

CHAPTER 20

Combination Therapies in Solid Tumour Oncology

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INTRODUCTION: A DEFINITION OF CANCER

Cancer is a generic term that designates a large group of diseases, characterised by the rapid generation of abnormal cells that disrupt tissue homoeostasis and grow beyond their typical boundaries. This escape to normal growth and apoptosis patterns provides cancer cells the ability to metastasise and spread to other organs beyond the organ of origin (Anonymous, 2018). With approximately 10 million deaths worldwide in 2018, cancer is a leading cause of death (Anonymous, 2018), and its annual economic burden is very high with a global estimated cost in 2010 of approximately US\$ 1.16 trillion (Stewart and Wild, 2014). There was in 2018 more than 18 million new cancer cases (Bray et al., 2018). The most common cancers in 2018 were lung (1.76 million deaths), colorectal (862,000 deaths), stomach (783,000 deaths), liver (782,000 deaths) and breast (627,000 deaths) (Fig. 20.1) (Anonymous, 2018; Bray et al., 2018). Cancer arises from the transformation of normal cells into tumour cells in a multistage process where a precancerous lesion transforms into a malignant tumour (Fig. 20.2), resulting from the interaction between genetic factors and external factors comprising physical carcinogens, chemical carcinogens and biological carcinogens (Anonymous, 2018). An example of tumour initiation mechanism is the activation by the expression of oncogenic mutant PIK3CA^{H1047R} of a multipotent genetic programme in normally lineage-restricted populations that not only initiates early stage tumour initiation as observed in breast cancer by triggering cell dedifferentiation into a multipotent stem-like state but also sets the stage for future intratumoural heterogeneity and tumour aggressiveness (Koren et al., 2015; Van Keymeulen et al., 2015). Notably, dedifferentiation is central to both tissue repair and stemness; it is a natural mechanism with obvious evolutionary advantages, albeit it coming at the cost of an intrinsic risk of cancer initiation. Phenotypic plasticity, illustrated by the epithelial-to-mesenchymal transition (EMT), has thus emerged as a new paradigm for understanding cancer initiation, progression, metastasis, and resistance to therapy (Gupta et al., 2019).

Tumours can be classified using molecular analysis tools. A majority of tumours fall in the category of endodermal/ectodermal tumours as the exposure to chemical

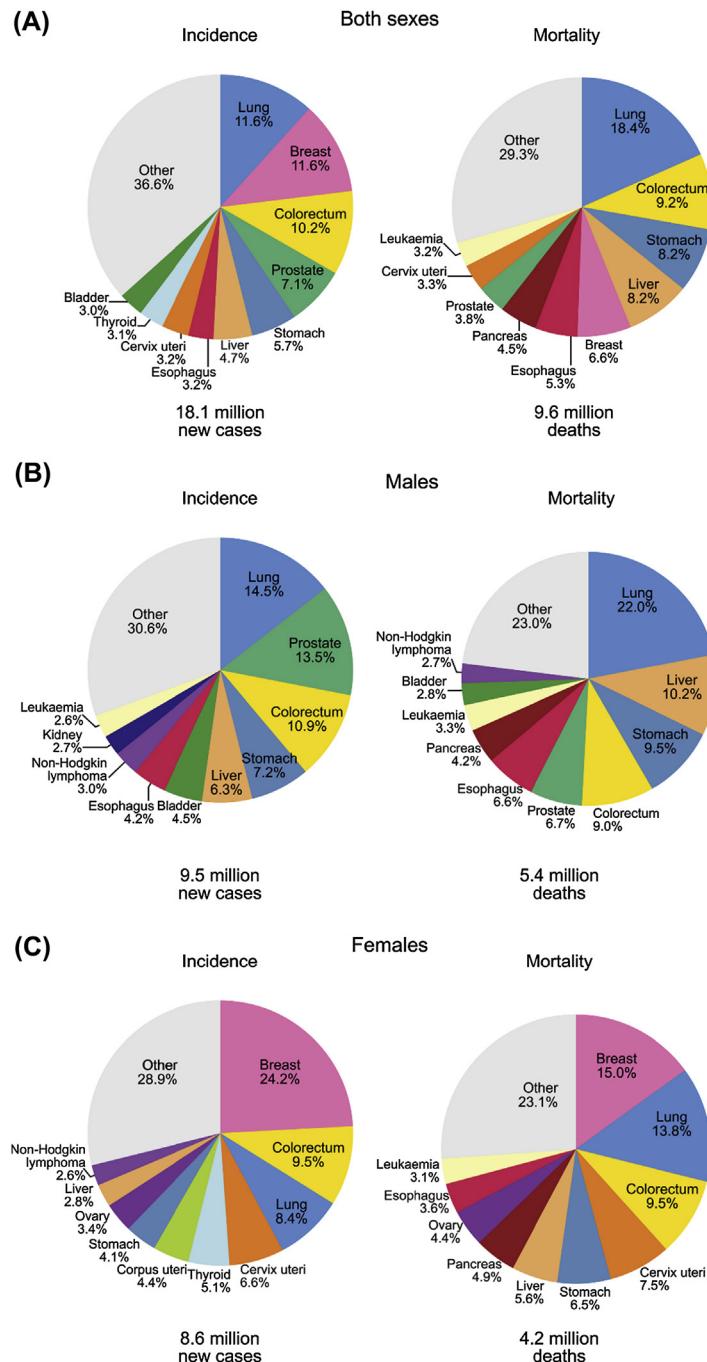


Figure 20.1 Incidence and mortality for the 10 most common cancers in 2018. The area of the pie chart reflects the proportion of the total number of cases or deaths; nonmelanoma skin cancers are included in the 'other' category. (Reproduced with permission Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.)

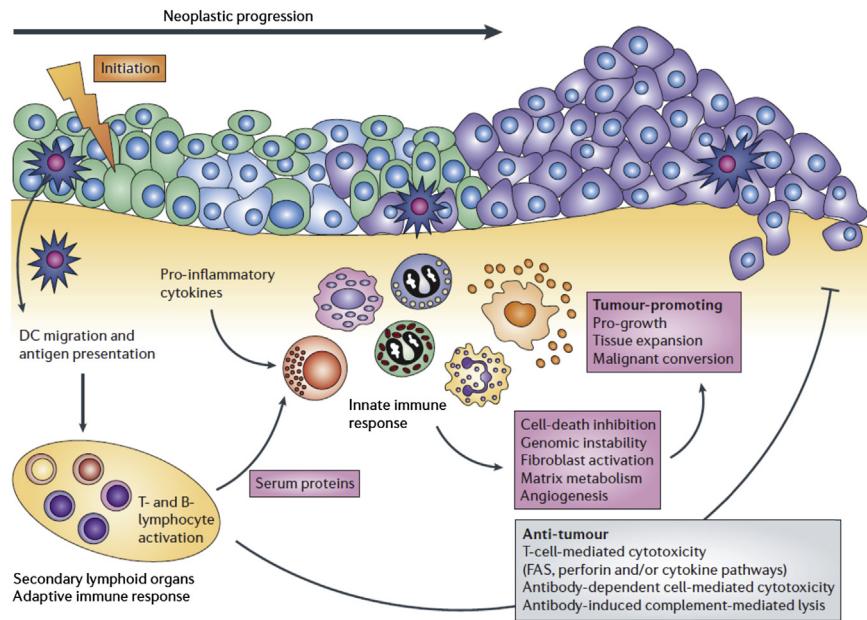


Figure 20.2 Inflammation and cancer: a model of innate and adaptive immune cell function during inflammation-associated cancer development. Antigens that are present in early neoplastic tissues are transported to lymphoid organs by dendritic cells (DCs) that activate adaptive immune responses resulting in both tumour-promoting and antitumour effects. The pathways that regulate DC trafficking during early cancer development and the exact nature of the antigen(s) remain to be established. Activation of B cells and humoral immune responses results in chronic activation of innate immune cells in neoplastic tissues. Activated innate immune cells, such as mast cells, granulocytes and macrophages, promote tumour development by the release of potent prosurvival soluble molecules that modulate gene expression programmes in initiated neoplastic cells, culminating in altered cell cycle progression and increased survival. Inflammatory cells positively influence tissue remodelling and development of the angiogenic vasculature by producing proangiogenic mediators and extracellular proteases. Tissues in which these pathways are chronically engaged exhibit an increased risk of tumour development. By contrast, activation of adaptive immunity also elicits antitumour responses through T cell-mediated toxicity (by induction of apoptosis antigen 1 (Fas), perforin and/or cytokine pathways) in addition to antibody-dependent cell-mediated cytotoxicity and antibody-induced complement-mediated lysis. (Reproduced with permission de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer* 2006;6:24–37.)

carcinogens occurs via the ectodermal skin or through the aerodigestive tract (that is, the lungs that are endodermally derived and the alimentary tract). Given that endoderm/ectoderm-derived cells are the most exposed to environmental carcinogens during a lifelong process, which combines with immunosenescence, their implications in tumours expectedly tend to increase in incidence with age (Berman, 2004).

It has long been recognised that chronic inflammation largely contributes in rendering patients susceptible to numerous forms of cancers (Dvorak, 2015). The tumour

microenvironment (TME) notably comprises inflammatory cells and mediators linking inflammation, carcinogenesis, and tumour progression (Mantovani, 2018). The link between inflammation and cancer has been demonstrated by various epidemiological studies and can notably be exemplified by the direct relationship that exists between obesity and cancer, chronic obstructive pulmonary disorder (COPD) and lung cancer in the absence of smoking, or ulcerative colitis and colorectal cancer (Houghton, 2013; Murphy et al., 2018). What is more, the infiltration and presence of a large number of macrophages in the TME of breast cancer, for example, has been shown to promote cancer growth and metastasis and to be linked to poor prognosis (Mantovani et al., 2007). All these observations promote the view that smouldering inflammation contributes to the proliferation and survival of malignant cells, angiogenesis and metastasis, as well as to the subversion of adaptive immunity, to reduced response to hormones and to chemotherapeutic agents and to the induction of genetic instability promoted by inflammatory mediators, which lead to the accumulation of random genetic alterations in cancer cells (Colotta et al., 2009; Coussens et al., 2013). This process is particularly driven by inflammatory cells and signalling molecules of the innate immune system and by specialised cells from the adaptive immune system, which direct the innate inflammation that promotes tumour progression (de Visser et al., 2006; Coussens et al., 2013). B lymphocytes and various serum factors appear to be essential for establishing chronic inflammatory states that are associated with premalignant progression and thus are involved in neoplastic pathways; this proceeds by B lymphocytes synthesising signals that trigger the inflammation via a mechanism driven by an antigen present in the environment that induces B lymphocytes to secrete antibodies targeting innate immune cells including macrophages, mast cells and neutrophils, thus setting and maintaining the inflammation (de Visser et al., 2006; Brodt and Gordon, 1982). On their activation, innate immune cells secrete an array of factors that promote cancer growth and metastasis (Anonymous, 2018; Anagnostou et al., 2017). The expression of CD68 (a protein of the lysosome-associated membrane protein family) and other genes associated with macrophage infiltration is typically associated with poor prognosis (McLemore et al., 2018). Furthermore, nonsteroidal antiinflammatory agents have been observed to protect against various tumours (Ulrich et al., 2006).

As described by de Visser et al. (2006), although cancers have at their roots cells that underwent genetic mutations, cancers are not simply autonomous masses of mutant cells, but they are first and foremost composed of a complex microenvironment comprised of numerous cell types including fibroblasts and epithelial cells, innate and adaptive immune cells, cells responsible for the formation of blood and of lymphatic vasculature and specialised mesenchymal cell types that are unique to each tissue microenvironment (Fig. 20.2). Considering that more than 90% of the anticancer therapeutics fail to receive approval, it is critical to have preclinical models that closely reflect this TME in cellular, biological and biophysical content (DiMasi et al., 2010; Hutchinson and Kirk, 2011;

Korstanje, 2003; Nair et al., 2017; Rubin and Gilliland, 2012). Low-passage, patient tumour-derived tissues embedded into basement membrane extract or ‘tumour organoids’ provide new means to perform pharmacologic testing in a three-dimensional assay that better recapitulates the biology of cancer than conventional preclinical models including mouse xenograft models (Onion et al., 2016; Skardal et al., 2015, 2016).

While tissue homoeostasis is maintained by the collaborative interactions between these various cell types mitigated by a variety of checking and balancing mechanisms, cancer development is enhanced when mutant cells leverage the collaborative capabilities of these cells to promote their own survival (deVisser et al., 2006). Putting all this together, the neoplastic process is characterised as follows (deVisser et al., 2006): (1) adaptive and immune cells regulate tissue homoeostasis and efficient wound healing; (2) chronic inflammatory disorders may arise due to altered interactions between adaptive and innate immune cells; (3) an abundance of infiltrating innate immune cells, comprising macrophages, mast cells and neutrophils, correlates with increased angiogenesis and poor cancer prognosis; (4) on the other hand, an abundance of infiltrating lymphocytes correlates with favourable prognosis; (5) chronic inflammatory conditions predispose to cancer development; (6) the long-term usage of nonsteroidal antiinflammatory drugs and selective cyclooxygenase-2 (COX-2) inhibitors is associated with reduced cancer incidence; (7) polymorphisms in genes that regulate immune balance influence cancer risk; (8) the immune status has been observed in both humans and in mouse models to affect the risk of cancer development in an aetiology-dependent manner; (9) the genetic elimination or the depletion of immune cells alters cancer progression in experimental models and (10) the activation of antitumour adaptive immune responses can suppress tumour growth.

The role of tumour-associated macrophages (TAMs) that constitute the major inflammatory component of the stroma of numerous tumours cannot be overemphasised (Sica et al., 2006). TAMs respond to conditions they meet in the TME such as hypoxia or the presence of lactic acid via a mechanism mediated by hypoxia-inducible factor 1 α : (HIF-1A) by secreting mitogens and an array of growth factors and enzymes that stimulate tumour angiogenesis (Bingle et al., 2002; Colegio et al., 2014). For example, the expression of arginase 1 induced by lactic acid favours tumour growth (Colegio et al., 2014). As TAMs are a distinct M2-skewed polarised myeloid macrophage population that promotes tumour progression by promoting angiogenesis, matrix remodelling and the suppression of adaptive immunity, these macrophages are potential targets of anticancer therapy, beyond conventional radio- and chemotherapies. For example, a selective PI3K- γ inhibitor was observed in preclinical studies to reprogramme macrophages from the M2 immune-suppressive to the M1 immune-activating phenotype and to promote the overcoming of resistance to checkpoint inhibitors. This compound exhibited in a Phase I/Ib 30–evaluable patient study appropriate tolerability and signals of efficacy with evidence of immune modulation when used in combination with nivolumab (NCT02637531) (Kanno et al., 2018). On the other hand, innovative therapies can also

be designed that make use of TAMs to stimulate host immune responses or to deliver therapeutic gene constructs to solid tumours (Bingle et al., 2002). Tumour associated neutrophils (TAN) also are recruited from the bone marrow and also infiltrate the TME with the outcome that they facilitate cancer immune evasion. For example, in pancreatic adenocarcinoma (PDAC), chemokine pathways have been shown to be co-opted to facilitate myeloid cell recruitment from the bone marrow to establish an immunosuppressive TME. Remarkably, targeting tumour-associated CXCR2⁺ TANs or CCR2⁺ TAMs alone has been observed to significantly improve antitumour immunity in pre-clinical models. However, as therapeutic resistance could be promoted by a compensatory influx of an alternative myeloid subset that would result in a persistent immunosuppressive TME, the dual blockade of both CCR2 and CXCR2 reduces total tumour-infiltrating myeloids, which in turn results in a more robust antitumour immune response and chemotherapeutic response in PDAC as compared with either strategy alone (Nywening et al., 2018).

