu- ture interest are related constructs designed to increase either the half-life or valency of bispecific targeting. One of these reagents, the dual affinity retargeting antibody (DART), uses two separate polypeptide chains with an interconnecting disulphide bridge (see Fig. 5.4.4). Larger tetravalent reagents have also been de- signed and have entered the clinic. In summary, the clinical effi- cacy of blinatumomab highlights the feasibility of drugs designed to localize a patient’s endogenous T cells to tumours regardless of the T cell’s specificity. The application of these technologies to more common solid tumours remains an open question, and may depend on the availability of suitable target molecules on the cell surface of tumour cells, as well as on efforts to surmount the chal- lenges inherent with CIV treatment regimens. Blocking the immune checkpoint molecule cytotoxic T lymphocyte antigen-4 (CTLA-4) Introduction and mechanism of action CTLA-4 is an immune checkpoint molecule present on the cell sur- face of some populations of T cells, which limits their function when engaged. Surprisingly, CTLA-4 is homologous to the T-cell stimula- tory receptor CD28, which, as discussed earlier, is important for full activation of T cells by binding to B7-1 and B7-2. Thus, it was ori- ginally postulated that CTLA-4 might also be a T-cell costimulatory molecule. These data were not clarified by multiple animal studies; for some time it was not obvious whether CTLA-4 transmitted a stimulatory or inhibitory signal to T cells. The pivotal event in these studies was the development of knockout mice lacking CTLA-4. Indeed, CTLA-4 knockout mice have a dramatic phenotype, with a progressive accumulation of activated T cells; these CTLA-4 knockout mice expire approximately 3–4 weeks after birth from progressive lymphoproliferative disease. These findings confirmed the notion that CTLA-4 transmits a negative or inhibitory signal Bispecific T-cell engager (BiTE®) • Single polypeptide chain • Single polypeptide chain • Chain dimerization VH VL VL VL VL VH VH VL VH VH VL VH VL VH VH VL • Two polypeptide chains • Interchain disulphide bridge Disulphide bridge CD19 CD3 CD19 Dual affinity retargeting (DART) Tetravalent tandem diabody (TandAb®) CD3 CD3 CD3 CD19 CD19 Fig. 5.4.4 T-cell redirecting engineered antibodies. These reagents aim to localize T cells to a tumour via interaction with the CD3 molecule on the surface of a T cell. Since the T-cell receptor which

conveys specificity is not involved, T cells can be localized to tumours regardless of their specificity. Cross-linking of CD3 leads to T-cell activation and acquisition of effector (killing) function. The short distance between the directing antibody and the anti-CD3 region serves to mimic a natural T-cell synapse and may facilitate target cell lysis. Shorter constructs are hampered by a short in vivo half- life: many additional constructs have been engineered in an attempt to overcome that limitation. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Clinical Oncology (Batlevi CL, et al., 2015, Novel immunotherapies in lymphoid malignancies. Nat Rev Clin Oncol, 13(1), 25–40), copyright © 2015.

480 SECTION 5 Principles of clinical oncology to T cells, and further suggested that blockade of CTLA-4 using a monoclonal antibody might augment a T-cell response, potentially even an antitumour T-cell response. Immunocompetent preclinical models confirmed this hypothesis, showing that CTLA-4 blockade was active in several tumour types. In terms of basic mechanism, it should be recalled that, for a T cell to become fully activated (and subsequently proliferate and mediate effector function) two receptor/ligand interactions are re- quired. The first of these occurs when the T cell's unique receptor (TCR) recognizes its specific ligand, a short peptide presented in the context of an MHC molecule. This interaction is specific, and if a good fit occurs, T-cell activation is initiated. However, full activa- tion of a CD4 or CD8 T cell requires a second signal transmitted by costimulatory molecules present on the same dendritic cell (DC) that expresses the peptide/MHC. This second signal is transmitted from the costimulatory molecules (B7-1 and/or B7-2) to the re- ceptor on T cells known as CD28. Only when both signals are re- ceived and integrated does an antigen specific T-cell proliferate, acquire effector function, and migrate to sites of antigen expression. Interestingly, CTLA-4 binds to B7 molecules with greater affinity than CD28 does, so when CTLA-4 is expressed, it essentially hijacks what would be a positive (activating) signal and converts it to an in- hibitory one. Blockade of the CTLA-4/B7 interaction with a mono- clonal antibody attenuates this inhibitory signal, resulting in T-cell activation, proliferation, and potentially the acquisition of effector function (see Fig. 5.4.5). Clinical considerations Melanoma Two anti-CTLA-4 antibodies have been studied in the clinic; these include the IgG2 antibody tremelimumab and the IgG1 antibody ipilimumab. Because ipilimumab was eventually approved by regu- latory agencies to treat melanoma we will focus on that regent here. After phase I studies involving patients with a variety of tumour types, two large, randomized phase III trials were launched in patients with advanced melanoma. In the first of these trials a total of approximately 700 patients with previously treated (second-line and beyond) advanced metastatic melanoma were randomized 3:1:1 to receive ipilimumab at a dose of 3 mg/ kg q 3 weeks plus a pep- tide vaccine directed against the shared melanoma antigen gp100, ipilimumab monotherapy, or a gp100 vaccine alone. Blocking CTLA- 4 with the monoclonal antibody ipilimumab resulted in a significant survival benefit: overall survival with single-agent ipilimumab was 10.1 months versus 6.4 months for patients treated with a the gp100 vaccine. The results of treatment with ipilimumab monotherapy was essentially identical to that observed with the ipilimumab/vac- cine combination (10.1 months vs. 10.0 months), showing that the vaccine appeared to add little in the way of a survival benefit in the second-line setting in melanoma. These data led to the United States FDA approval of ipilimumab in 2010, which was the first immune checkpoint blocking antibody to receive such a designation. The second large-scale study in melanoma extended these results to the first-line treatment setting; it enrolled 502 treatment naïve patients and randomized them to either ipilimumab plus chemo- therapy with dacarbazine, versus dacarbazine alone. Here the regimen including ipilimumab was again superior, with an overall survival of 11.2 months versus 9.1 months

