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The determinants of tumour immunogenicity

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Abstract

Many standard and targeted therapies, as well as radiotherapy, have been shown to induce an anti-tumour immune response, and immunotherapies rely on modulating the host immune system to induce an anti-tumour immune response. However, the immune response to such therapies is often reliant on the immunogenicity of a tumour. Tumour immunogenicity varies greatly between cancers of the same type in different individuals and between different types of cancer. So, what do we know about tumour immunogenicity and how might we therapeutically improve tumour immunogenicity? We asked four leading cancer immunologists around the world for their opinions on this important issue.

What are the most important determinants of tumour immunogenicity, in your opinion?

Thomas Blankenstein

Immunogenicity, which is the ability to induce adaptive immune responses, has been widely analysed by cancer cell transplantation experiments. Cancer cells that are rejected in naive syngeneic mice (which are known as regressors) are considered highly immunogenic. If rejection requires prior immunization, those transplanted cancer cells (which are known as progressors) are considered intermediate immunogenic. Failure to reject the tumour following immunization against trans-planted cancer cells classifies them as non-

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immunogenic. Low immunogenicity of cancer cells has often been attributed to selective immune processes in the primary tumour-bearing host. However, sporadic immunogenic tumours (which are regressors when transplanted) progress in the primary hosts, even though the primary host has $CD8^+$ T cells that are specific for the transplantation rejection antigen, which can induce life-long protection by prophylactic vaccination[1]. This indicates that the non-destructive immune response (that is, T cells that did not halt tumour growth) against immunogenic tumours can be converted into a destructive response on transplantation. Additionally, a progressor–regressor phenotype was shown to correlate with tumour growth kinetics but not with the absence or the presence of a rejection antigen[2]. Most transplanted cancer cells are adapted for atypical fast growth *in vivo*, which does not occur in the primary host. Therefore, cancer cell transplantation experiments do not recapitulate immune responses in the autochthonous host.

Tumours regularly induce adaptive immune responses in the autochthonous host, in cancer-prone mice and in humans. IgG antibodies specific for more than 2,000 antigens, which are often overexpressed by cancer cells and which are almost all selfantigens, have been detected in the serum of cancer patients (see the Cancer Antigen Discovery Program; see Further information). In mouse transplantation models, these antigens did not evoke rejection[3]. $CD8^+$ T cell-mediated responses against a number of tumour antigens, many of which were selfantigens, have been detected in tumours and in the blood of cancer patients[4]. In a mouse model of sporadic immunogenic cancer, rare stochastic tumour antigen (oncogene) expression induced immune tolerance in the premalignant phase, which was accompanied by the induction of tumour-reactive IgG antibodies, the expansion of anergic $CD8^+$ T cell populations and substantial infiltration of T cells into the lesions[1]. Immune dysfunction was observed in mice bearing immunogenic tumours but not in those bearing non-immunogenic tumours, probably because of chronic antigen stimulation[1]. Therefore, it seems that most tumours induce adaptive immune responses and, therefore, are immunogenic. The remaining issues are whether these immune responses are destructive or non-destructive for the tumour cells, and whether — and under which conditions — immune tolerance is preceded by a destructive T cell response, which is termed immunosurveillance. The substantial T cell infiltrate in premalignant lesions indicates that cancer cell-induced chronic inflammation is not a destructive response, because the host mice had already acquired immune tolerance[1].

Pierre G. Coulie

It is now widely accepted that human tumours are immunogenic, meaning that they elicit adaptive immune responses *in vivo*. These responses are mostly mediated by T cells. Spontaneous anti-tumour T cell responses occur frequently in cancer patients, and analyses of these patients led to the molecular identification of tumour antigens that are recognized by T cells. The presence of T cells within tumours, which is often associated with a more favourable clinical outcome, is probably a consequence of these spontaneous responses. The immunogenicity of a tumour depends on its antigenicity and on several other immunomodulatory factors that are produced either by tumour cells or by host cells in the tumour microenvironment. The rules governing the antigenicity of tumours towards T cells have been established by the work of T. Boon and colleagues[5]. T cells recognize peptides that are presented by human leukocyte antigen (HLA; also known as major histocompatibility complex (MHC)) molecules, and a few genetic mechanisms explain the existence of tumour-specific antigenic peptides: first, cancer-germline genes, such as members of the melanoma antigen (MAGE) A, B or C families and LAGE families, encode tumour-specific antigens in many types of tumour cells[6]. These antigens are tumour-specific because, normally, only male germline cells (spermatocytes and spermatogonia) express cancer-germline genes, but these cells do not carry HLA molecules and, therefore, do not