THE HALLMARKS OF CANCER

As a theoretical principle, the complexity of cancer biology suggests in itself that multiple interventions in parallel, or in sequence, and notably regarding solid tumours, are the key to controlling tumour growth and metastasis and to achieve tumour regression. A useful fundamental research and therapeutic R&D strategy is thus to approach the complexity of cancer biology, which again is notably characterised by dynamic changes in the cancer cell genomes with mutations producing oncogenes that drive dominant gains of function and suppressor genes that exhibit recessive loss of function, as well as high intratumoural heterogeneity (Bishop and Weinberg, 1996), by reducing it into interdependent underlying biological components. D. Hanahan and R. Weinberg have used this lens of analysis and proposed that tumour growth and metastasis all share fundamental hallmarks (Hanahan and Weinberg, 2000, 2011).

To use a 'chunk theory' method as a means to promote coherent cognition and to simplify the design of a therapeutic strategy for solid tumours (Gobet et al., 2001), the hallmarks of cancer comprise a variety of molecular mechanisms that can be consolidated in various biological capabilities acquired during the multistep development of tumours, in a process that typically has an age-dependent incidence involving several stochastic events (Fig. 20.3) (Hanahan and Weinberg, 2000; Renan, 1993). These hallmark capabilities include (1) sustaining proliferative signalling, (2) evading growth suppressors, (3) evading immune destruction, (4) enabling replicative immortality, (5) tumour-promoting inflammation, (6) activating invasion and metastasis, (7) inducing angiogenesis, (8) genomic instability and mutations, (9) resisting cell death and (10) reprogramming cellular metabolism to promote neoplastic proliferation. It is important to note that each of these hallmarks is underlined on the one hand by genome

instabilities that induce the genetic diversity that accelerates their acquisition and on the other hand by inflammation that fosters multiple hallmark functions (Hanahan and Weinberg, 2011). The role of the TME needs to be emphasised here again, with normal cells contributing to the acquisition of these hallmarks. These concepts that recapitulate the most important phenotypic characteristics of the neoplastic process and cancer progression are both simple and widely actionable to develop novel therapeutics and treatment regimens (Hanahan and Weinberg, 2011).

This conceptual structuration of the fundamental knowledge of cancer biology is particularly useful to categorise all cancer genes and their respective molecular contributions to the neoplastic and metastatic processes; however, it is also important to integrate the dynamics of cancer and its evolutionary mechanisms, whereby various cells are selected for clonal expansion (Horne et al., 2015). Cancer can thus be viewed not only as a single-cell mutational process but also as a macrocellular evolutionary process (Horne et al., 2015). In this light, cancer can be divided into two distinct evolutionary phases: (1) the punctuated stochastic phase and (2) the stepwise gradual phase. Punctuated phases are characterised by rapid genomic changes and high heterogeneity; they are caused by instability-mediated macrocellular evolution. Following selection pressure, including therapeutic selection pressure, a unique genomic backbone survives recapitulating many of the hallmarks of cancer discussed above. In this stepwise gradual phase, this new genome system is relatively stable over time, but it is subjected to microcellular changes enabling clonal expansion and diversification by continuing to acquire incremental low-level changes including gene mutations, epigenetic alterations and small traceable genome-level alterations that further facilitate the adaptation of cancer cells (Horne et al., 2015). The heterogeneity of tumours with respect to their genetic compositions is perhaps best exemplified by the high intratumoural/intrapatient heterogeneity of sporadic melanoma in that it is so high as it hinders the development of effective treatments (Takata et al., 2000). This intrinsic heterogeneity underlines the emergence of therapy resistances via target escape and clonal expansion of the therapy-resistant cells that are thus capable of expansion and metastasis; expectedly, this occurs through an evolutionary process that is similar to the well-documented phenomenon of acquired antibacterial resistances in microbial infections (Dexter and Leith, 1986; O'Donnell et al., 2016). Interestingly, microbial experimentations suggest that in the absence of selection pressure, chromosomal mutations that provide chemotherapeutic resistance typically have a fitness cost. However, when the selective pressure is removed, mutations that appear are adaptation to these 'costs of resistance' as observed in *Escherichia coli*, HIV and *Salmonella typhimurium* as the evolutionary process that takes place in the novel conditions typically results in mutations that reduce these costs, rather than in reversion to a higher fitness but drug-sensitive phenotype (Levin et al., 2000). Noteworthily, this ascent of intermediate-fitness compensatory mutants, rather than of high-fitness revertants, can be ascribed to higher rates of compensatory mutations relative to the rates of chemotherapeutic-sensitivity reversion (Levin et al., 2000). The notion that tumours

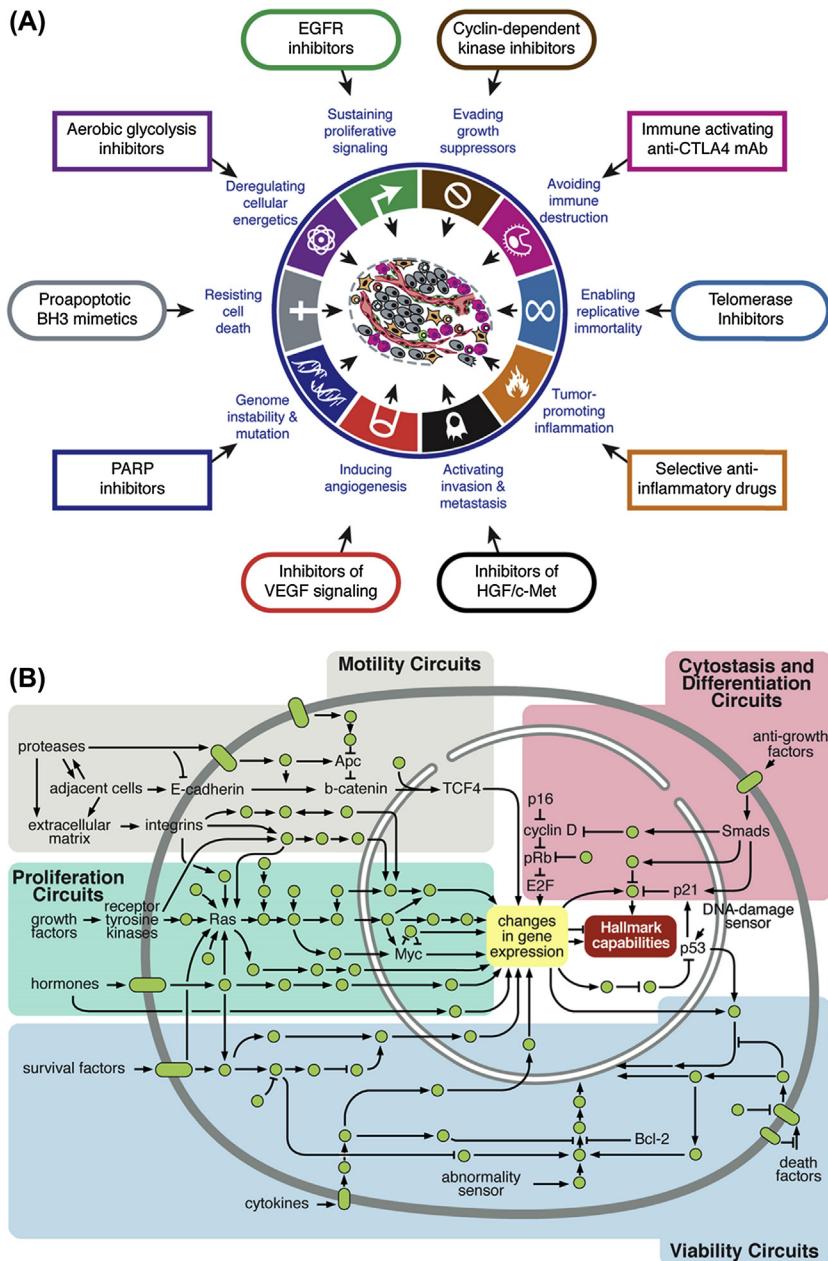


Figure 20.3 The hallmarks of cancer. In humans, tumourigenesis is typically a multistep process, with each of these steps resulting from genetic mutations. (A) The large variety of cancer cell genotypes suggests ten essential changes in cellular physiology that together drive the neoplastic process: (1) self-sufficiency in growth signals; (2) insensitivity to growth inhibitory signals; (3) the evasion of immunological destruction particularly by T and B lymphocytes, macrophages and natural killer cells; (4) limitless replicative potential; (5) promoting inflammation; (6) tissue invasion and metastasis; (7) sustained

constitute dynamic microcosms of evolution where a highly diverse pool of mutated cells compete for access to nutrients and to evade killing by the immune system is critical as it enables one on the one hand to explain how cancers arise and why they are hard to cure, and on the other hand, it enables one to leverage the toolbox of ecology and evolutionary biology that have been exquisitely developed in microbiology to generate novel therapeutic insights (Merlo et al., 2006). In cancer, dynamic changes in the cancer genome of a patient constitute a therapeutic hurdle as significant genomic changes may occur during the course of treatment and during the course of disease progression, albeit the underlying mutation being typically maintained in resistant tumours (Doebele et al., 2012; Li et al., 2013; Marusyk et al., 2012; Sequist et al., 2011; Tibaldi, 2014).

At the patient population level, it is critical to achieve appropriate stratification. For example, the implementation of the latest biomarker technologies including multiplex genotyping and high-throughput genomic profiling by next-generation sequencing technologies makes it now possible to analyse the cancer genome of patients from small tumour biopsies and thus to establish patient population stratification by grouping patients in molecularly well-characterised classes. This approach was for instance deployed in non-small cell lung cancer (NSCLC), an indication that could be viewed as a spectrum of rare diseases rather than as a single disease with a one-size-fits-all treatment (Fig. 20.4), with each subset being defined by molecularly validated targets (Li et al., 2013). However, the challenge remains for practicing oncologists to establish how most efficiently to select, interpret, and apply these new genetic and genomic assays and to develop tailored medicines to answer those unmet medical needs (Li et al., 2013).

These advances in multiplex genotyping technologies and high-throughput genomic profiling, both in terms of technical performance (speed and comprehensiveness) and cost and ease of implementation, have driven an important evolution in the standard of care of advanced-stage cancers, which have evolved from an empirical treatment based

angiogenesis; (8) genomic instability and mutations, (9) evasion of apoptosis; and (10) the capability to reprogramme cellular metabolism to most effectively support neoplastic proliferation. Importantly, each of these hallmarks is common to virtually all types of human tumours and represents the successful overcoming of hardwired anticancer defence mechanisms. Noteworthily, the relatively rare occurrence of cancer during an average human lifetime could be ascribed to this multiplicity of defences. What is more, inflammation mediated by innate immune cells can be diverted to support a number of these hallmarks, suggesting the tight link that exists between inflammation and cancer. (B) Cancer cells are regulated by a complex intracellular signalling network that is reprogrammed from the regulatory network present within normal cells. Regulatory subcircuits are represented as different ontological chunks regrouping different biological capabilities. Notably, there is significant cross talk between each subcircuit that in turn is sensing and responding to the signals originating from other cells from the tumour microenvironment. There is a deep pipeline of therapeutics with different molecular targets and mechanisms of action in development for most of these hallmarks. (Reprinted with permission Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70; Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–74.)

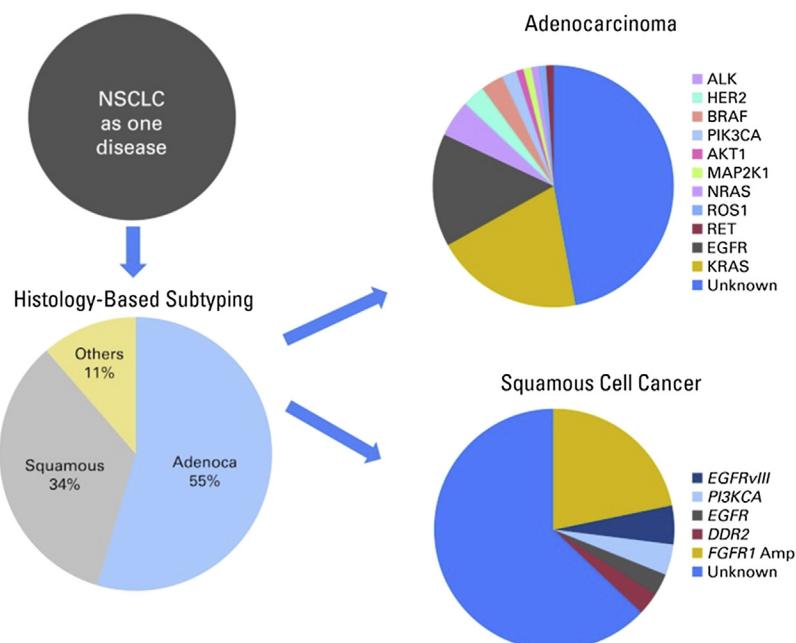


Figure 20.4 Viewing common diseases as a spectrum of rare diseases: subtyping NSCLC. Evolution of non-small cell lung cancer (NSCLC) subtyping from histologic to molecular based. EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; MAP2K1, mitogen-activated protein kinase 1. (Reprinted with permission Li T, Kung HJ, Mack PC, Gandara DR. Genotyping and genomic profiling of non-small-cell lung cancer: implications for current and future therapies. *J Clin Oncol* 2013;31:1039–49.)

on a disease categorisation to a precision oncology treatment strategy that is enabled by predictive biomarkers recapitulating the tumour molecular profile (Kalia, 2015). To stratify patient populations most effectively, multiple molecular quantities that underlie the complex phenotypes of drug response need to be measured in parallel. Beyond mutations in the DNA sequence and eventually the transcriptome, what needs to be monitored are the variations in the other systemic molecular dimensions including the metabolome, the interactome (that is, the network of underlying molecular interactions that occur in the cancer cell) and the secretome (Marusyk et al., 2012; Vucic et al., 2012). These different dimensions can notably be integrated using fuzzy logic and other computational methods to encompass multidimension and multinodal interactions, which incorporate randomness and uncertainty, to yield more biologically relevant predictions, an important step as the gain of a function of a gene or the loss of it is more precisely characterised by integrating different molecular measurements with biological knowledge than by analysing each data type separately (Huang et al., 2017; Keller et al., 2008; Pavel et al., 2016). Noteworthily, omics data complement, but do not substitute for, more conventional data such as imaging and electronic health record data (Huang et al., 2017).

Biomarker-based diagnostics are helpful to implement the personalised oncology medicine highlighted above by matching targeted therapies with patients, with the view to optimise clinical benefits by maximising efficacy and minimising systemic toxicities. On the other hand, prognostic biomarkers identify an array of quantities comprising somatic germ line mutations, changes in DNA methylation or in levels of microRNA and an elevation of circulating tumour cells in blood. Molecular diagnostics are already in use in clinical practice of personalised oncology medicine for several indications including chronic myeloid leukaemia, colon cancer, breast cancer, lung cancer and melanoma (Kalia, 2015). Novel biomarkers can be developed by profiling the high network diversity of cellular metabolism, and particularly cancer cell metabolism, using a hypothesis-free approach (Domenyuk et al., 2017, 2018).

CONVERGENCE INNOVATION IN INFLAMMATION AND ONCOLOGY

The typical first-line treatment options of a malignant solid tumour include tumour resection whenever applicable, chemo- and radiotherapy. To limit debilitating side effects of these physical or shotgun approaches to treating cancer, the hope has long been to develop safe and efficacious tumour-specific immunotherapies, reminiscent of the *Zauberkugel* (magic bullet) concept coined by P. Ehrlich, which was translated into a first product, arsphenamine, or Salvarsan, the first effective treatment against syphilis, which was commercialised in the early 1910s (Sörgel et al., 2004). To this end, various molecular classes and architectures have been tested in the past decades to achieve targeted therapy, starting with immunotoxins that combine the selectivity of a tumour-targeting ligand such as a monoclonal antibody (mAb) to the killing effect of a cytotoxic moiety (Alewine et al., 2015).