for chemotherapy alone. Perhaps most interesting are the results of long-term follow-up from the first trial, showing that approximately 15% of treated patients were alive 5 years post enrolment; such data support the concept that immunotherapy, when effective, may lead to long-term survival. Other tumour types Based on its activity in melanoma, and the notion that lung cancer-infiltrating T cells might be partially responsive to CTLA-4 blockade, the effect of ipilimumab was also explored in lung cancer. Here, an innovative phase II trial compared two different schedules of ipilimumab combined with taxane-based chemotherapy versus chemotherapy alone. In terms of an immunological rationale, one might postulate that taxane-based chemotherapy could potentially release tumour-associated antigens to help prime an antitumour response. Further, several preclinical studies showed that the rela- tive sequence of chemotherapy with immunotherapy can affect effi- cacy. In this randomized phase II trial, patients with advanced and previously untreated NSCLC were randomly assigned to standard chemotherapy (paclitaxel and carboplatin) or standard chemo- therapy plus ipilimumab (10 mg/kg) given according to two different schedules. In one arm (a 'phased' schedule), patients first received two cycles of chemotherapy followed by four cycles of ipilimumab plus chemotherapy. In a second (concurrent) arm, patients received all three drugs concurrently for four cycles, followed by two cycles PD-1 PD-L1 MHC Antigen CD28 B7 Tumour cell or antigen-presenting cell Tumour-specific T cell CTLA-4 anti-CTLA-4 anti-PD-1 T-cell receptor
Fig. 5.4.5 Immune checkpoint blockade. Several cell surface molecules inhibit the function of T cells in tumours, including CTLA-4, PD-1, and LAG-3. A series of antibodies that blocks transmission of an inhibitory signal has been developed. The first among these targeted the inhibitory molecule CTLA-4. Normal T-cell activation includes two steps: a first (antigen-specific) step involving the T-cell receptor, and a second signal transmitted from B7 molecules to CD28 on the T cell. When CTLA-4 is upregulated, it hijacks that second signal by binding to B7 molecules with higher affinity than CD28 does. Anti-CTLA-4 blocks this inhibitory pathway, leading to T-cell activation. An analogous pathway involves the transmission of a negative signal from PD-L1 on tumour cells to PD-1 on tumour-infiltrating T cells, so that blocking PD-1 (or PD-L1) can also result in T-cell activation and effector function. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Clinical Oncology (Drake CG, et al., 2013, Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. Nat Rev Clin Oncol, 11(1), 24–37), copyright © 2013.

 of chemotherapy alone. If patients had stable or responding dis- ease, they were permitted to continue on maintenance ipilimumab (once every 12 weeks) until disease progression. The primary end point of this randomized phase II study was progression-free survival. A total of 204 patients were enrolled, and the study met its primary end point of improved progression-free survival for the phased versus the control arm. In addition, overall survival dif- fered between arms, with a median overall survival of 12.2 months, 9.7 months, and 8.3 months in the phased, concurrent, and control arms, respectively, but these were not significantly different. In a preplanned subset analysis, patients with squamous cell histology showed a significantly improved progression-free survival as well as overall survival with the phased schedule versus control (chemo- therapy alone), although the patient numbers were relatively small. Taken together, these phase II data suggested that a phased treat- ment schedule could potentially provide clinical benefit in patients with lung cancer compared to chemotherapy alone. A phase III trial testing that hypothesis was initiated in 2011; it compares the phased schedule of ipilimumab with paclitaxel and carboplatin versus paclitaxel and carboplatin alone in patients with stage IV squamous- cell carcinoma. The primary end point of the trial is overall survival and, although enrolment has been completed, final clinical data have not yet been