present the cancer germline gene products as antigens to T cells. However, the presentation of some cancer-germline antigens by medullary thymic epithelial cells[7], which present selfantigens for T cell education in the thymus, might lead to some degree of T cell tolerance to these tumour-specific antigens. This tolerance can be overcome by the expression of the cancer germline genes by tumour cells, which thus promotes tumour antigenicity. Second, tumour-specific antigenic peptides can be produced by mutation (mostly point mutations), which leads to a modified peptide sequence or, rarely, to modification of the processing of the peptide. Third, oncogenic viruses (for example, human papilloma virus (HPV) and Epstein–Barr virus (EBV)) can encode antigenic peptides. Last, the expression of tissue-specific genes, notably in melanoma and prostate carcinoma, and the overexpression (compared with normal cells) of some genes in tumour cells, may all contribute to the presence of tumour-associated, but not truly tumour-specific, antigenic peptides.

Although probably all tumours carry antigens that can be targeted by T cells, some tumours express more antigens than others. In addition to differences in HLA expression, a few explanations for these differences can be proposed: DNA demethylation of cancer-germline genes, which induces their expression; loss of DNA mismatch repair, which leads to more 'antigenic' mutations in colorectal carcinomas[8]; and the expression of melanocyte-specific antigens, which could explain the greater clinical efficacy of various immune interventions in melanoma than in other cancers.

Many immunomodulatory mechanisms operate in tumours. Most of them inhibit anti-tumour immunity, and a few are mentioned below. One notable exception to this growing list is the observation that killing tumour cells with some chemotherapeutic drugs is followed by an anti-tumour immune response that is mediated by T cells, although this does not occur when using other drugs[9]. These results suggest that oncologists have been using immunotherapy without knowing it. They also open up interesting avenues for investigating chemotherapy plus immunotherapy combinations.

Eli Gilboa

Epidemiological studies have provided compelling, although perhaps not definitive, evidence that growing tumours in cancer patients can elicit a protective immune response, which can slow down — but not reverse — tumour growth[10]. Weak antigenicity is the root cause of why the immune system ultimately fails to control tumour growth; weak tumour antigens stimulate a weak, and thus slow, immune response that provides the opportunity and time for tumour cells to develop immune evasion mechanisms and to ultimately gain the upper hand. Of the two arms of the adaptive cellular response, $CD8^+$ T cells are thought to constitute the main effector arm of the anti-tumour immune response. Although the importance of $CD4^+$ T cells for generating $CD8^+$ T cell responses is well appreciated, accumulating evidence suggests that $CD4^+$ T cells can also exhibit effector functions against MHC class II moleculenegative tumours, and could in fact be superior to $CD8^+$ T cells at mediating anti-tumour immune responses[11,12].

Tumours develop a plethora of immunosuppressive mechanisms early in tumour development[13], and when that immunosuppression fails, resistant variants emerge such that antigen processing or the expression of dominant antigens are downregulated in the tumour cells[14]. Consequently, the local tumour immune response becomes increasingly compromised, and the immune cells exhibit reduced effector functions and limited persistence; this limited persistence is emerging as a key factor in the failure of the immune response to control tumour progression[15]. The tumour stroma has a pivotal role in establishing a hostile immune environment through the secretion of immunosuppressive factors and the recruitment of suppressive immune cells of myeloid and lymphoid origin. Tumour cells also directly contribute to immune resistance through the secretion of

immunosuppressive mediators, such as transforming growth factor-β (TGFβ) or natural killer (NK) cell receptor decoys, and through the expression of ligands for immune checkpoint (or co-inhibitory) receptors, such as programmed cell death 1 ligand 1 (PDL1). In addition — and just as important — is the limited access of the immune cells to the tumour cells. Cells and macromolecules cannot easily penetrate into the tumour microenvironment owing to physical barriers, such as a dense extracellular matrix (ECM) and a high interstitial fluid pressure that is caused by a lack of lymphatic drainage[16], as well as owing to cellular and molecular barriers that are expressed by endothelial cells, such as regulator of G protein signalling 5 (RGS5)[17] and endothelin B receptor (ETBR)[18].