A typical downside of the conventional first line of chemo- and radiotherapy treatments is that they are not selective for cancer cells as they typically target rapidly growing cells; they thus cause a range of side effects by killing healthy cells also and generally cause a transient immune suppression, which translates into an increased risk of (opportunistic or nosocomial) infections and in a decreased ability of the immune system to fight cancer. However, some antineoplastic compounds induce immunogenic (tumour) cell death (ICD) (Galluzzi et al., 2012; Tesniere et al., 2008), whereas other anticancer drugs alter the phenotype of surviving tumour cells by turning them into targets visible by immune cells (Garnett et al., 2008; Hodge et al., 2013). What is more, an array of molecules that regulate the inflammatory processes can be directly targeted such as transforming growth factor β (TGF- β), the inhibition of which enhances the cytotoxic T cell response thus contributing to preventing tumour metastasis (Ros and Vermeulen, 2018). Similarly, radiotherapy impacts cell-mediated immunity by suppressing antitumour immunity given among other mechanisms the radiosensitivity of lymphocytes, but the sensitivity of tumour cells that survive appears to be exacerbated, and radiations trigger an increased expression of proinflammatory cytokines comprising tumour necrosis factor (TNF) α and interleukin (IL) 1 β that recruit antigen-presenting cells (APCs)

(Chakraborty et al., 2004; Garnett et al., 2004; Ma et al., 2013; McBride et al., 2004; Nesselser et al., 2019; Pilones et al., 2015). ICD is triggered by the secretion of calreticulin (CALR), ATP, chemokine (C–X–C motif) ligand 10 (CXCL10) and high mobility group box 1 (HMGB-1) (Gebremeskel and Johnston, 2015). ICD can thus be stimulated by either high-dose radiotherapy alone or in parallel with chemoradiation regimens, which contribute to the establishment of a peritumoural proimmunogenic environment (Golden et al., 2014). These phenomena have implications regarding combination therapies, as patients who receive ICD inducers such as anthracyclines and oxaliplatin but do not upregulate CALR and patients who receive non-ICD inducers such as cisplatin, etoposide or mitomycin C may benefit from a combination therapy with endoplasmic reticulum stressors such as thapsigargin or tunicamycin or the administration of exogenous recombinant CALR (Gebremeskel and Johnston, 2015). Such approaches can be exemplified by the TONIC clinical trial (NCT02499367), a noncomparative 67-patient Phase II clinical trial in metastatic triple negative breast cancer; patients were randomised to (1) nivolumab without induction, (2) nivolumab with 2-week low-dose induction, (3) irradiation (3×8 Gy), (4) cyclophosphamide, (5) cisplatin or (6) doxorubicin, all followed by nivolumab therapy. The objective response rate (ORR) was 20%, with the majority of responses having been observed in the cisplatin arm (ORR of 23%) and the doxorubicin arm (ORR of 35%). Notably, an upregulation of immune-related genes involved in PD-1/PD-L1 and T cell cytotoxicity pathways were observed after short-term doxorubicin or cisplatin induction, an observation that was supported by enrichment after doxorubicin induction among upregulated genes related to inflammation, notably JAK–STAT and TNF- α signalling (Voorwerk et al., 2019). These observations promote the view that short-term doxorubicin or cisplatin conditioning therapy may induce a less immunosuppressive TME and thus increase the likelihood of response to PD-1 blockade in this indication and perhaps other cancer types (Voorwerk et al., 2019). ICD requires a functional T cell response and antigen presentation by DCs (reviewed in Chapter 18); therefore, patients afflicted by immunosuppression may not respond as well to ICD-inducing drugs. In those cases, combining those latter drugs with immune-stimulating drugs or inhibiting immunosuppressive cell populations might perhaps be a relevant therapeutic strategy. Immunostimulatory antibodies that either enhance stimulatory signals or block inhibitory ones include the following antagonists: anti-CTLA-4 (anticytotoxic T lymphocyte antigen 4), anti-PD1 (antiprogrammed death protein 1), anti-TIM-3 (anti-T cell immunoglobulin and mucin domain-containing protein 3) and the following agonists: CD40 agonists and OX40 agonists (Gebremeskel and Johnston, 2015). Furthermore, ICD inducers could be combined with immunostimulatory cytokines, as exemplified by oxaliplatin used in combination with IL-12 (Gonzalez-Aparicio et al., 2011). These encouraging results notwithstanding, some ICD inducers may induce suppressor cells, which results in inhibition of the immune response, indicating that it remains critical to determine the immunological profile of patients who are treated with ICD-inducing therapies (Gebremeskel and Johnston, 2015). The synergy with radiotherapy is worth emphasising with the observation that NKG2D-based chimeric antigen receptor T (CAR-T) cells and radiotherapy have shown synergistic efficacy in glioblastoma multiforme (GBM); this is

particularly interesting as the NKG2D ligands link the innate and adaptive immune responses by activating the receptors expressed on effector cells of both the innate (NK) and adaptive immune systems (CD8⁺ T cells), thus offering the possibility to enhance both innate and adaptive immune antitumour responses (Cho et al., 2010; Weiss et al., 2018).

As reviewed above, the complex cross talk between immunity and cancer cells may result in the inhibition or enhancement of tumour growth. It is the evasion by tumour cells of the killing by the immune response, which derives from the selection of tumour variants that are resistant to immune effectors and by the progressive formation of an immunosuppressive TME, which results in clinical cancer. A modern approach to cancer therapy proceeds from understanding the immunity–cancer interface and from developing pharmaceutical modalities that prevent tumour cells to either evade immune surveillance or that potentiate the immune response against cancer cells (Vinay et al., 2015). While the molecular mechanism is nowadays understood in some depth, observations of spontaneous cancer regressions associated with severe infections, and thus severe inflammation, were made as early as the 13th century (the St. Peregrine Laziosi case), albeit the beginning of the understanding of the phenomenon coming in 1891 when William B. Coley, then a surgeon at the New York Cancer Hospital (now Memorial Sloan-Kettering Cancer Center, New York, NY, USA), observed that patients who had infections after bladder cancer surgery typically had superior outcomes than those who had not suffered such postsurgery infections; Coley tested the concept and prepared doses of inactivated bacteria including *Streptococci*, *Serratia marcescens*, *Staphylococci* and *Escherichia coli*, known as ‘Coley’s toxins’, which resulted in complete disease remission in some patients (Říhová and Šťastný, 2015; Vernon, 2018). The deployment of these concepts using molecular biology techniques signalled the advent of immunooncology, that is, the harnessing of the capabilities of the immune system against cancer. The first embodiment of immunooncology was nonspecific immunomodulatory drugs comprising IL-2, IL α-2a and interferon (IFN) α-2b, which together represented global sales of US\$449 million when thalidomide and lenalidomide were launched in 2006 for the treatment of multiple myeloma (Haberman, 2007). The discovery in the late 1990s that PD-1 or CTLA-4 inhibition harnesses the immune system in a specific manner was a critical milestone that took the typical quarter of a century to be translated into commercial therapies (Freeman et al., 2000; Krummel and Allison, 1995; Leach et al., 1996; Zaidi and Jaffee, 2018).

For tumour cells, immune evasion or enhanced immune tolerance are selective survival advantages. Whether achieved directly or indirectly, these escapes promote their survival and outgrowth (Kim et al., 2007). Several immunotherapy product classes have been researched with the objective to achieve the controlled modulation of the immune system to fight cancer while avoiding autoimmunity or nonspecific inflammation: (1) transfer of immune effectors, (2) vaccination and (3) immunomodulatory therapy (Makkouk and Weiner, 2015; Riley et al., 2019). The field of immunotherapy thus comprises a variety of therapeutic modalities ranging from conventional small molecules and biologics, for example, to inhibit regulators of immune activation (immune checkpoint blockade) as these limit antitumour responses (Fig. 20.5) to multivalent antibodies,

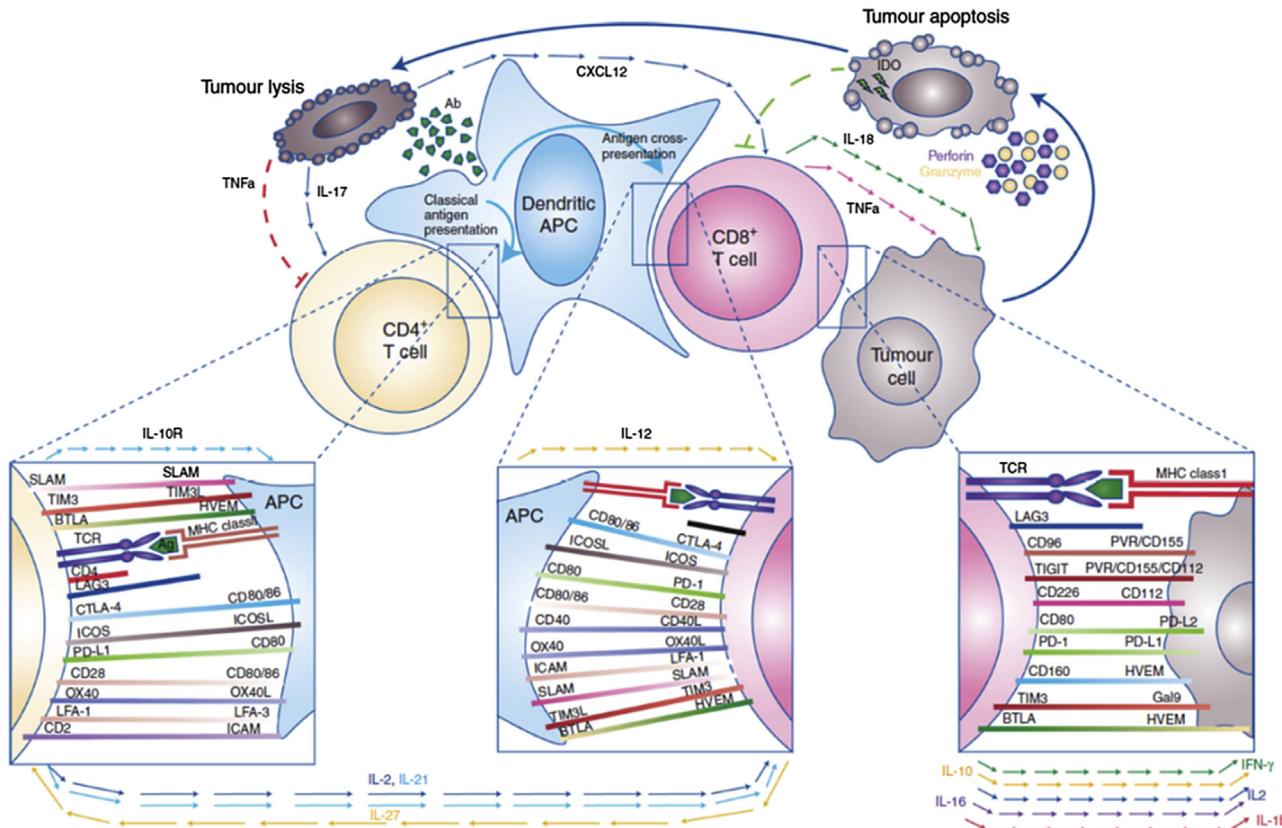


Figure 20.5 Oncology: the checkpoint blockade to enhance antitumour immunity. The cellular immune response to cancer is complex and involves an array of immunoregulatory interactions mainly involving antigen-presenting cells (APCs), T cells and tumour cells. The presentation of distinct antigen epitopes to CD8⁺ and CD4⁺ T cells in the context of major histocompatibility complex (MHC) class I (on APC or tumour cells directly) and class II (on APCs), respectively, facilitates tumour cell recognition, but numerous other molecular interactions (inset boxes) and input from paracrine and humoural factors (cytokines/chemokines, shown with arrowed lines) integrate to determine the ultimate outcome of immune recognition. The release of survival and inflammatory cytokines, such as interleukin-2 and interferon (IFN) γ , can promote a cytotoxic (CD8⁺) T cell response with consequent tumour-directed lytic activity mediated by release of cytotoxic granule contents (e.g., perforin and granzyme) as well as triggering of apoptotic pathways by tumouricidal cytokines (e.g., tumour necrosis factor α and IFN- γ) and death receptor ligation (e.g., FAS:FAS-L). Debris released from apoptotic or necrotic tumour cells may be taken up by APC and presented in a cycle of immunogenic cell death. However, prolonged immune activation is adaptively opposed by the upregulation of immune-inhibitory molecules (e.g., CTLA-4, PD-1, TIM3, TIGIT and CTLA-4) or their ligands, many of which may be subverted by tumour cells to escape immune attack. The release of antiinflammatory, immunoregulatory or Th2-skewed cytokines also contributes to dampening of the cellular response. (Reprinted with permission Cogdill AP, Andrews MC, Wargo JA. Hallmarks of response to immune checkpoint blockade. *Br J Cancer* 2017;117:1-7.)

bispecific T cell engagers (BiTEs) and engineered cell-based therapies such as recombinant T cells (e.g., CAR-T cells) and CAR-NK cells (CAR-natural killer cells) (Cogdill et al., 2017; Guillerey et al., 2016; Iwai et al., 2002; Ribas and Wolchok, 2018; Sadelain et al., 2017; Shao et al., 2019; Siegler et al., 2018; Zaidi and Jaffee, 2018).

Checkpoint blockade, that is, locking out immunoinhibitory molecules with an antibody (mAB) or a small molecule is a therapeutic strategy that has demonstrated clinical benefits, as exemplified by ipilimumab (Yervoy), an anti-CTLA4 mAb, as well as by nivolumab (Opdivo) or pembrolizumab (Keytruda), both anti-PD1 mAbs, alone or in combination with conventional chemotherapy in advanced NSCLC (Gadgeel et al., 2016; Hellmann et al., 2018a; Peters et al., 2018; Poole, 2014; Skalniak et al., 2017). While all these products are antibodies, small molecule inhibitors also are being researched, for example, inhibitors of the PD-1 interactions with its ligands (Guzik et al., 2017; Weinmann, 2016). However, not all patients respond positively to such therapies. As a result, not only are new biomarkers of response necessary, for example, to determine innate resistance due to the absence of the molecular target on the cancer cell surface but also novel ways of tackling adaptive escape and other therapeutic resistance mechanisms (Cogdill et al., 2017). Solid understanding of the mechanisms of adaptive resistance starts to emerge however. Notably, tumour-initiating stem cells (tSCs), which form no more than 2% of the initial tumour mass, have been observed to survive checkpoint blockade treatment by quieting the immune system through expressing the cell surface protein CD80, which is the receptor for CD28 and CTLA-4, and ultimately reconstituting a solid tumour. CD80 expression is triggered by TGF- β , and TGF- β -responding cells better resist killing from cytotoxic T cells by driving their exhaustion. However, blocking CTLA-4 or TGF- β , as well as CD80 ablation, renders tSCs vulnerable to adoptive T cell therapy, ultimately resulting in diminishing tumour relapses. As a result, TGF- β blockers, antibodies or small molecules represent exciting agents to be used in combination therapies and particularly in combination with adoptive T cell therapy or with checkpoint inhibitors (Miao et al., 2019).

Current work is focused on how to maintain durable responses to checkpoint inhibitors as well as to sensitise those tumour types that are immune insensitive at baseline to checkpoint blockade therapy. Combining checkpoint inhibitors with other therapeutic modalities is a logical step forward and notably with drugs that target immunosuppressive cells in the TME. Signals of efficacy have already been attained in preclinical and early clinical studies aimed at testing this treatment concept (Zaidi and Jaffee, 2019).