reported. Early phase I studies suggested that CTLA-4 blockade might have some activity in prostate cancer, and based on the notion that (at the time of study initiation) men with advanced prostate cancer had few treatment options, two large randomized phase III studies of CTLA-4 blockade were initiated in prostate cancer. The first of these focused on late-stage patients; men who had progressed on or after completion of docetaxel-based chemotherapy. This trial also included a low dose of radiation therapy (8 Gray) to between one and five bone fields, based on preclinical data suggesting that irradi- ation of murine tumours might release tumour antigens and thus 'prime' an antitumour immune response. Approximately 800 men with metastatic, castration-resistant prostate cancer were random- ized 1:1 to ipilimumab at a dose of 10 mg/kg every 3 weeks versus IV placebo. The trial's primary end point was overall survival, which was numerically but not statistically significant: overall survival was 11.2 months in the ipilimumab group as compared to 10.0 months in the placebo group (HR 0.85, $p = 0.053$). A second trial was initi- ated in patients with less advanced prostate cancer: here approxi- mately 500 men who had castration-resistant disease but who had not yet received chemotherapy were randomized 2:1 to ipilimumab at a dose of 3 mg/kg versus placebo. That prechemotherapy trial was also reported as negative for its primary overall survival end point. Toxicity and adverse events As the immune checkpoint molecule CTLA-4 likely evolved to pro- tect organs from autoimmune attack, it is not surprising that clinical trials of anti-CTLA-4 were uniformly associated with an approximate 20–25% incidence of grade 3 and 4 immune-related adverse events. The most common of these are inflammation of the skin (derma- titis) as well as the gut (colitis), but inflammatory pathology has been reported to occur in almost all organ systems. These toxicities range from mild to life-threatening, and represent a significant bar- rier to widespread adoption of ipilimumab therapy. Treatment al-gorithms for autoimmune toxicity have been developed, and the rapid induction of immunosuppressive therapy coupled with dis- continuation of ipilimumab renders most adverse events relatively manageable. Initial treatment generally involves treatment with corticosteroids, but refractory cases occasionally require treatment with TNF-blocking drugs, which nearly always effective. Blocking the immune checkpoint molecule programmed death–1 (PD-1) Introduction/Mechanism of action Another immunological checkpoint which has achieved major clin- ical relevance is that mediated by the interaction between the mol- ecule known as programmed death-1 (PD-1) and its ligands PD-L1 and PD-L1. PD-1 was initially identified in a library-based screen of CD8+ T cells, but at that time its function was obscure. Subsequent work identified the ligand for PD-1 as PD-L1 (also known as B7- H1) and showed that the interaction between PD-1 and B7-H1 leads to a marked inhibition of T-cell function. In animal studies, PD-1 blockade was shown to potentiate an antitumour immune response. To further clarify the role of PD-1 in immunity, PD-1-deficient animals were developed; these mice show a mild degree of strain-specific autoimmunity which is strikingly mild as compared to the early lymphoproliferative death that characterizes Ctla4-knockout mice). Perhaps most clinically relevant, human studies showed that increased expression of B7-H1 was associated with a poor clin- ical outcome in several tumour types, most notably in renal cell carcinoma. Early development of PD-1 blocking antibodies The first monoclonal antibody that blocks the interaction between the immune checkpoint molecule PD-1 and its ligand PD-L1 (nivolumab) entered clinical trials in cancer patients in late 2007. Preclinical data available at that time showed relatively modest effi- cacy for single-agent PD-1 blockade in mouse tumour models—thus, clinical expectations were not high, especially given the relatively advanced cancer patients who often enrol in phase I trials of a new agent. Interestingly, evidence of clinical activity was noted even in this initial dose escalation study, in which the drug was adminis- tered in a fairly intermittent schedule, with a first dose followed by two additional doses

given at 3- and 4-month time points. Objective responses were noted in patients with melanoma and RCC, con- sistent with prior experience with cytokine administration showing that these are immune-responsive tumour types. Responses were also noted in a patient with colorectal cancer, and a mixed response was observed in a patient with NSCLC; both of these tumour types were generally considered to be nonimmunogenic. Toxicity in this trial appeared to be less than that previously observed for CTLA-4 blockade, with no grade 3 or 4 adverse events reported. In a second, larger, phase Ib trial, the interval between doses was decreased to q 2 weeks, grossly consistent with the serum half-life reported in the phase I study. Here, objective responses were ob- served in approximately 30% in patients with melanoma or kidney cancer, and approximately 20% in patients with non-small-cell lung cancer. Responses were often rapid and durable: almost half of the responding patients achieved a partial response or complete re- sponse within 8 weeks of treatment initiation, and median duration