Elizabeth M. Jaffee

Tumour immunogenicity is simply defined as the ability of a tumour to induce an immune response that can prevent its growth. But our understanding of the mechanics of this complex process is still evolving. In 1909, Paul Ehrlich was the first to put forth the hypothesis that the immune system has the ability to repress cancer growth. Burnet later provided a mechanism for this, theorizing that tumours express neo-antigens that induce immune responses that are capable of eliminating developing tumours[19]. Lewis Thomas[20] took an evolutionary view, theorizing that organisms have well-developed mechanisms that are similar to graft rejection mechanisms that protect against cancer formation. These theories led to the concepts of cancer immunosurveillance and immunoediting[21]. The mechanism that was originally thought to explain tumour immunogenicity was that the immune system discriminates between self-antigens (antigens that are expressed by normal tissue) and non-self antigens (distinct antigens that are unique to tumour cells), and thus rejects tumours that have escaped immune recognition[22]. Although some evidence supports this proposed mechanism, it does not explain tumours that continue to progress and that are not rejected by the host immune system despite the expression of non-self antigens, nor does it explain the presence of many foreign antigens expressed by bacteria that reside symbiotically in the gut.

To explain these inconsistencies, Pradeu and Carosella[23] suggested that immunogenicity does not depend on whether an antigen is detected as non-self, but rather, that immunogenicity depends on the tumour cell presenting new antigen epitopes that are different from what the immune receptors have regularly experienced. They referred to this as an ‘abrupt’ change in epitope presentation. However, to explain immune tolerance, Pradeu and Carosella also correctly stated that a change in epitope presentation is not sufficient for immunogenicity[23]. How the new antigen is presented in the context of the frequently presented antigens will determine whether immunity or tolerance is induced. If the new antigen is presented in low quantities and with small progressive modifications, then immune tolerance occurs. Thus, taking into account recent observations and experimental data[24,25], at a minimum, immunogenicity requires that tumour cells express adequate levels of antigens that are uniquely expressed by the tumour cells relative to the normal cell from which the tumour was derived, and that the tumour cells effectively present antigens in a form that leads to immune activation instead of immune tolerance.

What are the possible mechanisms by which tumour immunogenicity is regulated?

T.B.

The mechanisms that regulate tumour immunogenicity are different for tumourspecific (non-self) antigens (TSAs) and tumour-associated (self) antigens (TAAs). High-avidity T cells that are specific for TAAs are usually deleted in the thymus, and low-avidity T cells (of uncertain therapeutic value) remain. Because T cell-mediated responses are similarly

detected against TSAs and TAAs, the classical definition of immunogenicity — self (not-to-be-ridded) versus non-self (to-be-ridded) discrimination[26] — does not apply to spontaneous T cell-mediated immune responses against tumour cells. Tumour immunogenicity can be better explained by an antigenic discontinuum[23]. According to this model, antigens that are produced in sufficient quantities and that are released with appropriate kinetics, as occurs for TAAs during malignancy and accompanying cell death, induce adaptive immune responses, notably within the narrow range of the T cell repertoire that is normally skewed against self-antigens. Because tumours progress despite measurable B cell and T cell responses, both in mice[1] and in humans[27], antigenic discontinuum probably induces non-destructive immune responses. This is often misleadingly referred to as tumour immunity.