The treatment of numerous cancers has undergone a paradigm change embodied by the clinical successes attained by deploying conventional biologics to achieve the locking of immune checkpoint receptors or their ligands and thus block their corresponding signalling pathways. However, as highlighted earlier, only a minority of patients with advanced cancers durably respond to the immunotherapies, albeit some being maintained several years after the treatment has stopped, whereas a fraction of the responders relapse (Borcoman

et al., 2019; Kim et al., 2018a). On the other hand, the large majority of target-positive patients do not respond; regarding pseudoprogression defined as the tumour regressing after an initial progression, it occurs in no more than 10% of the patients and as a result the majority of patients who exhibit disease progression do not respond to the immunotherapy treatment (Borcoman et al., 2019). This lack of durability is due to the immunosuppressive environment of the tumour inhibiting those adaptive responses that are essential for efficacy, typically outside of the pathway of the targeted checkpoint (Borcoman et al., 2019; Kim et al., 2018a; Dougan et al., 2019; Wilkinson and Leishman, 2018).

As a result, novel therapeutic strategies need to be explored to treat the nonresponders and the patients who relapse. Several research pathways are being explored, including to (1) discover novel effective checkpoint blockades, (2) deploy cell-based therapies as a means to bypass the endogenous immunity, (3) develop novel ways to stimulate adaptive anti-tumour responses that have not yet been harnessed using, for example, vaccines, adjuvants or combinations with cytotoxic therapies, (4) inhibit innate immune suppression and (5) modulate the protumour metabolism within the TME, or (6) boost endogenous professional antigen presenting cells (macrophages, B cells, DCs) (Fig. 20.6) (Dougan et al., 2019; Kvistborg and Yewdell, 2018; Wculek et al., 2019; Wilkinson and Leishman, 2018).

CHALLENGES IN SOLID TUMOUR ONCOLOGY

With the outstanding progress achieved in some liquid cancers such as acute lymphoblastic leukaemia (ALL), the therapeutic potential of engineered cell-based therapies as well as their adoptability by regulatory agencies have been unambiguously demonstrated (Frey, 2019; Rosenbaum, 2017). The adoptive transfer of such living drugs complements in active T cells the natural immune defences. Similarly, immunooncology drugs of the checkpoint inhibitor class aim at boosting the efficacy of natural immune defences. Although significant progress regarding the treatment of liquid tumours and their relapses is still required to respond to patients' medical needs for appropriately treating liquid cancers, the greatest opportunity in oncology, and certainly also its greatest challenge, lies in developing safe and effective treatments for solid tumours.

CAR-T cells (discussed in Chapters 3, 4, 12, 19, 23, 26 and 28) and CAR-NK cells (Chapter 5) are targeted anticancer therapeutics based on natural immune effector cells: the cells are conferred antigen specificity against cancer cells by a molecular architecture, a CAR that is typically delivered by a virus-based vector and that comprises an antibody or any other form of ligand-derived targeting ectodomain that is fused with a hinge, a transmembrane domain and intracellular T cell signalling domains. The killing function is performed by the immune effectors T or NK cells by way of their native mechanisms, thereby replicating the 'magic bullet' design of the immunotoxin where any ligand on the target cell surface can be targeted, except that here the cytotoxic function is performed by a live cell. Given this ligand–antibody approach that departs from nature's way

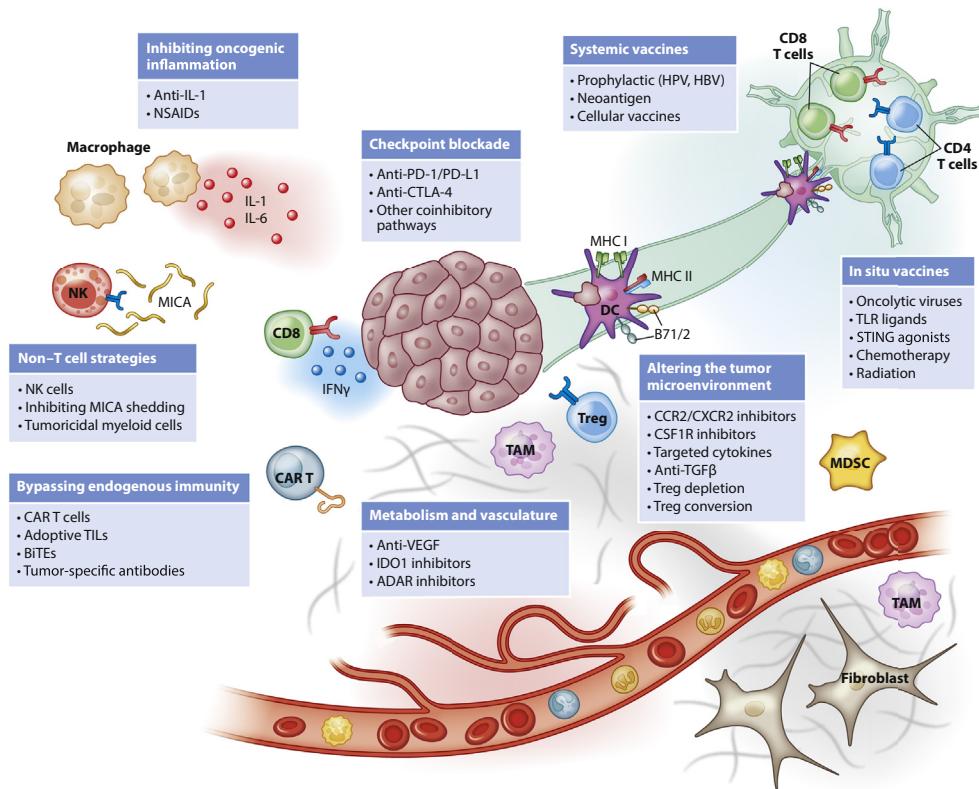


Figure 20.6 Oncology beyond the checkpoint blockade to enhance antitumour immunity. Building on the success of checkpoint blockade, new strategies to overcome the immunosuppressive pathways that protect tumours from antitumour immunity are being explored. These include both systemic and in situ vaccines to activate naïve T cells, prophylactic vaccines and blocking oncogenic innate inflammation. Moreover, the TME itself is being targeted through multiple mechanisms to (1) reduce the number of regulatory adaptive and innate cells, (2) block immunosuppressive metabolites and cytokines (3) disrupt tumour vasculature and (4) activate tumouricidal macrophages or NK cells. What is more, tumours can also be directly targeted by therapeutic antibodies and cell-based therapies to bypass endogenous responses either through the ex vivo expansion of anti-tumour T cells or infusion of engineered T cells, including CAR-T cells that directly recognise tumour-expressed targets. ADAR, adenosine deaminase acting on RNA; BiTE, bispecific T cell engager; CAR, chimeric antigen receptor; DC, dendritic cell; HBV, hepatitis B virus; HPV, human papilloma virus; IDO1, indoleamine 2,3-dioxygenase 1; IFN, interferon; IL, interleukin; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; MICA, MHC class I chain-related protein A; NK, natural killer; NSAID, nonsteroidal antiinflammatory drug; STING, stimulator of interferon genes; TAM, tumour-associated macrophage; TIL, tumour-infiltrating lymphocyte; TLR, toll-like receptor; Treg, regulatory T cell; VEGF, vascular endothelial growth factor. (Reprinted with permission Dougan M, Dranoff G, Dougan SK. *Cancer immunotherapy: beyond checkpoint blockade*. *Ann Rev Cancer Biol* 2019;3:55–75.)

of T cell receptor (TCR)-driven recognition, which is dependent on the MHC, any ligand for which an antibody can be generated constitutes a potential target, thus interestingly including lipids or carbohydrates and glycans, which opens wide the feasibility of targeting cancer-specific neoantigens resulting, for example, from aberrant glycosylations that differentially occur in cancerous cells, such as the O-glycosylation of mucin 1 (MUC1), which is a common marker of cancer linked to changes in cell adhesion, tumour growth and poor prognosis (Chmielewski et al., 2013; Jackson et al., 2016; Kumaresan et al., 2014; Rodríguez et al., 2018; Steentoft et al., 2018; Wilkie et al., 2008).

Although, as highlighted earlier in this Chapter as well as in other Chapters (Chapters 3, 4, 12, 23 and 28), outstanding remission rates have been achieved in ALL using CAR-T cell products, these engineered immune effectors have met little success to this date for the treatment of solid tumours, given specific challenges that facilitate the evasion by malignant cells from the patient's immune defences, a process that is facilitated by immune checkpoint molecules such as PD-1 and CTLA-4: (1) intratumoural heterogeneity and macrocellular evolutionary mechanisms; (2) immunosuppressive TME including cells (regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs)), soluble factors (e.g., TGF- β) and immune checkpoints (PD-1, CTLA-4, lymphocyte activation gene (LAG)-3 protein); (3) a metabolically hostile microenvironment that is hypoxic, lactate-rich and nutrient-poor; (4) few tumour-associated antigens (TAAs) targets; (5) limitations in the ability of CAR-T cells to persist and expand in vivo following adoptive transfer and lack of survival and growth factors such as IL-2; (6) insufficient migration of CAR-T cells to tumour sites and insufficient infiltration of solid tumours by CAR-T cells, with the abnormal vasculature exacerbating the physical barrier of the tumour-associated stroma; (7) enhanced killing capabilities are needed and (8) safety profile needs to be improved to limit tumour burden-related cytokine release syndrome (CRS) and neurotoxicities (Horne et al., 2015; Gonzalez-Aparicio et al., 2011; Rodríguez et al., 2018; Steentoft et al., 2018; Alatrash et al., 2019; Bagley et al., 2018; D'Aloia et al., 2018; Kakarla and Gottschalk, 2014; Mirzaei et al., 2017; Sterner et al., 2018). However, in spite of these difficulties, encouraging signals of efficacy have been observed in solid tumours such as GBM (cf. Chapter 19), an indication heretofore recalcitrant to known treatments (Bagley et al., 2018; Brown et al., 2016).

Intratumoural Heterogeneity and Macrocellular Evolutionary Mechanisms

Intratumoural heterogeneity, including metastasis heterogeneity, and the macrocellular evolution of tumours that occurs within a patient, discussed in a preceding paragraph, are the primary causes of target escape and cancer relapses. Strategies to overcome this therapeutic hurdle include to target multiple antigens and to use multispecific CAR-T cells, as well as to implement proactive patient monitoring for tumour antigens (Horne et al., 2015; Takata et al., 2000; Dexter and Leith, 1986; Merlo et al., 2006; Marusyk et al., 2012).

Immunosuppressive Tumour Microenvironment

Up to one-third of patients receiving immunotherapy exhibit disease progression after a period of objective response (Restifo et al., 2016; Vijayan et al., 2017; Wang et al., 2017a). The relatively high frequency of such adaptive resistance indicates that an array of redundant and nonredundant immunosuppressive mechanisms coexist within the TME leading to treatment escape (Vijayan et al., 2017). A first strategy to address the immunosuppressive TME that is the focus of the checkpoint blockade strategies pursued with, for example, anti-PD-1 or anti-CTLA-4 drugs, as discussed in a preceding paragraph, is to implement combination therapy with soluble molecules to interrupt inhibitory pathways. Among the enzymes central to immune escape for which small molecule or biologics inhibitors can be developed are indoleamine-2,3-dioxygenase (IDO) encoded by the gene *ido1* and tryptophan 2,3-dioxygenase (TDO). Notably, dual covalent IDO/TDO inhibitors likely represent the best approach as compared with the single inhibition of IDO as IDO inhibition alone was observed not to be sufficient to prevent treatment escape; the dual inhibition approach indeed enables to prevent pathway redundancy in the tryptophan-kynurenine pathway in the TME (Kim et al., 2018b; Tomek et al., 2017). Beyond dual IDO/TDO inhibitors, inhibitors of nodal downstream effector pathways such as aryl hydrocarbon receptor blockade, a receptor that has been linked to Treg differentiation, also are worth considering (Tomek et al., 2017; Muller et al., 2019). This view is particularly reinforced by the disappointing results of the ECHO-301 Phase III clinical trial (NCT02752074) combining the IDO inhibitor epacadostat (INCB024360) with pembrolizumab (Muller et al., 2019). Glucocorticoid-induced tumour necrosis factor receptor (GITR) is another attractive immunooncology target as it promotes the functions of effector T cells (Teff) but lowers Tregs-mediated suppression. Therefore, agonists of GITR, exemplified by TRX518 that was observed to be well tolerated (NCT01239134), are currently being tested in combination with anti-PD1 therapy (NCT02628574) (Koon et al., 2016; Zappasodi et al., 2019). Similarly, mucin domain-3 protein (TIM-3) is being explored as a target to rescue anti-PD-1 inhibitor resistance. Similar to PD-1, TIM-3 is an immune checkpoint receptor that is expressed on tumour-infiltrating lymphocytes (TILs). PD-1 blockade of human head and neck squamous cell carcinoma TILs has been observed to result in TIM-3 upregulation thereby supporting a circuit of compensatory signalling resulting in anti-PD-1 blockade escape in a mechanism driven by the phospho-inositol-3 kinase (PI3K)/Akt complex downstream of TCR signalling. These observations promote the view that the dual targeting of PD-1 and TIM-3 or the PI3K/Akt complex could result in more effective cancer immunotherapies (He et al., 2018; Riley, 2009; Shayan et al., 2017). This hypothesis is being tested in several clinical trials, exemplified by trial NCT03099109 conducted by Eli Lilly and Company (Indianapolis, IN, USA) in patients with advanced or refractory solid tumours combining an anti-PD-1 compound with an anti-TIM-3 one. Similar combinations of immunosuppressant inhibitors are being explored in a

variety of other clinical trials; however, a typical drawback of immunooncology therapy combinations is that the eventual gain in response might come at the expense of increased toxicities, as was observed in a melanoma study of immunotherapy combinations, Checkmate 069, where nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) were assessed in combination as compared with ipilimumab alone, or in an NSCLC study ([NCT02039674](#)), where pembrolizumab (anti-PD-1) was tested in combination with chemo- and immunotherapies comprising ipilimumab, paclitaxel, carboplatin, bevacizumab, pemetrexed, erlotinib or gefitinib ([Niyongere et al., 2018](#)). On the other hand, engagement of the receptor PD-1 by its ligands modulates signalling networks downstream of the TCR other than PI3K/Akt, including mTOR, which ultimately promotes tumour growth ([Riley, 2009](#); [Kleffel et al., 2015](#)). As a result, and as oncogenical activation of the AKT–mTOR pathway facilitates immune escape by promoting the expression of the ligand PD-L1, it is interesting to consider combinations of immunotherapies with targeted cancer therapies such as mTOR or PI3K/Akt small molecule inhibitors that have been developed, in spite of their recognised toxicities, or to use a dual-specificity inhibitor that inhibits the kinase domains of both PI3K and mTOR as these are similar in structure ([LoRusso, 2016](#); [Martins et al., 2013](#); [Mayer and Arteaga, 2016](#); [O'Donnell et al., 2018](#)). Notably, the combination of an mTOR inhibitor with an anti-PD-1 antibody has been demonstrated in a mouse model not only to decrease tumour growth but also to increase tumour-infiltrating T cells and to decrease Treg levels ([Lastwika et al., 2016](#)). An additional possible target is prostaglandin E2 (PGE2), which is both an essential homoeostatic factor notably via inducing antiinflammatory M2 macrophage polarisation and a key mediator of immunopathology in chronic infections and cancer; PGE2 has been shown to induce myeloid-derived suppressor cells (MDSCs), thus promoting tumour growth ([Sinha et al., 2007](#)). As a result, the selective inhibition of COX-2 as a PGE2-forming enzyme or achieving the overexpression of the PGE2-degrading enzyme 15-hydroxyprostaglandin dehydrogenase have been proposed as novel approaches to regulate both PGE2 levels and PD-L1 expression with the goal to stimulate antitumour immune response and its converse, to alleviate immune suppression ([Prima et al., 2017](#)). The adenosinergic pathway constitutes another major immunosuppressive mechanism, the inhibition of which is also worth exploring. This pathway is mediated by high concentrations of extracellular adenosine in the tumour tissue by way of a complex interplay with adenosine receptors, particularly the A_{2a}, A_{2b} and A₃ receptors, to develop primary tumours and metastases and is activated within hypoxic tumours through tumour- and host-mediated mechanisms ([Vijayan et al., 2017](#); [Gessi et al., 2011, 2018](#)). Adenosine receptor small molecule drugs or biologics antagonists have proven useful in preclinical studies, and clinical trials are underway with adenosine antagonists alone or in combination with anti-PD1 or anti-PD-L1 compounds ([Vijayan et al., 2017](#)). There are numerous other potential molecular targets that can be inhibited to address the immunosuppressive TME with the view to generate a synergistic effect with immunotherapies; another