 of response was over 100 weeks. Some responses persisted even after therapy was discontinued. Overall, the drug was well-tolerated with a 5% rate of grade 3 or 4 adverse events at a follow-up greater than one year. The clinical activity of antibodies blocking the PD-1/ PD-L1 interaction was soon confirmed in clinical trials of a second anti-PD-1 antibody, pembrolizumab. In pretreated melanoma pa- tients, across all dose levels, the confirmed objective response rate to pembrolizumab was approximately 40%, similar to that ob- served with nivolumab. Both agents were similarly well-tolerated, with grade 3 or 4 adverse effects observed in about 15% of patients. Interestingly, this trial included patients who had been previously treated with the CTLA-4 blocking antibody ipilimumab, and there were no significant differences in rates of response or toxicity be- tween ipilimumab-naïve patients and those who received prior ipilimumab. These data suggest that the immunosuppressive path- ways mediated by CTLA-4 and PD-1 are mechanistically distinct. This second trial also introduced the notion that PD-L1 expres- sion on tumour cells might serve as a predictive biomarker for PD- 1 activity. This makes immunological sense, since PD-1-blocking antibodies likely function by blocking the interaction between PD-1 on tumour-infiltrating T cells and PD-L1 on either the tumour cells themselves or on tumour-associated myeloid cells. Using an in-house PD-L1 staining assay, the absence of PD-L1 staining was found to correlate strongly with a lack of clinical benefit. The presence of PD- L1 staining was associated with response, but expression was not absolutely required for activity. These initial efforts spawned at least three proprietary biomarker assays, which, as will be seen next, have been evaluated prospectively in several large-scale trials, and PD-L1 positivity has been required for trial entry in several studies. Clinical considerations Melanoma Given the impressive activity of PD-1 blockade noted in the ini- tial phase I and Ib trials, it is not surprising that early develop- ment focused strongly on melanoma. One factor that complicated early development was the then-recent approval of agents that in- hibit the activity of a mutant kinase (BRAF V600E) that drives the malignant phenotype in about a third of melanoma patients. The key phase I trial of the PD-1 blocking antibody enrolled approxi- mately 150 patients with advanced melanoma and who must have had either one or two prior lines of therapy. Separate cohorts in- cluded patients who had received anti-CTLA-4 (ipilimumab) as a prior therapy. Clear evidence of activity was noted at both a 2 mg/ kg and a 10 mg/kg dose (every 2 weeks), with an objective response rate of approximately 25%. These responses were generally durable, with most persisting at least one year. Pembrolizumab was granted accelerated approval by the US FDA in 2014. Later that same year, nivolumab was also granted approval in advanced melanoma, based on similar data from a 120-patient cohort from the phase Ib trial, coupled with safety data from an

ongoing trial randomizing patients to either nivolumab or chemotherapy. These two approvals repre- sented a landmark in cancer immunotherapy, as they were the first approvals of PD-1 blocking agents. A subsequent monotherapy study in melanoma extended the in- dication for one of these agents to first-line patients. In a randomized phase III study, nivolumab was compared to dacarbazine chemo- therapy in previously untreated patients who lacked the BRAF V600E mutation that would render their disease treatable by kinase inhibitors that block the activity of this driver mutation. The response rate to nivolumab in these untreated patients was 40%, as compared to 14% for chemotherapy. At one year, a clear survival benefit was demonstrated, with 73% of patients alive in the nivolumab arm, as compared to 42% in the chemotherapy arm. So, for patients without the BRAF V600E mutation, nivolumab represented a superior first- line treatment option. The utility of PD-L1 staining as a predictive biomarker was also evaluated here. Nivolumab was superior to dacarbazine chemotherapy in patents with either PD-L1 positive or negative tumours, but objective responses were more prevalent in the patients whose tumours were positive for PD-L1 versus those that were negative or indeterminate (53% versus 33%). But even in the PD-L1 negative/intermediate group, nivolumab was superior to dacarbazine in both response rate and progression-free survival, meaning that the biomarker has comparatively little clinical utility in the first-line setting for BRAF V600E negative patients. Kidney cancer As discussed in this chapter already, the first phase I and phase Ib trials showed clear evidence of clinical activity of PD-1 blockade in RCC. Those data were confirmed in a 160-patient dose-finding study, which surprisingly showed that q 3-week dosing with nivolumab at 0.2, 2, and 10 mg/kg resulted in an objective response rate of ap- proximately 20% which was not at all affected by dose levels. An extensive biomarker analysis confirmed this result, showing essen- tially no meaningful difference in any parameter examined between doses. A phase III trial was eventually launched, consistent with the regimen used in melanoma, a dose of 3 mg/kg q 2 weeks was chosen. This trial randomized approximately 800 RCC patients who had progressed on one or more lines of prior antiangiogenic therapy to either the anti-PD-1 antibody nivolumab or to the mTOR in- hibitor everolimus. The trial met its overall survival end point, with an overall survival of 25 months in the nivolumab arm as compared to 20 months for everolimus. Objective responses were also more common in the anti-PD-1 arm at 25% versus 5% for everolimus. Interesting, there was no difference in radiographic progression- free survival between the arms. Toxicity was quite similar to that observed in lung cancer and melanoma, with approximately 20% of patients having a grade III or IV that required intervention. Subgroup analysis showed benefit across multiple categories, with the fascinating exception that there appeared to be a greater benefit of nivolumab (versus everolimus) in patients with a poor MSKCC prognostic score. Based on these data, nivolumab was approved by the US FDA in late 2015. Non-small cell lung cancer Given the known efficacy of other immunological modalities in mel- anoma and kidney cancer, the activity of PD-1 in those two cancers was perhaps not particularly surprising, although the rate of re- sponses clearly exceeded prior data with cytokines and PD-1 blockade was obviously much better tolerated. What was perhaps surprising was the activity of PD-1 blockade in lung cancer, a tumour type that was previously thought to be nonimmunogenic. As described, clear activity was noted in the phase Ib trial of nivolumab, leading to fur- ther studies in both squamous and nonsquamous disease. The first regulatory approval for a PD-1 blocking antibody in lung cancer came in early 2014 in metastatic squamous NSCLC, and was based