However, antigenic discontinuum of TAAs cannot explain why tumours progress despite the expression of TSAs and in the presence of TSA-specific T cell-mediated immune responses[4]. Both the inflammatory conditions and when TSA recognition occurs during tumour evolution probably influence the outcome of T cell activation. Under acute inflammatory conditions, as occurs during infection with tumour-associated viruses, T cells are efficiently activated and either prevent tumour development or select ‘immune escape’ variants[28]. Initial tumour antigen recognition under resting conditions or under conditions of chronic inflammation results in immune tolerance[1]. Some researchers suggested that autochthonous, methylcholanthrene-induced tumours induce a destructive T cell response[29]. However, these data were not reproducible when appropriate control mice were used[30,31]. Convincing evidence from experimental cancer models that non-pathogen-associated autochthonous tumours induce a destructive tumour antigenspecific T cell response is still lacking. This is compatible with the observation that only virus-associated cancers (all of which may not yet be known) — and not the most common forms of cancer, such as breast, prostate, lung and colon carcinomas — occur with increased frequency in immune-suppressed patients[32]. It seems that viral infection induces a to-be-ridded immune response, whereas, TSAs, even though they are truly foreign antigens, induce a not-to-be-ridded immune response. How frequently somatic mutations in cancer cells create novel T cell epitopes is currently unknown. One should not exclude exceptions from the rule — for example, melanoma. However, studies in humans remain descriptive, and current experimental models rarely recapitulate the sporadic nature of cancer.

P.G.C.

Numerous immunomodulatory mechanisms have been described that either render tumour cells less sensitive to immune attack (resistance) or inhibit anti-tumour immune responses (immunosuppression). Tumour cells that either directly suppress anti-tumour immune responses or induce other cells to do so are probably selected for by the spontaneous occurrence of anti-tumour immune responses.

The most common cause of resistance is decreased antigenicity owing to HLA class I molecule downregulation or loss, which frequently occurs in human tumours[33]. Some HLA molecule defects are reversible with cytokines, such as interferon-γ (IFNγ). Defects in the antigen-processing machinery have also been observed.

A plethora of immunosuppressive mechanisms has been described in tumours, and very well reviewed[34]. The immunosuppressive factors that are contributed by tumour cells include the expression of programmed cell death protein 1 (PD1) ligands; local tryptophan depletion through indoleaminepyrrole-2,3-dioxygenase (IDO)[35] or tryptophan-2,3-dioxygenase (TDO)[36,37]; and the production of galectin 3, which reversibly impairs T cell activation[38], lactic acid, adenosine, prostaglandins and transforming growth factor-β (TGFβ). I limited this list to mechanisms that could be reversed by pharmacological agents or antibodies. The immunosuppressive mechanisms that are contributed by non-tumour cells include regulatory

T (T_{Reg}) cells that could be attracted to some tumours by chemokines[39,40], and myeloid-derived suppressor cells and their contact-dependent immunosuppression, which involves nitrogen oxide (NO) and reactive oxygen species (ROS)[41].

Notably, all of these mechanisms act locally. Therefore, drugs, antibodies or cytokines that block them could be administered locally, provided that relieving suppression activates systemic immune effector cells that eliminate other, non-treated tumour cells at distant sites and that prevent the appearance of metastases. The analysis of a few patients who responded clinically to cancer vaccines suggested that this is indeed possible, and that it involved either priming of new T cells through antigen spreading or the re-stimulation of pre-existing tumour-specific T cells[42,43].

E.G.

The main culprits for failed anti-tumour immune responses are the professional (dendritic cells) and semiprofessional (macrophages) antigen-presenting cells (APCs) in the tumour milieu that suboptimally activate their cognate T cells. This leads to reduced effector functions of T cells, which consequently fail to persist and expand, and which can even acquire immunosuppressive properties.

So, what controls the APCs? Initially, weak antigenicity elicits a suboptimal inflammatory response that leads to reduced expression or to the secretion of ‘danger’ signals to fully activate the APCs. At this early stage though, the immune system still has the upper hand, as epidemiological studies show[10]. Subsequently, epigenetic immunosuppressive mechanisms are activated to defeat this slow-acting immune response. Tumours will express (for example, PDL1) and secrete (for example, TGFβ) immunosuppressive products and will coordinate a tumour milieu with immunosuppressive properties that is characterized by the infiltration of immunosuppressive cells of myeloid and lymphoid origin.

Key unanswered questions are, which are the upstream tumour cell-expressed mediators that initiate the cascade of events that lead to immunosuppression? And how common are they among different tumours? Probably at a much later time, when immunosuppression fails, resistant tumour cell variants subsequently emerge that have lost dominant antigens or that harbour defects in antigen-processing pathways[14]. Although there is compelling evidence that both epigenetic and genetic mechanisms contribute to immune evasion, I suspect that the epigenetic mechanisms are the immediate and primary mechanism. The upregulation of PDL1 on the surface of tumour cells, the secretion of TGFβ and the infiltration of immunosuppressive cells into the tumour milieu occur much earlier than genetic resistance emerges, which is an inherently slow process[44]. The penetration of immune cells into the tumour, as well as tumour hypoxia, also represents important impediments to immune-mediated control of tumour growth[16]. Whether the tumour actively regulates these processes for the purpose of preventing immune-mediated elimination is, however, unknown.