relevant example is the combination of a TGF- β inhibitor with a PD-1 inhibitor or a PD-L1 blocker, as a synergistic upregulation of the proinflammatory IFN- γ has been observed with the simultaneous blockade from binding of the PD-1 ligand B7-H1 (i.e., PD-L1) and TGF- β , the expression of which is largely increased in numerous cancers, with such increase, as emphasised in a preceding section, being linked to the loss of responsiveness of cancer cells to TGF- β -mediated growth inhibition, a mechanism the consequences of which are exacerbated by the angiogenic and context-dependent (TGF- β has been observed to be proinflammatory in the presence of IL-6) immunosuppressive effects of this cytokine through the inhibition of tumouricidal natural and lymphocyte-activated killer cells (Gold, 1999; Sanjabi et al., 2009; Wei et al., 2008). What is more, inhibiting the IL-6/JAK/STAT-3 signalling cascade, which is hyperactivated in numerous cancers and typically associated with poor prognosis, in combination with PD-L1 antibody blockade was reported to reduce tumour progression in murine models of pancreatic cancer and represent a promising therapeutic approach: IL-6 activates the signal transducer and activator of transcription 3 (STAT-3) by acting upon Janus kinases (JAKs) which phosphorylate STAT-3 to activate it, leading particularly to suppressing T cell-mediated immunity by downregulating MHC Class II (Johnson et al., 2018; Kitamura et al., 2017; Mace et al., 2018). This concept was notably tested in in vitro models in combination with an oncolytic vesicular stomatitis virus expressing IFN β (VSV:IFN- β) that has been shown to have inhibitory effects on various cancers including NSCLC. The JAK(JAK-1/JAK-2)/STAT inhibitor, ruxolitinib (Jakafi), was used in combination with VSV:IFN- β ; ruxolitinib was observed to inhibit STAT-1 and to a lesser extent STAT-3 and prevented NSCLC cells from enhancing PDL-1 in response to the oncolytic virus therapy (Patel et al., 2019). Furthermore, in a Phase Ib multicentre, open-label study in which the immune effects of the inhibitors were assessed using multiple biomarker technologies, JAK1 (itacitinib) or PI3K δ (INCB050465) inhibitor therapy was combined with pembrolizumab therapy in patients with advanced solid tumours. While in the conditions tested itacitinib combined with pembrolizumab resulted in detrimental changes in the TME and peripheral immune profile, the combination of parsaclisib (INCB050465) and pembrolizumab was observed to lead to more favourable changes in the TME and peripheral T cell activation (Kirkwood et al., 2018). The safety and efficacy of itacitinib in combination with pembrolizumab is tested in a single-centre, single-arm Phase II study in patients with metastatic PD-L1 positive NSCLC (NCT03425006). However, the complexity of treating solid tumours is highlighted by the JANUS 1 Phase III clinical trial failure of the combination of ruxolitinib and capecitabine (Xeloda) as a second-line treatment of patients with advanced or metastatic pancreatic cancer; interim analyses demonstrated that the combination did not show a sufficient level of efficacy to warrant continuation (Hurwitz et al., 2018). There are, however, other target options in the JAK/STAT pathway that can be explored, comprising the family of suppressor of cytokine signalling (SOCS) proteins. Members of this protein

family are inducible inhibitors of cytokine receptors that activate the JAK/STAT pathway; these could be the basis of a novel mechanism of action of targeted drugs with unique modes of action to inhibit JAK/STAT pathway while avoiding the toxicities and limitations of JAK/STAT inhibitors (Durham et al., 2019).

It is worth highlighting here again that radio- and chemotherapies induce ICD, thus enabling ‘*in situ* vaccination’ and impacting cell-mediated immunity. Moreover, it was demonstrated that conventional chemotherapies and targeted drugs improve the efficacy of immunotherapies including cytokine-mediated immunostimulation and cancer vaccines (Galluzzi et al., 2012; Tesniere et al., 2008; Nesselser et al., 2019; Pilones et al., 2015). This apoptotic phenomenon is represented in Fig. 20.7. Notably, a deep understanding of the complex cross talk between cancer cells, stromal cells and immune cells will likely permit the identification of new targets for anticancer therapy (Galluzzi et al., 2012). Given the high complexity of the biology of cancer and of the mammalian cell, as discussed in Chapter 7, artificial intelligence tools and novel disease taxonomy (nosotaxonomy) and refined cytokine and cellular signal transduction network modelling would be instrumental in generating novel hypotheses for preclinical and clinical validation.

Metabolically Hostile Microenvironment

As previously discussed, the TME is typically hypoxic, lactate-rich and nutrient-poor. Cancer cells thus face a strong competition from nontumour cells for nutrients. T cell metabolism is dynamic and depends on the TME context and particularly on the immune response; it notably impacts both cellular signalling and epigenetics. Moreover, direct alterations in the fluxes of metabolic pathways appear to be sufficient to modulate the longevity and proliferative capacities of T cells. As a result, an interesting strategy to improve the efficacy of engineered live immune effector cells is to harness cellular metabolic pathways (Kishton et al., 2017). Similarly to manufacturing technologies deployed for the production of mesenchymal stem cells (MSCs) with tailored efficacy properties, the metabolism of antitumour T cells can particularly be modulated at the ex vivo manufacturing stage to promote their ‘on delivery to patient’ tumour killing activities (de Witte et al., 2015; Rosenberg and Restifo, 2015). For example, reducing glycolysis by inhibiting the enzyme hexokinase by the simple addition in the culture medium of the nonmetabolisable analogue 2-deoxyglucose, or using small molecule inhibitors by targeting the serine/threonine kinase Akt (i.e., protein kinase B) that stimulates aerobic metabolism, results in improved antitumour efficacy against established and vascularised cancers (Crompton et al., 2015; Sukumar et al., 2017). In addition, and as alluded to above, reducing the metabolism of ex vivo expanded CAR-T cells can also be achieved by adding rapamycin to inhibit the mTORC1 pathway, which ultimately reduces the mitochondrial membrane potential but promotes antitumour activity (Sukumar et al., 2016). Another noteworthy example of successful culturing conditions is the addition in the culture medium of c-myc inhibitors, as c-myc signalling activity drives anabolic

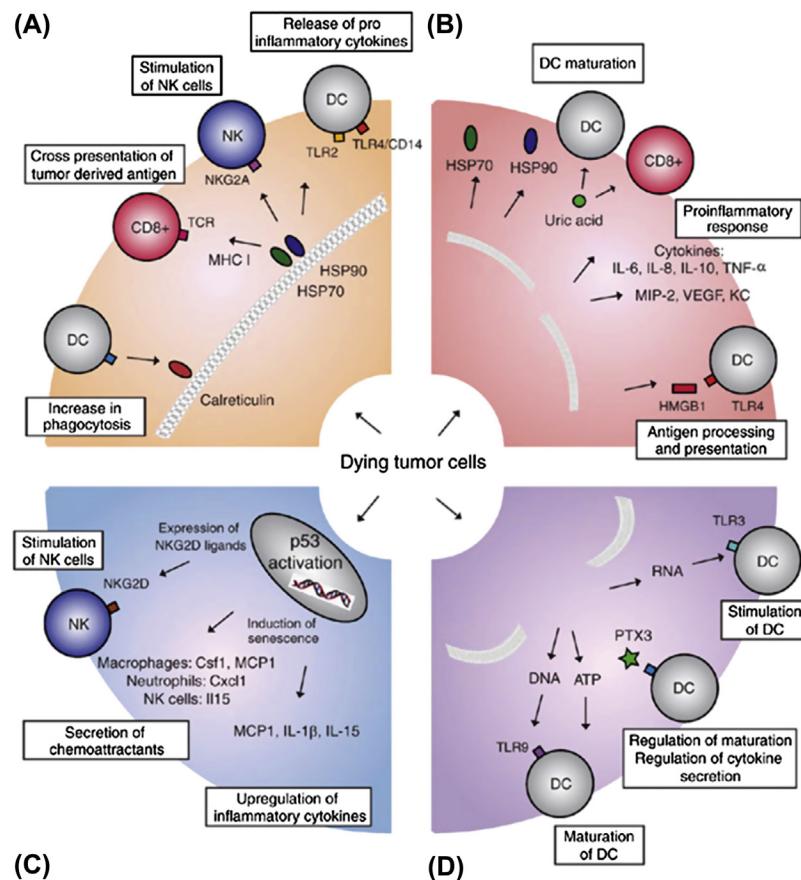


Figure 20.7 Immunogenic tumour cell death. Representation of the main immunogenic determinants of dying tumour cells. (A) Plasma membrane characteristics of immunogenic cell death. On apoptotic cell death induced by anthracyclines or γ -irradiation, calreticulin is translocated to the membrane, thereby dictating the immunogenicity of tumour cell death by determining the phagocytosis by dendritic cells (DCs). During tumour cell death, HSP70 and HSP90 can be transferred to the plasma membrane and cross-present tumour-derived peptides to CD8 $^{+}$ T cells, leading to a cytotoxic response. HSP70 and HSP90 also stimulate (natural killer) NK cell lysis, through NKG2A ligands. In response to HSP family members, DCs are able to mature and release proinflammatory cytokines. (B) Inflammatory cytokines released from dying cells. Necrotic cell death is associated with the release of numerous proinflammatory cytokines, including HSP family members, uric acid, which stimulates DCs and increases cytotoxic response, interleukin (IL) 6, IL-8, IL-10, tumour necrosis factor (TNF) α and also high-mobility group box 1 (HMGB1), which binds to toll-like receptor (TLR) 4, and regulates antigen processing and presentation. Of note, some of these components, such as HMGB1, can also be released during apoptotic cell death, leading to the induction of an immune response. (C) End-stage degradation products with proinflammatory properties. During late stages of cell death, cellular components with proinflammatory properties can be released from the cell debris. These include RNA molecules, which are recognised by TLR3 and lead to DC stimulation, DNA molecules, which interact with TLR9, or nucleotides. Importantly, late-stage apoptosis can be associated with the induction of PTX3, which interacts with the immunological synapse formed by DCs and apoptotic bodies. PTX3 modulates the immune response triggered by dying cells. (D) P53 activation in cancer cells and its immunological consequences. The DNA damage response pathway includes the activation of the protein p53, which leads not only to tumour cell apoptosis but also to stimulation of the immune system through expression of NKG2D ligands, triggering NK-mediated lysis. Activation of p53 is also associated with the induction of tumour cell senescence, which is accompanied by secretion of various chemoattractants and upregulation of inflammatory cytokines. (Reproduced with permission Tesniere A, Panaretakis T, Kepp O, Apetoh L, Ghiringhelli F, et al. Molecular characteristics of immunogenic cancer cell death. *Cell Death Differ* 2008;15:3–12.)

growth and metabolism in activated T cells thereby improving in vivo persistence and anti-cancer function (Kagoya et al., 2016). Remarkably anti-PD-L1 therapy directly inhibits the glycolytic activity of tumours cell, which translates into a competitive advantage for T cells, and immune checkpoint inhibitors may directly promote T cell anabolic metabolism. Nonetheless, and despite numerous challenges remaining to implement these concepts therapeutically, it is clear-cut that the balance of T cell metabolic activity and the expandability, longevity and durability of these cells are key regulators of their therapeutic characteristics. Consequently, ex vivo or in vivo pharmacological interventions, and perhaps genetic ones as well, to modulate antitumour T cell metabolic properties have the potential to generate improved anticancer living drugs (Kishton et al., 2017).

Few Tumour-Associated Antigens Targets

A prerequisite to developing safe and efficacious engineered live immune effector cells is to identify appropriate antigens that are tumour-associated and on the one hand ideally expressed by a wide range of tumours, and on the other hand ideally instrumental for tumour aggression to limit tumour escape, but restricted to tumours or eventually to nonvital or temporally dispensable tissues to avoid on-target/off-tumour toxicities. Importantly, CAR-directed living drugs will be particularly efficacious in cancers with low mutational burden and represent a critical option in cancers where treatment by checkpoint blockade fails (Sadelain et al., 2017). Of particular interest are cancer stem cells markers such as CD133 or CD44 or their spliced variants that are present in tumour tissues but with a low level of expression in normal tissues for those markers that are ubiquitously expressed such as CD44 (Atanackovic et al., 2016; Wakamatsu et al., 2012). Amongst solid tumour antigens that are currently being treated using CAR-T cell therapies are CD171, epidermal growth factor receptor (EGFR) vIII, carbonic anhydrase IX, α -folate receptor, HER2, α HER2/CD3, carcinoembryonic antigen, IL13R α 2, GD2, Erb2 + MUC1, vascular endothelial growth factor (VEGF) receptor, FAP, mesothelin and NKG2D (Sadelain et al., 2017; Mirzaei et al., 2017). In addition, mutation-derived antigens, or neoantigens, are actively being researched as they constitute very attractive immune targets given their selective expression on tumours, which may not only minimise immune tolerance but also minimise the risk of autoimmunity (Yarchoan et al., 2017). Many neoantigens are intracellular. However, these antigens can also be addressed with living drugs, as exemplified by an exploratory treatment directed against NY-ESO-1, which is an intracellularly expressed TAA. Two different strategies can be employed here: (1) an anti-NY-ESO1 mAb can be combined with anticancer drugs that facilitate the release of this antigen from dying tumour cells; in contrast to either drug alone, this combination therapy induces a strong antitumour effect accompanied by the development of NY-ESO-1-specific effector/memory CD8 $^{+}$ T cells (Noguchi et al., 2012); (2) mimicking the natural immune mechanism by combining NY-ESO-1:CAR-T cells that function as a mimetic of TCR for the recognition of the NY-ESO-1 antigen

in the context, for example, of human leucocyte antigen (HLA) A2 and subsequently adapting HLA-A2⁺ T cells such that they function as APCs by expressing the NY-ESO-1 antigen (Patel et al., 2016). Neoantigens can be identified notably by crunching next-generation sequencing data generated from a large array of tumour biopsies and other technology platforms of similar design (Bais et al., 2017; Boyle et al., 2017; Nogueira et al., 2018). Remarkably, the neoantigen landscape evolves during adaptive resistance to immune checkpoint blockade therapies with some of the new tumour neoantigens being recognisable by T cells (Anagnostou et al., 2017). These neoantigens should be included in the neoantigen pool of therapeutic interest that could be deployed for personalised oncology treatments; remarkably, oncolytic virus therapy is an emerging means to harness them. Targeting multiple antigens has the potential to avoid antigen-null tumour cells and antigen escape and to address the heterogeneity of tumours. What is more, dual receptor strategies have been successfully implemented to avoid on-target/off-tumour effect through the necessary binding of two tumour-borne antigens for the CAR-T cell to be active (Zhang et al., 2018). TCR-engineered T cell therapy is a relevant approach to address neoantigens and was reviewed in Chapter 13.