 on data from a single-armed phase II study as well as preliminary data comparing nivolumab versus docetaxel in second-line disease. The randomized

second-line study was stopped early because an in- terim analysis determined that it met its primary overall survival end point, with an overall survival of 9.2 months for nivolumab, versus 6 months for docetaxel chemotherapy. Prospective evalu- ation of PD-L1 status as a predictive biomarker for response was not revealing, overall survival was improved in the nivolumab arm re- gardless of PD-L1 status. In late 2015, pembrolizumab was also ap- proved in advanced NSCLC. Interestingly though, this approval was granted only in concert with the use of a companion diagnostic (i.e. for patients with PD-L1 positive disease). Subsequently, nivolumab was also approved for nonsquamous NSCLC, again without the use of a companion diagnostic. So, at the current time, both PD-1 blocking antibodies are approved in second-line lung cancer under appropriate conditions. Clinically, use of the companion diagnostic is likely to delay treatment in certain patients, but conversely may help to insure a greater risk/benefit ratio for these drugs. Ongoing studies of PD-1 blocking agents in combination with chemotherapy are underway in the first-line setting, potentially extending the spec- trum of eligible patients. Hodgkin's lymphoma In Hodgkin's lymphoma, a genetic amplification of a region of chromosome 9 results in upregulation of the expression of the lig- ands PD-L1 and PD-L2. The amplified region also includes the signal transduction molecule JAK2, resulting in upregulation of PD-1 as well. These data suggested then Hodgkin's lymphoma might be susceptible to PD-1 blockade, and upregulation of both ligands meant that it would be more logical to block PD-1 rather than to attempt to block both ligands. Data supporting this hypothesis was first reported in a relatively small 23 patient trial of patients with refractory Hodgkin's lymphoma; the resulting data are perhaps the most impressive demonstration of PD-1 blockade to date, with a re- ported response rate of 87%, with 17% of patients demonstrating a complete response (CR). A second larger trial demonstrated similar activity, leading to a provisional approval for classical Hodgkin's lymphoma that has progressed after autologous haematopoietic stem cell transplantation and post-transplant brentuximab vedotin. This approval was granted regardless of PD-L1 status. Although PD-1 blockade was well-tolerated in this setting, it was noted that patients who received an allogeneic haematopoietic stem cell trans- plant after nivolumab may be at greater risk of graft-versus-host dis- ease and other transplant-related complications, prompting further study in that setting. Bladder cancer The interaction between PD-1 on T cells and PD-L1 on tumour cells can also be blocked by antibodies directed against PD-L1. Indeed, in animal models of chronic infection, self-tolerance and cancer blocking PD-L1 can be as efficacious as blocking PD-1. So, a human anti-PD-L1 monoclonal antibody was tested in a phase I trial that enrolled approximately 200 patients; the trial included 75 patients with metastatic NSCLC and 52 with metastatic melanoma. The antibody was administered once every 2 weeks in 6-week treat- ment cycles. In general, PD-L1 blockade was well-tolerated with a grade 3 and 4 treatment-related toxicity rate of only 9%. However, the activity of this particular PD-L1 blocking antibody (MDX-1105) appeared to be less than that observed with the PD-1 blocking agents highlighted earlier. In NSCLC patients, for example, the response rate was approximately 10%. While further development of MDX-1105 was not pursued, sev- eral additional PD-L1 antibodies have entered the clinic, both alone and in combination with additional chemotherapy or immuno- therapy agents. The first of these agents to achieve regulatory ap- proval, atezolizumab, was developed most rapidly in urothelial bladder cancer. This is because multiple studies showed that urothelial bladder cancer expresses PD-L1 at similar levels to other tumours (approximately 10–20% of tumour cells) with increased levels of PD-L1 expression seen in more advanced and metastatic tumours compared to early-stage disease. Moreover, increased PD-L1 expression in these tumours has been associated with reduced overall survival and recurrence-free survival following cystectomy. These data supported the notion that bladder tumours may