E.M.J.

Several mechanisms are known to regulate both antigen expression and antigen presentation, which are the two major criteria that dictate tumour immunogenicity[45]. Genetic and epigenetic mechanisms have been shown to regulate the expression of some tumour antigens. As one example, neo-antigens are produced as a result of genetic alterations, including mutations in oncogenes (such as *KRAS*) and chromosome rearrangements (such as *BCR-ABL1*)[25,46]. As a second example, cancer testis antigens (also known as cancer-germline antigens), which are a family of antigens that have limited expression in non-cancer tissues (male germline cells, some ovarian tissue and trophoblasts), are expressed by tumour cells from a number of cancers, and this expression is often induced by either

hypomethylation or histone acetylation of the encoding genes[47]. As a third example, annexin A2, which is normally involved in embryogenesis, was identified as an antigen that is recognized by vaccine-induced antibodies in responding patients with pancreatic cancer. Annexin A2 expression is upregulated in pancreatic cancer cells relative to normal pancreatic tissue, and this upregulation is associated with its relocation from the cytosol to the cell surface owing to phosphorylation at Tyr23, which facilitates its recognition by antibodies[48].

There are also known mechanisms that regulate antigen processing and presentation. Mechanisms that regulate antigen processing and presentation in APCs alter the form and the quantity of the epitopes that are presented for immune recognition. For example, alterations in epitope processing can occur owing to changes in proteasomal or post-proteasomal machinery, and these changes result in altered epitope binding to MHC molecules. As another example, both genetic and epigenetic alterations can regulate MHC class I molecule availability for presenting processed epitopes on the tumour cell surface[49]. These cellular changes affect the affinity and abundance of epitopes presented for immune recognition, which ultimately affects the quality of the immune response. But other factors within the tumour and its microenvironment are just as crucial for determining the immunogenicity of tumour antigens. For example, immune checkpoint signalling pathways are increasingly being identified that regulate the effector function of lymphocytes. APCs within the tumour microenvironment and cancer cells have been shown to express inhibitory ligands that provide signals that induce immune tolerance in effector cells[50]. In addition, regulatory cells (T_{Reg} cells, macrophages and subsets of dendritic cells) provide inhibitory signals or secrete inhibitory cytokines that suppress the immunogenicity of a tumour and promote immune tolerance and tumour progression[47]. Further elucidation of these complex interactions will identify opportunities for new therapeutic interventions that alter the balance in favour of tumour immunogenicity.

How might we therapeutically modulate tumour immunogenicity and what do we need to understand to move forwards?

T.B.

Cancer vaccines usually aim to revert tolerance against TAAs after cancer is established, which is a difficult task that has not yet been achieved in either mice or humans. The question is whether self-reactive T cells that survived mechanisms that induce immune tolerance will ever be able to reject established tumours? It is hoped that combinatorial treatments with agents that lower T cell activation thresholds (for example, by blocking PD1 or cytotoxic T lymphocyte-associated antigen 4 (CTLA4) signalling) will increase T cell-mediated responses to TAAs, even though the nonspecific induction of self-reactive T cells brings with it the risk of autoimmunity without tumour regression. If tumour-induced immunosuppression in humans is a symptom rather than a cause of cancer[1], if self-reactive T cells are epigenetically imprinted for a tolerant state[51], if local induction of tolerance impedes systemically functional T cells[52] and if a recent claim of improved survival in prostate cancer following vaccination is not caused by specific T cells but by flaws in clinical study design[53], then cancer vaccinologists are in trouble.