Limitations in the Ability of CAR-T Cells to Persist and Expand in Vivo following Adoptive Transfer and Lack of Survival and Growth Factors Such as Interleukin-2

Persistence, activation and in vivo expansion are critical parameters to both CAR-T cells efficacy and to reduce the rates of relapses or the rates of severe CAR-T treatment-related toxicities. Similarly to safety, persistence is strongly influenced by the CAR architecture or the type of T cells and can be enhanced, for example, by optimising the combination of specific intracellular signalling domains (Sadelain et al., 2017; Guedan et al., 2018; Halim et al., 2018). Moreover, functional moieties can be evolved towards greater functional fits by genetic engineering techniques including by assay-based directed evolution or knowledge-based directed mutagenesis. This approach can be illustrated by the optimisation of CAR-T cell activation and persistence via mutating the CD28 costimulatory domain based on a deep understanding of the mechanism of CD28-dependent exhaustion in CAR-T cells (Boucher et al., 2018). On the other hand, pharmacological intervention also is useful to this end, as exemplified by the combination of PD-1 inhibitor and CAR-T cells that was observed in a mouse model to strongly influence the efficacy of CAR-T cell therapy, as this combination results in a significant decrease in the percentage of Gr1⁺ CD11b⁺ MDSCs present in the TME (John et al., 2013a). Following the same rationale, CAR-T cells could be genetically engineered to knock out PD-1 or CTLA-4 (Mirzaei et al., 2017). An additional approach is to engineer CAR-T cells such that they locally synthesise various helper immunostimulatory cytokines, such as IL-15 that enhances T cell expansion and persistence in vivo by increasing the expression of antiapoptotic compounds, or IL-7 that enhances the

expansion of CAR-T memory stem cells, or IL-12 that supports T cell activation and expansion and is used at high doses in ex vivo manufacturing processes (Mirzaei et al., 2017; Dudley et al., 2003). Specifically regarding IL-2, the expression of this cytokine enhances the Th1 immune response, reverses anergy in tumour-infiltrating cells, inhibits the Treg-mediated suppression of the antitumour effector functions of T cells, reduces the synthesis of immunosuppressive cytokines including IL-10 or TGF- β by tumour-associated myeloid cells and enhances antitumour activity and expectedly enhances systemic IFN- γ (Mirzaei et al., 2017).

Insufficient Migration and Infiltration of CAR-T Cells to Tumour Sites

Trafficking of CAR-T cells deep into the tumour mass is a mechanical and a mechanistic hurdle that can be circumvented by a variety of approaches. First of all, the capability to degrade the extracellular matrix and target tumour-associated stromal cells can be conferred to live immune effector drugs to facilitate their infiltration into solid tumours, for example, by engineering heparanase (HPSE) expression (Scarfó and Maus, 2017). Another interesting ‘mechanical’ approach worth envisaging is the in situ delivery of CAR-T or CAR-NK cells, for example, at the time of tumour resection; this strategy was tested in glioblastoma with encouraging results (Nellan et al., 2018; Priceman et al., 2018; Sridhar and Petrocca, 2017). What is more, the homing capabilities of CAR-T cells to tumour sites can be increased by engineering. An innovative ex vivo enzymatic approach is to decorate the surface of CAR-T cells with fucosyl residues forming the Sialyl Lewis X moiety on P-selectin glycoprotein ligand-1 as these are involved in the recruitment of circulating cells in chronic inflammation sites or cancers and in the native rolling, adhesion and transmigration mechanisms of T cells and other cells; this molecular machinery has been described in detail for MSCs (Khaldoyanidi, 2008). In nature, the enzyme fucosyltransferase controls the synthesis of E-selectin ligands in human T cells (Alatrash et al., 2019; Knibbs et al., 1996; Mondal et al., 2018; Wolpe et al., 2019). This concept was particularly validated with Tregs in a preclinical model of GvHD (graft-versus-host-disease) where fucosylated Tregs persisted longer in vivo and showed increased efficacy at a lower dose, as well as in mouse models of leukaemia, breast cancer and melanoma where fucosylated T cells exhibited enhanced homing to cancerous tissues, ultimately boosting the antitumour activity of the antigen-specific cells (Alatrash et al., 2019; Parmar et al., 2015; Vertès, 2015a). Furthermore, T cell migration to tumours is mediated by tumour-expressed chemokines and chemokine receptors on the surface of T cells. Following this rationale, the overexpression of CXCR2, CCR2b or CCR4 that recognise, respectively, CXCL1, MSLN or CCL2, or CCL17 on the surface of tumour cells was observed to enhance T cell trafficking to tumour sites (Mirzaei et al., 2017). Numerous other strategies are being explored to augment CAR-T cell trafficking to tumour sites including, for example, by blocking protein kinase A localisation or expressing IL-7 and CCL19 as these factors are critical for the maintenance of T cell

zones in lymphoid organs (Adachi et al., 2018; Newick et al., 2016). Notably, the delivery of oncolytic viruses represents another attractive strategy to attack solid tumours by synergising with oncolysis as tumours treated with these therapeutic agents have been demonstrated to augment both the infiltration of activated macrophages and cytotoxic T cell as well as proinflammatory cytokines; what is more, tumour lysis debulks tumours and releases TAAs that in turn can drive an antitumour adaptive response with the potential to mitigate tumour antigen escape (Guedan and Alemany, 2018; Rosewell Shaw and Suzuki, 2018). Remarkably, oncolytic viruses can impact uninfected tumour sites as well through the cross-presentation of neoantigens, i.e., through *in situ* vaccination, from established tumours (Bommareddy et al., 2018). Oncolytic viruses can be engineered to selectively replicate within tumour tissue and to selectively destroy tumour tissue while enhancing antitumour immunity; however, to this date, oncolytic viruses have only shown moderate antitumour effects in the clinic (Rosewell Shaw and Suzuki, 2018; Twumasi-Boateng et al., 2018). For example, deploying bispecific T cell engagers (BiTEs) expressed in a replication-dependent manner by an oncolytic virus in combination with CAR-T cell therapy was demonstrated to overcome, particularly by way of an increased BiTE-mediated T cell activation, the typical limitations in solid tumours of CAR-T cells or of BiTEs (poor delivery kinetics and penetration into tumours and on-target off-tumour activity) as monotherapies (Scott et al., 2018; Wing et al., 2018). Noteworthily, several molecular tools of this diverse toolbox could be implemented in parallel in the search for synergies to achieve improved tumour tissue penetration.

Enhanced Killing Capabilities Are Needed

Increasing the potency (and safety) of CAR-T cells can be achieved by implementing novel superior architectures including novel antigen specificities, novel costimulatory signalling domains, novel T cell activating functional groups, and designing synthetic receptors, switches or circuits to control the anticancer activities of CAR-T cells in terms of their location, duration and strength (Sadelain et al., 2017; D'Aloia et al., 2018). Here, and akin to rationale-based design in the field of combinatorial logic circuits with the potential of biological computational circuits to custom-design cell signalling having been demonstrated in principle (Wang et al., 2011), systematically mapping the effects of various functional elements that govern different signalling domains has been proposed as a useful first step to identify functional building blocks, with the objective to define optimal CAR architectures through a combinatorial strategy where these elemental functions are deployed (Chakravarti and Wong, 2015). Another axis of development is to engineer the expression of novel killing machinery or to engineer the expression of 'helper' proteins. Enhanced efficacy can thus be achieved by optimising various parameters including (1) addressing the immunosuppressive TME to prevent multiple intratumour T cell inhibitor mechanisms (see paragraphs above); (2) achieving superior T cell stimulation by signal amplification, for example, by using armoured T cells secreting as

helper proteins various cytokines such as IL-12 as discussed in a preceding section as the local accumulation of this IL was shown to attract an innate immune cell response towards cancer cells that are invisible to CAR-T cells; (3) developing biomarkers and implementing companion diagnostics based on the aetiology of differential response; (4) leveraging neoantigens and particularly intracellularly derived cancer antigen peptides; (5) addressing intratumour heterogeneity by using multiple antigen or antigen pools; (6) achieving greater but controlled persistence, for example, using a drug-inducible growth switch to limit the severity of cytokine release syndrome (CRS); (7) using next-generation receptors, multi-specific mAbs or Fc variants; (8) using optimised modules including optimised linker and transmembrane domains and (9) optimising conditioning regimens. Notably, beyond the increased (constitutive or inducible) expression of IL-12, the increased expression of IL-2, CD40L and 4-1BBL have proven to enhance the efficacy and persistence of CAR-T cells (Chmielewski et al., 2013; D'Aloia et al., 2018; Yeku and Brentjens, 2016). A remarkable example of a novel architecture that combines CAR-T cells therapy and checkpoint blockade is that of the targeted delivery of a PD-1-blocking scFv by CAR-T cells based on the affinity interactions with the tumour cells enabled by the CAR; this novel construct was shown in an in vivo preclinical study to significantly enhance antitumour efficacy (Rafiq et al., 2018). The therapeutic potential of oncolytic viruses as a combination therapy with CAR-T cells for solid tumour is worth highlighting again, given that the mechanism of action of both these therapeutic modalities are different and complementary, as viruses are (1) lytic; (2) immunogenic and (3) amenable by genetic engineering techniques to selectively deliver to the TME molecules that can promote the killing capabilities of engineered immune effector cells such as CAR-T cells (Guedan and Alemany, 2018; Rosewell Shaw and Suzuki, 2018; Ajina and Maher, 2017). Oncolytic viruses have been reported to enhance the efficacy of adoptive CAR-T cell therapy. For example, the intratumoural delivery of oncolytic vesicular stomatitis virus was reported to promote the infiltration of CD28⁺ cells in a melanoma mouse model, thereby significantly increasing the median survival time (Rosewell Shaw and Suzuki, 2018).

Improved Safety Profile

Adoptive cell therapy has demonstrated stunning efficacy in several life-threatening indications for more than 50 years since the advent of haematopoietic stem cell transplantation with to this date more than 1 million patients dosed, for example, in inherited anaemias, immunodeficiencies or haematologic malignancies or more recently the adoptive therapy of allogeneic MSCs in GvHD (Vertès, 2015a). Similarly, adoptive CAR-T cell therapy has demonstrated stunning efficacy in ALL, but the current CAR architecture may trigger severe treatment-related adverse events, most commonly including severe to fatal tumour load-dependent CRSs in about a third of the treated patient population and severe neural toxicities (Frey, 2019; Anagnostou et al., 2019; Brudno and

Kochenderfer, 2016; Jain and Davila, 2018). CRS is a systemic inflammatory response caused by massive T cell expansion and by cytokines, including IL-6, IFN- γ , IL-15, IL-8, IL-10 and IL-2, which are observed at elevated levels in patients treated with CAR-T cells, leading to severe, however typically reversible, organ dysfunctions. Neurotoxicities on the other hand proceed from a different pathophysiology than CRS, a hypothesis is that they are mediated by myeloid cells that cross the blood–brain barrier (Sterner et al., 2018) and thus require different therapeutic management (Brudno and Kochenderfer, 2016; Sharma et al., 2018). A metaanalysis reviewing the outcomes of anti-CD19 CAR-T cell therapies in ALL by architectural type remarkably demonstrated that significant differences in efficacy and toxicity exist between different CAR constructs, with CD28 leading to lower efficacy but (expectedly, as linked to T cell expansion) also to lower toxicity. This metaanalysis included data from 573 patients (309 adults and 264 children). The pooled complete remission rate was comparable between adults and children (84% and 83%, respectively), with the efficacy varying from 86% for 4-1BB-costimulated CAR-T cells to 74% for CD28-costimulated CAR-T cells of the same architecture generation, with pooled 1-year progression free survival being 53% for adults and 56% for children (Anagnostou et al., 2019). A first step to achieve short-term and long-term safety is to prevent the occurrence of CRS and to better manage them. The availability of a biological ‘AND gate’ based on a disease signal (in the case of cancer, the tumour antigen) and on a therapeutic signal (preferentially an oral small molecule) is in theory an excellent option to engineer safe living drugs (Wu et al., 2015). Enhanced safety of the CAR-T cell therapy can thus be approached along the following axes: (1) improving control, for example, by separating the costimulatory domain from the antigen recognition domain via a separate molecular switch controlled by an oral molecule that is delivered either systemically or locally and with varying dosages for refined temporal and spatial control of living drug therapy; (2) availability of an antidote as an orally delivered molecule that induces a CAR-T cell suicide switch; (3) reducing side effects via inhibitory CARs using signalling domains from inhibitory pathways that suppress the activity of the CAR-T cells that are bound to antigens from healthy cells; (4) enhanced tumour selectivity by making use of two antigen receptors in parallel and (5) avoiding HAMA (human anti-mouse antibody) and HACA (human antichimeric antibody) immune responses by using fully humanised components, thus notably avoiding typical limitations of the first immunotoxins. An example of a molecular ‘ON’ switch is rimiducid; in this innovative CAR-T/small molecule couple, the CAR-T cell harbours two engineered antigens: one to direct it to the cancer cell and another to bind to rimiducid to exert a costimulatory effect (Kingwell, 2017). Such biological Boolean logic has been successfully implemented to reduce toxicities by combining CARs of two different specificities: here two CARs are coexpressed, providing on one the primary CD3 ζ signal and providing on the other the costimulatory CD28 signal. Simultaneous signalling through both chains is required to fully induce T cell activation. Given this constraint,

both antigens must be engaged. This biological 'AND gate' was demonstrated for targeting: (1) ErbB2 and Muc1, (2) mesothelin and folate receptor- α and (3) PSCA and PSMA (Holzinger and Abken, 2019). More recently, the new anti-CD19 CAR molecule CD19-BBz(86) that bears the costimulatory domains 4-1BB and CD3 ζ was observed to result on the one hand in the production of lower levels of cytokines and the expression of higher levels of antiapoptotic molecules than CD19-BBz CAR-T cells; moreover, they proliferated more slowly but retained potent cytolytic activity, as suggested from the data of a 25-patient Phase I clinical trial in B-cell lymphoma (NCT02842138), where 54.5% of the treated patients exhibited complete remission (6 of 11 patients) but neither neurological toxicity nor CRS greater than grade 1. In addition, no elevated serum cytokine levels after the CAR-T therapy cell infusion was detected including in those patients who achieved complete remission; remarkably, CD19-BBz(86) CAR-T cells persistently proliferated and differentiated into memory cells in vivo (Ying et al., 2019). This development demonstrates that subtle changes in the architecture of CARs can result in significant improvement of the intrinsic attributes of a living drug. Regarding neurotoxicities, GM-CSF neutralisation by lenzilumab might result in enhanced CAR-T cell antigen-specific proliferation in the presence of monocytes and thus was proposed as a potential preventive treatment of neurotoxicities that would proceed via monocyte control (Sterner et al., 2018).

SUCCESSES IN CHECKPOINT INHIBITORS

Immunooncology and checkpoint inhibitor blockade constitute game-changing fundamentals in terms of the understanding and innovation for the treatment of cancers. These major advances expand the array of therapeutic modalities beyond the horizon of targeted oncogene pathway inhibition that was in itself a major advance as compared with conventional surgery or chemo- and radiotherapies (Bild et al., 2006).