evade the immune system by up-regulating PD-L1 expression. Following intriguing activity observed in a phase I study, a mid-sized phase II nonrandomized study was launched in patients with metastatic bladder cancer. Two cohorts were included; the first was a 300- patient cohort who had progressed after platinum-based chemo- therapy (i.e. a standard second-line bladder cancer population). An additional, smaller cohort was enrolled simultaneously; this was a group of patients who were not eligible for platinum-based chemotherapy because of either poor performance status or because of decreased renal function. In the larger, post-platinum cohort, an overall response rate of approximately 15% was reported, with higher rates of response in patients with PD-L1 expression in the tumour or in tumour-infiltrating immune cells. Impressively, 12- month overall survival was approximately 40% for these patients, comparing favourably with prior studies of chemotherapy in this setting. Based on those data, the PD-L1 antibody atezolizumab was approved by the US FDA for the treatment of metastatic bladder cancer. The first-line data from the platinum-ineligible cohort were also noteworthy, with a response rate of approximately 24%. There are multiple ongoing studies of PD-1 and PD-L1 blockade in bladder cancer and it appears likely that additional agents may receive regu- latory approval within the next several years. Toxicity and adverse events In multiple trials, with several different blocking antibodies, and in multiple tumour types the toxicity of PD-1 and PD-L1 blockade ap- pears to be fairly similar. A common low-grade toxicity is fatigue, which has been reported in 25–40% of treated patients across trials. Fatigue has been reported in both advanced and less advanced dis- ease settings, suggesting that this side effect of PD-1 blockade is not simply a reflection of the underlying cancer pathology. Endocrine toxicities are also relatively common, with hyperthyroidism occurring in approximately 10% of treated patients across series. Grade III/IV hypothyroidism has also been noted, although this is considerably less common. Acute hyperthyroidism usually re- solves within 4–8 weeks, but hypothyroidism may persist long term, and require ongoing replacement. Rash and/or other skin toxicity is also fairly common, reported in approximately 10–15% of pa- tients; these adverse events are generally mild although more ad- vanced cases may require treatment with oral corticosteroids. The incidence of PD-1-related diarrhoea varies considerably among trials, with some studies reporting low (<5%) incidence, and others

 a higher (20%) rate of this adverse event, which is by contrast quite common with CTLA-4 blockade. Perhaps the most worrisome ad- verse event mediated by PD-1 blockade is inflammation of the lung (pneumonitis) which was responsible for three deaths in the phase Ib study of nivolumab. Subsequent studies showed that this was a true side effect, but with a real rate that is likely less than 3–5%. Still, distinguishing worrisome pneumonitis from disease progression can be challenging—particularly in patients with lung cancer or with other cancers metastatic to the lungs. Management of immune- related toxicities is facilitated by several widely available treatment algorithms which mostly involve a stepwise progression from fre- quent monitoring, followed by increasing doses of corticosteroids and hospitalization if the patient fails to respond adequately after the adverse event. Immune agonist approaches Introduction In addition to immune checkpoint molecules, whose engagement downregulates T-cell function, there are several molecules that pro- vide an activating signal to T cells. So, the ultimate outcome of a T cell's interaction with a dendritic cell involves an integration of both the positive and negative signals present during that interaction. While still in early stages of development, agonist antibodies that activate immune cells have tremendous potential in cancer immun- ology, and are briefly discussed here to provide a context for future developments. OX40 on T cells OX40 (CD134) is a member of the tumour necrosis factor (TNF) receptor