In adoptive T cell therapy, self versus non-self discrimination is of pivotal importance for efficacy. Transferred allogeneic, but not syngeneic, T cells exerted efficient graft-versus-leukaemia effects, because they recognized foreign (minor histocompatibility) antigens[54]. However, the lack of cancer cell specificity often causes graft-versus-host disease. Methods have been developed to isolate human T cell receptors (TCRs) that are specific for almost any human TAA from the repertoire of non-tolerant T cells[55,56]. The expression of such

TCRs by T cells from patients[57] will generate T cells that recognize the self-antigens that the TCRs are specific for as foreign, and, following transfer into hosts with lymphopenia, these might be as effective as allogeneic T cells. Because TAAspecific T cells of high avidity are usually deleted, as they pose an autoimmunity risk, the most important issue to address is which antigen to target[58]. Therefore, analysis of low, so far unrecognized, expression of TAA on vital organs, or better still, at the single cell level, is important.

P.G.C.

In immunotherapy, the simplest way to circumvent the problems of decreased tumour immunogenicity is to act early, before the selection of immune resistant or immunosuppressive tumour cells. Currently, the options are passive immunization through adoptive T cell transfer, active immunization through vaccination, and blocking co-inhibitory signalling of tumourspecific T cells (for example, through the use of CTLA4 or PD1 antagonistic antibodies). These immunotherapies can be combined with each other or with various forms of immunomodulation, including some classical anticancer therapies.

The first problem to overcome is the selection of the tumour antigen or other antigens to target in a given patient. What eventually matters is the number of complexes of a given peptide and a given HLA molecule present on the tumour cell surface that are available for detection by cytolytic T lymphocytes. We do not yet have reliable methods to assess these numbers. Levels of expression of HLA and peptide-encoding genes provide indications, but antigen processing is enormously important and remains generally unpredictable. To detect tumour peptide–MHC complexes we need a technological breakthrough comparable to that of the development of tetramers for T cell detection. Currently, when we observe no clinical benefit in a cancer patient who is passively or actively immunized against a defined tumour antigen, we have no good methods to ascribe the failure to insufficient tumour cell antigenicity or to local inefficiency of the induced or transferred lymphocytes, or to both.

This brings me to the second problem: the immunomodulatory mechanisms that operate in tumours. We need to know more about which mechanisms are relevant in which types of human cancers, and when and where several mechanisms operate simultaneously; we also need to find reliable biomarkers of their activity, and develop non-toxic means of blocking these mechanisms.

E.G.

Vaccination protocols are currently designed to elicit immune responses against antigens expressed by tumour cells. However, recent studies have shown that vaccination against products expressed by the normal constituents of the tumour milieu, such as endothelial cells, macrophages or fibroblasts, is also capable of engendering protective anti-tumour immunity, in the absence of substantial autoimmunity[59,60]. Targeting stromal cell-expressed antigens, therefore, deserves consideration because, unlike tumour cell-expressed antigens, stromal cell-derived antigens are universal, and the problems associated with the emergence of tumour cell variants that have lost expression of the target antigen would be minimal. Also, given the emerging evidence for the pivotal role of $CD4^+$ T cells as effectors in the anti-tumour immune response[11,12], the development of vaccination strategies should be redirected from the current (almost) exclusive focus on stimulating $CD8^+$ T cell responses towards also, or instead, promoting this arm of the immune response. Vaccination protocols target tumour-resident antigens with the aim of stimulating immune responses that will offset the inherently weak antigenicity of the tumour. An alternative, and arguably preferable, approach would be to express new, and thereby potent, antigens in the disseminated tumour lesions of the patient. An approach to achieve this has recently been described[61].

Recent studies have underscored the importance of the persistence of the immune response in protective immunity. Notwithstanding the success of ipilimumab (which antagonizes CTLA4) highlighting the importance of manipulating co-stimulation– co-inhibition signals to control the shortterm persistence of activated T cells, the treatment-associated adverse effects argue that targeting such drugs to the tumour will be useful, if not necessary, as recently suggested[62]. Perhaps even more important will be the ability to promote the long-term persistence of (vaccine-induced) tumour immunity, namely immunological memory[15]. Recent studies have shown that this may be accomplished using pharmacological inhibitors — for example, TSW119, which is an inhibitor of glycogen synthase kinase-3β (GSK3β)[63] — although given their pleiotropic effects, targeting such drugs to the appropriate immune cells may be necessary.