Among the checkpoint inhibitors, the CTLA-4 inhibitor ipilimumab was first approved in 2011 by the US FDA (Food and Drug Administration) for the treatment of melanoma. In the 676-patient Phase III clinical trial of unresectable stage III or IV melanoma with disease progression while receiving therapy for metastatic disease, the observed overall survival was 10 months; grade 3 or 4 immune-related adverse events occurred in 10%–15% of patients and 14 deaths were reported related to the study drugs (2.1%), 7 of which were associated with immune-related adverse events (Hodi et al., 2010). The most common adverse events were immune-related events; they occurred in approximately 60% of the treated patients with 10%–15% being grade 3 or 4 immune-related adverse events that mostly affected the skin and the gastrointestinal tract with all these occurring during the induction and reinduction periods (Hodi et al., 2010).

The PD-1 inhibitor pembrolizumab was first approved by the US FDA in 2014 for the treatment of melanoma. Common side effects that were attributed to pembrolizumab

were fatigue, pruritus and decreased appetite, with no clear difference according to dose or schedule (Garon et al., 2015; Shaverdian et al., 2017). The interim analyses of the KEYNOTE-006 study, an 834-patient multicentre, open-label, randomised Phase III clinical trial, revealed that pembrolizumab shows superior overall and progression-free survival as compared with ipilimumab in patients with advanced melanoma. The median overall survival was not reached in the pembrolizumab groups but was 16.0 months with ipilimumab, suggesting that pembrolizumab provides superior overall survival versus ipilimumab, with no difference between pembrolizumab dosing schedules (Schachter et al., 2017). Pembrolizumab exhibits significantly superior Kaplan–Meier estimates of overall survival in metastatic melanoma patients receiving first-line treatments (Baynes, 2019). Notably, biologically informed screening Phase II programmes have identified broad-spectrum monotherapy activity of pembrolizumab in more than 25 major cancer types; as a result, pembrolizumab received more than 20 US approvals in a variety of indications and tumour types (Baynes, 2019).

The immune checkpoints CTLA-4 and PD-1 function at different phases of the effector T cell activity (Figs 20.5 and 20.8), respectively, cytotoxic T-lymphocyte-associated protein 4, or CD152 (its ligands are CD80, i.e., B7-1, and CD86, i.e., B7-2), and programmed cell death protein 1, or CD279 (its ligands are PD-L1, i.e., CD274 or B7-H1–B7 homologue 1, and PD-L2) (Postow et al., 2015). The interactions between these immune checkpoints and their ligands occur at multiple steps of an immune response; for example, in the case of PD-1, on the one hand PD-L1 or PD-L2 expressed on an APC negatively regulates T cell activity through PD-1 and through an interaction between B7 and PD-L1, and on the other hand, in the TME where tumour-expressed PD-L1 interacts with PD-1 on T cells to suppress T cell effector function (Postow et al., 2015). Considering that these coinhibitory molecules are mostly expressed on T cells after their activation (Messel et al., 2011; Sansom, 2000), a reasonable hypothesis is that combining their inhibitors could increase T cell activation and tumour killing, albeit with the risk of an increased toxicity (Floudas et al., 2019). Such a PD-1/CTLA-4 combination was tested in a Phase III clinical trial in advanced melanoma where the combination of nivolumab with ipilimumab resulted in an overall survival rate at 3 years of 58% (314 patients), whereas it was 52% in the case of the nivolumab monotherapy (316 patients) and 34% for the ipilimumab monotherapy (315 patients); however, treatment-related adverse events of grade 3 or 4 occurred in 59% of the patients receiving the combination therapy, in contrast to 21% for those receiving nivolumab monotherapy and 28% for those receiving ipilimumab monotherapy (Wolchok et al., 2017). Similar assessments are conducted in hepatocellular carcinoma (HCC) to compare the safety and the efficacy of combinations of nivolumab with ipilimumab, or durvalumab (Imfinzi) (PDL-1 blocker) with tremelimumab (anti-CTLA-4) (Floudas et al., 2019).

A variety of approaches are being explored to enhance the efficacy of immune checkpoint inhibitors. The epigenetic regulatory family of histone deacetylases (HDAC)

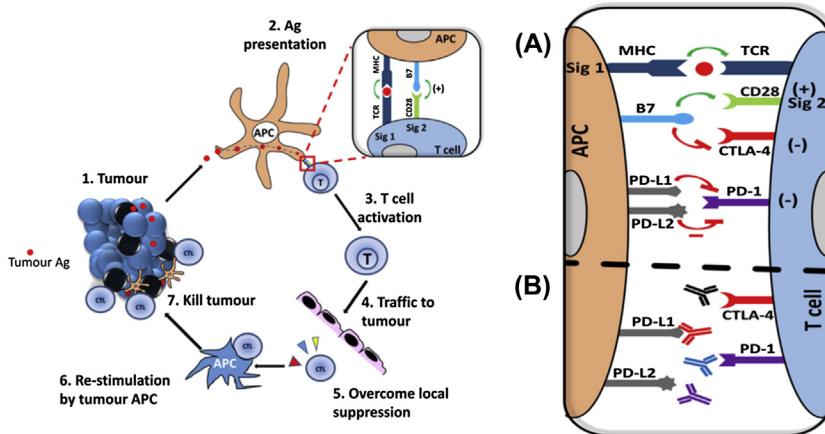


Figure 20.8 Mechanism of immune checkpoint blockade. **Left panel:** Tumour antigen cross-presentation and induction of antitumour immunity (Adachi et al., 2018). Tumour antigen release: tumour-specific antigens (red dots) are picked up by professional antigen-presenting cells (APCs), such as dendritic cells, and migrate from the tumour site to the draining lymph node (Ajina and Maher, 2017). Antigen presentation: As the APCs migrate, they mature during the time in which the tumour antigen is processed into small peptides, which are then presented on the surface of the APC in the context of a major histocompatibility complex (MHC)-peptide complex to lymphocytes (Al-Lazikani et al., 2012). T cell activation: Recognition of the MHC peptide complex by cognate T cell receptor constitutes signal 1. However, a second costimulatory signal (CD28:B7.1/B7.2) is required before a naïve T cell can become fully activated cytotoxic CD8 T lymphocytes (CTL). T cell activation can be enhanced by CD4 helper T cells (Altrash et al., 2019). T cell trafficking: Activated T cells leave the dLN and traffic through the peripheral blood vessels back to the tumour site (Albain et al., 2009). Overcoming local suppression: Activated CTLs need to be able to overcome tumour immunosuppressive mechanisms in the tumour microenvironment (Albiges et al., 2015). APC restimulation: Tumour resident APC restimulates antigen-specific CTLs (Alewine et al., 2015). Tumour killing: Antigen-specific CTLs recognise and kill antigen bearing tumour cells. **Right panel:** T cell activation is ultimately dictated by the balance of costimulatory versus coinhibitory signals. (A) On T cell activation (signal 1 + signal 2), coinhibitory receptors such as CTLA-4 and PD-1 are upregulated. CTLA-4 binds with greater avidity to the B7 molecules CD80/CD86 and outcompetes binding of CD28 leading to inhibition of T cell activation. (B) Monoclonal antibodies directed against inhibitory receptors block their ability to bind to their respective ligand, favouring the costimulatory signal and thus prolonging T cell activation and the antitumour immune response. (Reproduced with permission Steven A, Fisher SA, Robinson BW. *Immunotherapy for lung cancer. Respirology* 2016;21:821–33.)

constitutes a relevant target here as the inhibition of these enzymes that are dysregulated in numerous cancers has met initial clinical success in some haematologic malignancies (Mottamal et al., 2015). HDAC inhibitors exhibit not only direct tumour cytotoxicity but also they alter their immunogenicity and enhance antitumour immune responses. The observation that Class I HDAC inhibitors upregulate the expression of PD-L1 and PD-L2 in melanomas has moreover suggested to combine HDAC inhibition with PD-1 blockade, a hypothesis that was validated in a mouse model whereby mice treated with the

combination therapy exhibited slower tumour progression and increased survival as compared with mice from the control or the monotherapy treatment groups (Woods et al., 2015). There are numerous clinical trials testing immune checkpoint inhibitors in combination with an array of different therapeutic modalities, comprising oncolytic viruses or tyrosine kinase inhibitors (TKIs). As discussed earlier, TKIs including EGFR and JAK have pleiotropic effects on immune pathways and the immunosuppressive TME (Paul and Mukhopadhyay, 2004). Among the TKIs, sorafenib and lenvatinib are tested in combination with nivolumab (respectively, NCT03439891 and NCT03418922) and pembrolizumab (respectively, NCT02988440 and NCT03006926) and avelumab (Bavencio) with axitinib (NCT03289533) (Floudas et al., 2019). Other drug classes that are being explored as combination with immune checkpoint inhibitors are VEGF inhibitors, as tumour-secreted VEGF supports tumour progression by inducing neovascularisation and by acting as an immunosuppressive factor; effects of VEGF inhibition include the enhancement of both antigen presentation and intratumoural T cell infiltration (Wallin et al., 2016). This concept was tested in a Phase I/II clinical study by the combination of atezolizumab (Tecentriq) (anti-PD-L1) and bevacizumab (Avastin) (anti-VEGF), which resulted in partial responses in 62% of patients with treatment-naïve advanced HCC (Wallin et al., 2016; Stein et al., 2018).

As discussed, there are numerous new therapeutic strategies to fully harness the potential of immune checkpoint inhibitors, including drugging with small molecules novel immune checkpoint proteins such as LAG-3, OX40, TIM-3, and B7-H3 or HHLA2 from the B7 immune checkpoint family, or as previously discussed GITR (GITR-related protein), given that this protein promotes Teff functions and hampers Tregs suppression; another option yet is to target aberrant tumour-expressed glycosylations (Rodriguez et al., 2018; Zappasodi et al., 2019; Smith et al., 2019; Tang et al., 2017; Yan et al., 2019). What is more, the blockade of the PD-1/PD-L1 axis in combination with other coinhibitors, and particularly TIM-3, CTLA-4 and LAG-3, has been reported to exert synergistic effect in reversing T cell exhaustion (Chen and Flies, 2013) and that of CTLA-4 with agonist anti-GITR molecules was shown to completely abrogate ex vivo the immunosuppression mediated by human liver tumour-derived Tregs (Zappasodi et al., 2019; Pedroza-Gonzalez et al., 2015). Other approaches again include exploring the combination with CAR-T or CAR-NK immune effector cell-based therapies or with therapeutic agents that stimulate the immune system such as dendritic cell cancer vaccines, adjuvants or oncolytic viruses. A critical challenge is to identify, from the huge space of such possibilities, what are the optimal therapeutic strategies to overcome the complex barriers to achieving effective antitumour responses with the objective to maximally harness the immune system to fight cancer while avoiding inducing autoimmunities; nevertheless, rational choices can be made from a deep understanding of the molecular actors of cellular regulatory networks and of cancer biology, as well as of the complex mechanisms that enable neoplastic cells to evade immune system-mediated killing (Dougan et al., 2019; Andersen, 2019).

COMBINATION THERAPIES: SEQUENTIAL VERSUS PARALLEL VERSUS CONDITIONING INDUCTION REGIMENS

There is a long history of use of combination therapy in cancer, with the isobole equation and the additivity concept having been formally formulated in 1928 by Siegfried Loewe (Fig. 20.9). Beyond efficacy, a critical concern is of course limiting toxicities to healthy tissues including on-target toxicities and pharmacokinetic interactions. The first clinically successful combination chemotherapy was performed in 1965 with the coadministration of methotrexate (an antifolate), vincristine (Rinca, which is an alkaloid tubulin inhibitor), 6-mercaptopurine (a purine nucleotide synthesis inhibitor) and prednisone (a steroid) to treat childhood ALL (Fig. 20.1) (Al-Lazikani et al., 2012; Frei and Freireich, 1965). Since that time, cytotoxic agents have been demonstrated to be most effective when they are given in combination for promoting synergistic or additive effects (Al-Lazikani et al., 2012). Remarkably, simulation experiments of the dynamics of therapeutic resistance comparing synergistic combinations with additive combinations, at the same combination of effective doses, suggest that synergistic combinations, referred to as 'proefficacy', or 'offensive' strategies, are superior in delaying the onset of resistance through an early dramatic decrease in the number of cancer cells, but antagonistic combinations, referred to as 'antiresistance' or 'defensive' strategies, appear superior in suppressing the expansion of resistant subclones by preventing the clonal expansion of singly resistant cells and thus provide a long-term defence against treatment escape (Saputra et al., 2018). As a result, and given the very high intratumoral genotype heterogeneity, delivering rational combinations of targeted therapies has been suggested as the appropriate strategy (Al-Lazikani et al., 2012). In practice, first-line cancer chemotherapy typically comprises a combination therapy, as exemplified in NSCLC, with bevacizumab (Avastin, a VEGF inhibitor preventing neovascularisation) approved by the US FDA in 2006 to be administered in combination with carboplatin (e.g., Paraplatin, which generates lesions in the DNA thus preventing replication and transcription) and paclitaxel (e.g., Taxol, which blocks mitosis by targeting tubulins), for the initial treatment of patients with unresectable, locally advanced, recurrent or metastatic, nonsquamous NSCLC, with the approval having been based on a significant improvement in overall survival (Cohen et al., 2007). Targeted therapies aim at exploiting the phenomenon of oncogene addiction by targeting a specific oncogene that is overexpressed and that is critical for the cancer cells to retain their malignant phenotype or by remedying the inappropriate expression of a tumour-suppressor gene. This class of therapeutics can be exemplified by TKIs targeting EGFR such as gefitinib (Iressa) and erlotinib (Tarceva), used in EGFR-mutant NSCLC (Al-Lazikani et al., 2012). However, responses are in many cases only transient due to multiple mechanisms of resistance including (1) alterations in the addiction pathway, (2) activation of parallel signalling pathway bypasses, (3) oncogenic signal transduction pathway-independent routes driven by the EMT and gain in stem cell-like properties, (4) changes in the TME and (5) the presence of multiple malignant mutations within the same tumour (Al-Lazikani et al., 2012; de Bono and

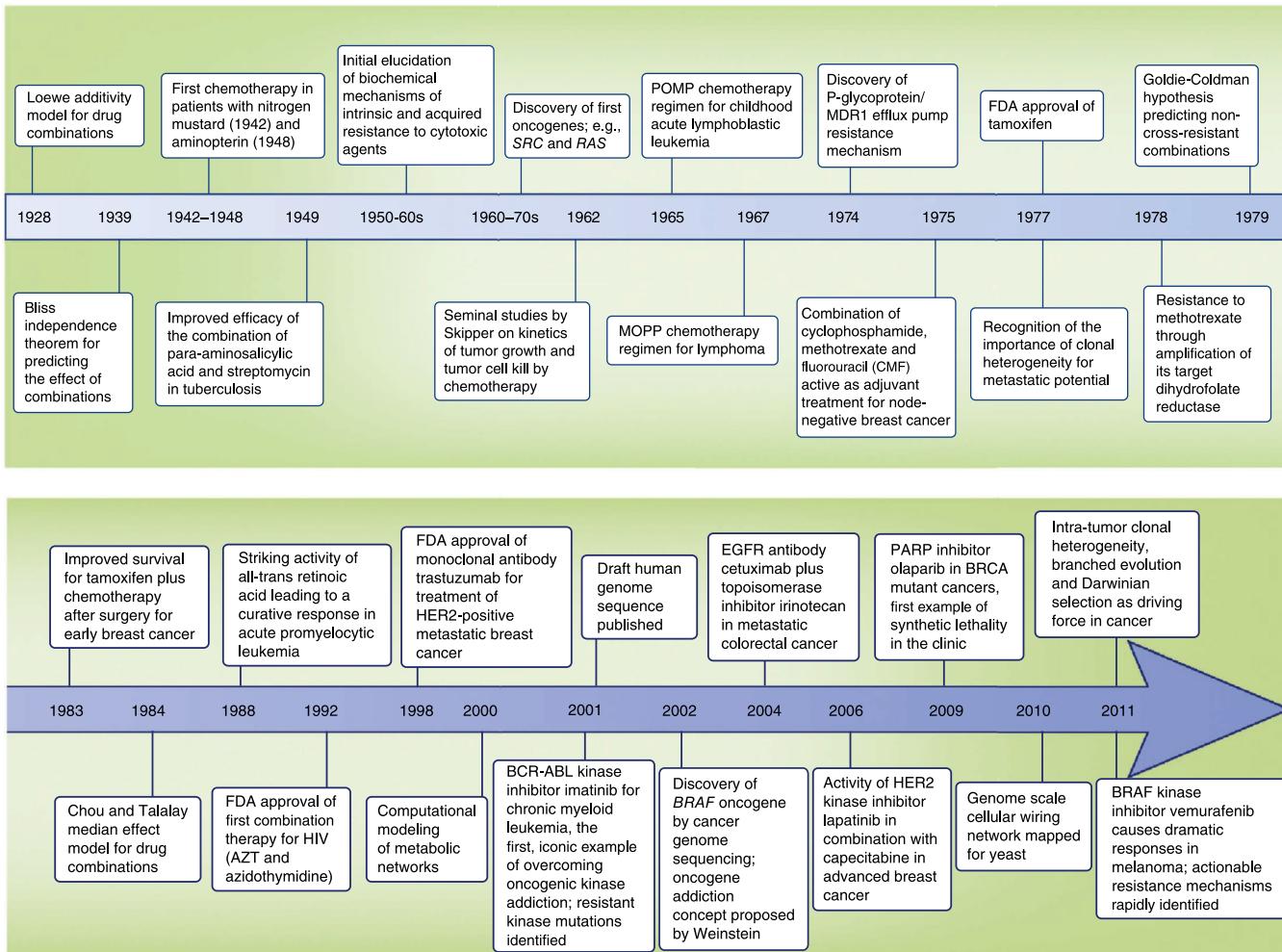


Figure 20.9 History of combination therapies in oncology: 1928–2011. POMP: procarbazine, vincristine (Oncovin), nitrogen mustard (mustine) and prednisone. MOPP: nitrogen mustard, vincristine, prednisone and procarbazine. (Reproduce with permission Al-Lazikani B, Banerji U, Workman P. Combinatorial drug therapy for cancer in the post-genomic era. *Nat Biotechnol* 2012;30:679–92.)