superfamily, and is a cell surface molecule expressed after either CD4 or CD8 T cells recognize their specific antigen. Engagement between OX40 on a T cell and OX40 ligand on an ad- jacent dendritic cell provides a powerful costimulatory signal to the T cell. Additional data show that OX40 engagement might also pro- vide an inhibitory signal to regulatory T cells, mitigating their sup- pressive function and driving them towards an effector phenotype. In a phase I study of a murine anti-OX40 in patients with advanced cancer, the drug had a reasonable safety profile and induced the re- gression of at least one metastatic lesion in perhaps 40% of patients. This murine anti-OX40 is now in clinical trials in combination with chemotherapy and radiation several tumour types.
A second anti- OX40 antibody also is undergoing phase I testing; the remarkable activity of OX40 blockade in multiple murine tumour models ren- ders these trials of special interest. 41BB/CD137 on T cells Like OX40, 4-1BB is also a member of the TNF receptor super- family, and is expressed on natural killer cells and activated T cells. Natural killer cells are a population of lymphocytes that kill their targets using similar mechanisms to CD8 T cells, but recognize their targets based on loss of MHC molecules and other manifestations of cellular stress. The ligand of 4-1BB (4-1BBL) is expressed on ac- tivated dendritic cells, and the signal transmitted from 4-1BBL to 4-1BB leads to increased T-cell proliferation and the expression of antiapoptotic molecules. In an early phase I dose escalation study, an agonist 4-1BB antibody (urelumab) was well-tolerated in patients with metastatic melanoma, and while only 6% of patients had a par- tial response, 17% of patients showed stable disease at 6 months. Based on those encouraging results, a phase II trial was launched but several patients experienced severe hepatitis and the trial was discontinued.
A different 4-1BB antibody is currently in three phase I trials in combination with pembrolizumab in advanced solid tu- mours, and in combination with the anti-CD20 antibody rituximab in patients with Hodgkin’s lymphoma. Urelumab has also re-entered clinical testing and is now in multiple phase I and II clinical trials in a variety of haematological and solid malignancies; these trials generally involve lower doses of anti-41BB than were explored in the earlier phase I studies. CD40 on dendritic cells Unlike OX40 and 4-1BB, CD40 is predominantly expressed on den- dritic cells, and ligation of CD40 by its ligand CD40L leads to stimu- lation and maturation of DCs. The ligand, CD40L, is expressed on activated CD4 T cells, and is one way in which ‘help’ is transmitted from CD4 T cells to other cells in the immune system. DCs that re- ceive a CD40 signal become much more potent in terms of being able to subsequently activate CD8 T cells; thus, this is an indirect mechanism by which full CD8 T-cell activation can be achieved. As is the case for OX40, preclinical studies using anti-CD40 in murine models has been quite compelling with CRS reported in several ag- gressive murine tumour types. In a phase I study in patients with metastatic solid tumours, an anti-CD40 agonist antibody was well- tolerated with 14% of patients showing a partial response. As is the case for the other agonist antibodies, multiple ongoing trials are underway. Immunotherapy combinations Combining checkpoint blockade with VEGF-targeted agents Vascular endothelial growth factor (VEGF) plays a key role in tu- mour progression by promoting angiogenesis; this generation of new vasculature is critical in facilitating tumour growth. Several agents that block VEGF signalling are used in cancer treatment, these include the monoclonal antibody bevacizumab which binds to VEGF and prevents it from binding to its receptors, as well as small molecule tyrosine kinase inhibitors commonly used to treat kidney cancer. Mechanistically, the antitumour effects of VEGF inhibition include the inhibition of new vessel formation, the ‘pruning-back’ of recently formed vasculature, and a normalization of the structure of existing intratumoural blood vessels. The last of these is of particular interest from an immunological perspective, as the abnormal vasculature that predominates in many tumour types is hostile to T-cell emigration, so the normalization of that vasculature facilitates the egress of CD8 T cells from the circulation into

the tumour bed. VEGF likely plays several other roles in sup- pressing an antitumour immune response: (1) it inhibits the matur- ation of DC, leading to decreased presentation of tumour antigens to T cells; (2) these immature DC drive the differentiation of regulatory T cells, which directly inhibit antitumour responses; and (3) VEGF signalling promotes the induction of a population of myeloid cells with regulatory function, so-called myeloid suppressor cells, which also inhibit an antitumour immune response. There is thus a solid

5.4 Cancer immunity and immunotherapy 485 rationale for combining immunotherapy agents with inhibitors of the VEGF pathway. In RCC, where angiogenesis plays a critical role in tumour pro- gression, VEGF inhibition (with first-generation tyrosine kinase in- hibitors) was combined with the PD-1 blocking antibody nivolumab in several early phase trials. While a high percentage of responses were noted, the combination resulted in what appeared to be addi- tive toxicity, so further development of those combinations was halted. Later-generation tyrosine kinase inhibitors (TKIs), axitinib for example, appeared to be more amenable for development in combination with PD-1 or PD-L1 blockade, and several randomized phase III trials comparting conventional treatment to the combin- ation of TKI plus immunotherapy are currently underway. Similar combinations based on the anti-VEGF monoclonal antibody have also been evaluated; these also showed good tolerability and a high rate of objective response, although the clinical experience to date is rather limited. Perhaps most encouragingly, patient samples from trials of bevacizumab plus the anti-PD-L1 antibody atezolizumab showed increased post-treatment CD8 T-cell infiltration, consistent with the proposed mechanism of action, and several ongoing phase III trials are comparing the atezolizumab/bevacizumab combination to standard of care treatment in patients with RCC. Similar combin- ations are being explored in NSCLC and other diseases as well. Combining CTLA-4 and PD-1 blockade CTLA-4 is highly expressed on the regulatory CD4 T cells that in- filtrate tumours, whereas most CD8 T cells within tumour express significant levels of PD-1. These observations suggested that com- bining PD-1 blockade with CTLA-4 blockade could lead to an improved antitumour T-cell response; indeed several preclinical animal models confirmed a dramatic synergy for this combination. Sequential treatment was less effective, it appeared that simultan- eous blockade of both checkpoints was important for efficacy. These results were confirmed in patients, initially in a relatively small series of patients with melanoma where the combination of the anti-PD- 1 antibody nivolumab with the anti-CTLA-4 antibody ipilimumab led to a response rate of approximately 50%, which is greater than that observed for either agent alone. Similar high response rates have also been reported in patients with kidney cancer, as well as in several additional tumour types. Notably, most objective responses associated with combined checkpoint blockade occur rapidly (i.e. within the first 2–4 months of treatment). These data led to a phase III trial in patients with metastatic melanoma; this two-armed, 142- patient trial randomized patients 2:1 to receive either ipilimumab plus nivolumab, or ipilimumab alone. The trial showed a signifi- cant increase in the overall response rate for combined treatment (60% vs. 11%), the increase in objective response rate was paralleled by a similar increase in progression-free response and duration of response. Based on those data, the combination was approved by the US FDA to treat BRAF V600 wild-type melanoma. Multiple ongoing trials are evaluating various anti-PD-1 or anti-PD-L1 plus anti-CTLA-4 combinations in a variety of tumour types including non-small cell lung cancer, kidney cancer, bladder cancer, and sev- eral others. Enthusiasm for the impressive clinical responses that occur in response to combined PD-1/CTLA-4 blockade must be tempered by the high rate of serious toxicity reported whenever the combin- ation has been tested. In general, the rate of serious (grade III or IV) adverse events has been in the range of 50–70%; these events are