Arguably, countering tumour-induced immunosuppressive mechanisms, which is reliant on identifying the upstream and the dominant immunosuppressive cells and/or mediators that operate in tumours, is likely to be highly beneficial. It will also be important to improve the access of immune cells to the tumours; for example, by blocking RGS5 (REF. 17) and ETBR[18] in endothelial cells, by using Hedgehog pathway inhibitors to reduce desmoplasia[64] or by using CendR motifcontaining peptides[65]. Finally, the future of cancer therapy will witness the combination of immunotherapy with chemotherapies that promote immunogenic tumour cell death[66]. Indeed, combination strategies in cancer (immuno)therapy are a must. The challenge is to identify which of the many choices to pick.

E.M.J.

The first vaccine and the first immunomodulating agent were recently approved by the US Food and Drug Administration (FDA) for the treatment of prostate cancer (sipuleucel-T)[67] and melanoma (ipilimumab)[68], respectively. These two clinical milestones are paving the way for the development of additional vaccines and immunomodulating agents that can enhance the function of effector cells and that can enhance the immune recognition of tumour cells that express immunogenic antigens. The success of future immunotherapy strategies will partly depend on the identification of additional immunogenic antigens that can serve as the best tumour-rejection targets. Although the immunogenicity of an antigen is crucial, it is also important to target antigens that are biologically important to tumour progression. Therefore, we need to better understand how potentially immunogenic antigens are linked to the biology of the cancer. Therapeutic success will also depend on developing the best antigen delivery systems. Issues concerning the optimal vaccine platform (peptide, DNA, protein, viral, bacterial and so on) and the number of antigens that should be simultaneously targeted still need to be addressed. Finally, therapeutic success will also depend on the elucidation of the entire network of immune signalling pathways that regulate immune responses in the tumour microenvironment. These regulatory mechanisms are probably influenced by the specific type of cancer, which has its own unique set of genetic, epigenetic and inflammatory changes that evolve with the advancement of disease. Understanding these networks will provide new targets for modulation and, ultimately, will result in improved combinatorial immunotherapies for the successful treatment of cancers.

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Glossary

Adaptive immune responses	Responses mediated by antigen-specific lymphocytes and antibodies, they are highly antigen-specific and include the development of immunological memory
Allogeneic	From different individuals of the same species
Anergic	A state in which T cells are unresponsive and cannot be activated by antigen
Antigenicity	The ability to be recognized by the immune system by binding to T and B cell receptors, although this might not result in overt responses
Autochthonous	Formed from endogenous tissue in the correct anatomical location
Cancer-germline genes	Embryonic genes that are normally only expressed in male germline cells that become expressed in cancer. Can also be described as cancer-testis genes
Desmoplasia	The growth of fibrous or connective tissue
DNA mismatch repair	DNA repair mechanism that corrects mispaired nucleotides that originate during DNA replication and recombination
Graft-versus-host disease	Inflammatory and tissue-destructive immune reactions that result from the attack on host tissues by infused allogeneic lymphocytes
Graft-versus-leukaemia	Following allogeneic transplantation of bone marrow or blood stem cells, donor T cells may recognize peptides on leukaemia cells that result in beneficial immune attack
Human leukocyte antigen	Cell-surface molecules that are encoded by the major histocompatibility complex. These molecules present antigenic peptides to T cells. HLA class I molecules present antigen to $CD8^+$ T lymphocytes, and HLA class II molecules present antigen to $CD4^+$ T lymphocytes
Immunoediting	Describes the complex relationship between a developing tumour that is under constant pressure from the host immune system. Cancer immunoediting consists of three phases: elimination (that is, cancer immunosurveillance), equilibrium and escape
Lymphopenia	Reduced numbers of lymphocytes, commonly following radiotherapy or chemotherapy
Minor histocompatibility	Polymorphic peptides derived from normal cellular proteins that can be recognized in the context of major histocompatibility complex molecules. Immune responses against these polymorphic antigens can result in graft-versus-host reactions, graft rejection or beneficial anti-tumour responses
Regulatory T (T_{Reg}) cells	A T cell subpopulation that suppresses the activation of other T cells and that maintains immune system homeostasis and peripheral tolerance to self-antigens
Syngeneic	Genetically identical

Tolerance	The process that ensures that repertoires of B cells and T cells are biased against self-reactivity, which reduces the likelihood of autoimmunity

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