associated with a high rate of treatment discontinuation. Many of the reported adverse events are similar to those previously re- ported with anti-CTLA-4 (ipilimumab) monotherapy, but of greater severity. Thus, colitis is common, as is hepatitis. Endocrinopathies, including thyroid dysfunction and hypophysitis, are more prevalent than observed with either monotherapy. Clinically, these toxicities are usually manageable by initiating treatment with corticoster- oids, and escalating treatment to include other immunosuppressive agents like TNF-α-blocking drugs. If treated early and aggressively, most of these grade III and IV immune-related events resolve, but conditions reminiscent of chronic autoimmunity have been re- ported in successfully treated patients. As is the case for immune checkpoint blockade monotherapy, treatment algorithms are avail- able to assist in the management of immune-related toxicity. If such combinations become commonplace in cancer treatment, medical oncologists will need to expand their skills beyond typical chemo- therapy related complications like cytopenias, infection, and nausea/ vomiting to become facile at managing autoimmune side effects of immunotherapeutic drugs. Additional immunological combinations The relatively benign toxicity profile of agents that block the PD-1/ PD-L1 pathway fuelled enthusiasm for combining these drugs with a wide variety of other agents. Combinations involving conventional chemotherapy are attractive, especially given medical oncologists' familiarity with standard chemotherapy drugs. But it should be ap- preciated that conventional chemotherapy agents are frequently immunosuppressive; so combinations involving the sequencing of chemotherapy with immunotherapy might prove more logical than simple concomitant administration. Given the wide range of chemotherapy regimens already in common use for the more preva- lent cancers, it is not surprising that much late stage development involves chemotherapy/immunotherapy combination regimens. Combinations of multiple immunotherapy drugs are also of interest, especially given the potentially synergistic efficacy seen with com- bined PD-1/CTLA-4 blockade. Attractive partnerships in that regard include additional checkpoints like LAG-3, TIM-3, and others, as well as combining PD-1 blockade with agonist antibodies intended to stimulate an antitumour response. One particularly interesting series of combination involves agents that inhibit the enzyme indolamine 2,3 deoxygenase (IDO). Within the tumour microenvir- onment, IDO catabolizes the degradation of tryptophan which is important because this amino acid is essential for T-cell survival and function. The upregulation of IDO in tumours is a good example of subversion of a physiological pathway; this pathway is critical in maintaining maternal-fetal tolerance during neonatal development. Several drugs that inhibit IDO have been clinically evaluated, and while monotherapy has generally been disappointing, early results suggest that IDO blockade may prove additive or synergistic with immune checkpoint blockade in several tumour types. Summary/Future directions Cancer immunity is a field that is evolving extremely rapidly, and by the time this chapter is published it is highly likely that add- itional monotherapy and combination regimens will be approved

 in multiple tumour types. Nevertheless, the basic immunological principles outlined at the start of this chapter are unlikely to evolve significantly, hence understanding the basic mechanisms underlying an adaptive antitumour immune response should be valuable in understanding future agents, as well as their toxicities. Looking ahead further, it seems likely that most tumour types will eventually be treated with immunotherapy of some sort, potentially with the type of durable long-term response seen occasionally with current regimens. FURTHER READING Batlevi CL, Matsuki E, Brentjens RJ, Younes A (2016). Novel immuno- therapies in lymphoid malignancies. Nat Rev Clin Oncol, 13, 25–40. Boutros C, et al. (2016). Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination. Nat Rev Clin Oncol, 13, 473–86. Disis ML, Bernhard H, Jaffee EM (2009). Use of tumour-responsive T cells as cancer

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