[Ashworth, 2010](#); [Gerlinger et al., 2012](#)). Conceptually, two main combination strategies exist: (1) within the same pathway (i.e., a 'vertical' combination), targeting, for example, a receptor and a molecular actor situated downstream of the signalling pathway controlled by the said receptor and (2) multipathway (i.e., a 'horizontal' combination), targeting independent molecular actors of different signal transduction pathways. An interesting vertical combination is that of an mTOR inhibitor of the PI3K inhibitor class with trastuzumab, as a mechanism of trastuzumab resistance in breast cancer is the activation of the PIK3CA oncogene coding for a subunit of PI3K or the loss of the tumour suppressor gene PTEN that codes for the opposing phosphatase ([Al-Lazikani et al., 2012](#)). Interestingly, mTOR inhibition has been observed to ameliorate immunosenescence ([Mannick et al., 2014](#)). The class of small molecule mTOR inhibitors, given at an appropriate dose to leverage synergic or additive effects with other drugs of a combination, could thus represent a platform combination drug in spite of the typical adverse events of products of this class, including stomatitis, diarrhoea, nausea, cytopenia, hyperlipidemia and hyperglycemia.

The power of combination therapy is a typical part of the development strategy of the oncology therapeutic areas of large pharmaceutical companies. As highlighted earlier, after having solidly established Keytruda (pembrolizumab) as a monotherapy in multiple cancer indications, Merck (Merck Sharp & Dome, Merck & Cie, Inc., Kenilworth, NJ, USA) is deploying this drug as a broad-spectrum foundation for combination therapies ([Baynes, 2019](#)). An illustration of this is the approval in Spring 2019 by the US FDA of the anti-PD-1 Keytruda in combination with the receptor TKI (including vascular endothelial growth factor receptors) Inlyta developed by Pfizer (Pfizer Inc., New York, NY, USA) (axitinib) as first-line treatment for patients with advanced renal cell carcinoma (RCC). Inlyta monotherapy was previously shown to trigger partial responses in clinical trials in RCC and several other oncology indications and in a 723-patient Phase 3 clinical trial to be superior to sunitinib monotherapy as a second-line treatment with the benefit of a longer progression-free survival ([Anonymous, 2019a](#); [Motzer et al., 2013](#); [Rini et al., 2011a](#)). This approval was supported by results from the 861-patient, randomised, multicentre, open-label Phase 3 KEYNOTE-426 clinical trial in advanced RCC. Estimated overall survival rates were significantly improved in patients who received the Keytruda–Inlyta combination compared with sunitinib (another multitargeted receptor TKI) (respectively, 90% versus 78%) with an ORR of 59% for patients who received the Keytruda–Inlyta combination and 36% for those who received sunitinib, with a complete response rate of, respectively, 6% and 2% and a partial response rate of 53% and 34% ([Anonymous, 2019a](#)). Remarkably, the anti-PD-L1 Bavencio (avelumab) developed by Merck KGaA (Darmstadt, Germany) also was approved in Spring 2019 as first-line treatment of patients with RCC in combination with Inlyta developed by Pfizer Inc. (New York, NY, USA), demonstrating that both immune checkpoint receptors and their ligands have a potential for combination immunotherapy ([Anonymous, 2019b](#)).

The approach of cancer immunotherapy relies on mitigating numerous molecular mechanisms that mediate immune tolerance to self-antigens. There has been tremendous convergence innovation in recent years on the one hand between the fields of oncology and immune tolerance that culminated in the development of immune checkpoint inhibitors, and on the other hand, in the fields of HIV and cancer for the development of adoptive therapy of engineered immune effector cells such as CAR-T cells. The advantage of such living drugs is that they bypass many mechanisms of immune tolerance by the simple fact that they are expanded *ex vivo* and delivered in large numbers, albeit persistence and expansion *in vivo* remain critical parameters for the success of these novel therapies. It is worth emphasising again that combining living drugs with checkpoint inhibitors to block detrimental and immunosuppressive signalling pathways constitute an exciting novel approach of conducting combination therapies. The concept was demonstrated in a mouse model where the combination of a PD-1 inhibitor with CAR-T cells resulted in enhanced efficacy mediated by a significant decrease in the TME in the percentage of Gr1⁺ CD11b⁺ MDSCs (John et al., 2013a). What is more, *in situ* immunisation aiming at breaking tolerance to neoplastic cells is another particularly attractive complementary therapeutic avenue, which can be achieved by a variety of means including vaccination with whole tumour cells, protein, peptide or dendritic cells, not to forget that the phenomenon of ICD can be leveraged by radio- or chemotherapy to have apoptotic cancerous cells serve as accessible pools of neoantigens or even oncolytic viruses that lyse cells and make intracellular neoantigens available. Such an *in situ* vaccination approach can be illustrated by the combination of Flt3L (ligand of the Flt3 receptor tyrosine kinase) to recruit DCs to the tumour, low-dose radiotherapy to release TAAs and intratumoural poly-ICLC administration as an Flt3L agonist to activate tumour-antigen loaded DCs, a combination aiming at triggering in patients who previously did not respond to PD-1 blockade the development of a population of PD1⁺CD8⁺ T cells that would make them respond to the checkpoint inhibitor therapy (NCT01976585) (Hammerich et al., 2019). It is also worth noting here that mTOR functions as a mediator of Flt3L signalling promoting the development and homoeostasis of CD8⁺ and CD103⁺ DCs (Wu, 2010).

Advancing in understanding and harnessing all these mechanisms collectively is critical to overcome immune tolerance in cancer (Mantovani, 2018). Combination trials recorded as of the end of 2017 are skewed towards PD-1/PD-L1 inhibitors, with the majority of the combinations tested including targeted therapies, chemotherapies, radiotherapies, chemo-radiotherapy or multiway combination therapies and the PD-1/PD-L1 inhibitors approved by the end of 2017: pembrolizumab (399 trials), nivolumab (340 trials), durvalumab (161 trials), atezolizumab (124 trials) and avelumab (32 trials) (Fig. 20.10); however, the top strategies currently being explored are combinations with other immunooncology drugs, targeted therapies and chemotherapies (Tang et al., 2017). By Spring 2019, anti-PD-1/PD-L1 therapies have been approved in numerous cancers, including in melanoma,

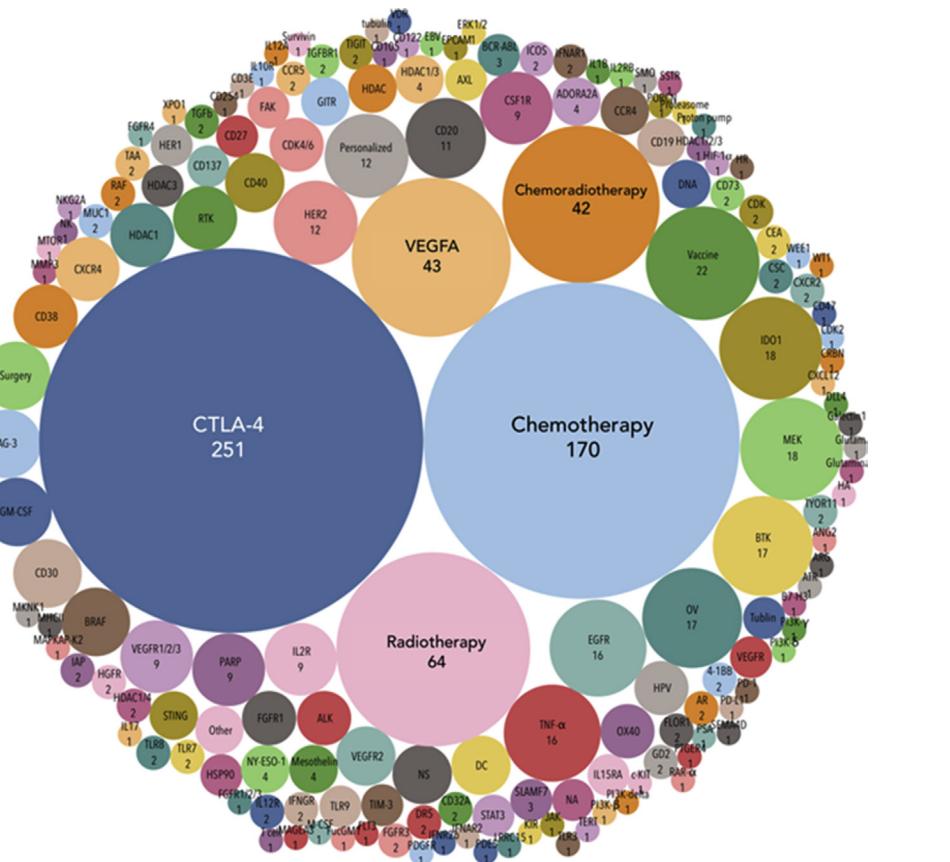


Figure 20.10 2017 landscape analysis of targets of anti-PD-1/L1 combination trials. The size of the bubble correlates to the number of trials. Number of trials using common combination strategies: anti-CTLA-4 agents: 251; chemotherapies: 170; radiotherapies: 64; anti-VEGFA agents: 43; chemoradiotherapy combinations: 42. There was a very rapid increase of new anti-PD-1 or anti-PD-L1 combination trials between 2009 and 2017, increasing from 1 trial in 2009, 5 in 2010, 13 in 2012, 58 in 2014, 190 in 2015, 329 in 2016 and 469 in 2017 (52,539 new patients enrolled in 2017 alone). (Reproduced with permission Tang J, Shalabi A, Hubbard-Lucey VM. Comprehensive analysis of the clinical immuno-oncology landscape. *Ann Oncol* 2017;29:84–91.)

NSCLC, RCC, urothelial bladder cancer, Hodgkin's lymphoma, head and neck cancer, Merkel cell carcinoma, microsatellite instability-high or mismatch repair deficient cancer, colorectal cancer, gastric cancer, HCC, cervical cancer, primary mediastinal large B-cell lymphoma, small cell lung cancer, cutaneous squamous cancer and breast cancer.

Targeted therapies aim chiefly at triggering the apoptosis of malignant cells or the arrest of their cellular cycles. Considering the complexity of the pathobiology of cancer, a logical evolution in developing therapeutic regimens with long-term clinical benefits is to deliver combinations of drugs that address several, if not all, of the hallmarks of

cancer and thus to break multiple mechanisms of resistance. This trend is already signalled by the broad use of VEGF inhibitors to prevent tumour angiogenesis in first-line chemotherapies. Combining checkpoint inhibitors to remedy the tumour suppressive TME, VEGF inhibitors to starve fast growing cancer cells and engineered immune effector cells to specifically kill malignant cancer cells appears a rational therapeutic strategy. Beyond efficacy, a critical question remains that of toxicity, an issue that was highlighted by the Phase I clinical trial failure in metastatic RCC of the combination between sunitinib and the CTLA-4 inhibitor tremelimumab due to dose-limiting toxicities related to acute kidney injury (Rini et al., 2011b). Nevertheless, it is worth noting here again that it has already been demonstrated that combining PD-1 inhibitors and CAR-T cells could potentially lead to superior clinical benefits than either therapy alone (John et al., 2013a,b). Here, designer CAR-T cells, for example, PD-1 or CTLA-4 deletion mutants, to prevent the toxicities of anti-PD1 or anti-CTLA-4 therapies, could provide appreciable advantages for solid tumour therapy (Mirzaei et al., 2017). This approach would be facilitated by implementing the so-called 'universal' cells, the HLA genes of which have been inactivated to reduce their alloreactivity and enable their allogeneic use (Gornalusse et al., 2017). Remarkably, it was reported that CAR-T cells that do not overexpress PD-1 on their stimulation due to the ablation of the corresponding genes exhibit enhanced T cell activation by the upregulated expression of CD137 (4-1BB) and are characterised by significantly increased persistence at the tumour site as well as enhanced antitumour activity compared with conventional CAR-T cells (Menger et al., 2016; Ren et al., 2017). Following the same rationale, multidrug-resistant TCR- $\alpha\beta$ -deficient CAR-T cells were generated and observed not only to display efficient antitumour activity but also to proliferate in the presence of purine and pyrimidine nucleoside analogues, compounds that are typically used as preconditioning lymphodepleting regimens (Valton et al., 2015). Similarly, the combination of conventional immunotherapy or chemotherapy and macrophage-modulating therapy may be effective to control or eradicate metastatic cancer; Similarly, low-dose irradiation was observed to polarise TAMs, which are primarily M2-polarised and immunosuppressive, towards proinflammatory M1-like macrophages, thus inducing the secretion of proinflammatory cytokines such as IL-12 and increasing nitric oxide output on the one hand, and supporting anti-tumour TH₁ responses, endothelial activation and cytotoxic T-lymphocyte recruitment to tumours on the other (Bronte and Murray, 2015; Kitamura et al., 2015; Zheng et al., 2017). Another approach worth noting is the targeting of T cells against TAMs using a CAR directed against a macrophage-specific protein, such as folate receptor β that is highly expressed by TAMs (Cassetta and Kitamura, 2018). On the other hand, macrophages have, in contrast to T cells, a high ability to infiltrate almost every tissue and are often recruited by solid tumours. This property makes them attractive vehicles for CAR therapy. As a result, monocyte-derived human macrophages (MOTO-CAR) cells have been developed by engineering macrophages for the expression of a synthetic

tumour-targeting receptor and to secrete cytokines, ligands or chemokine receptors (Velazquez et al., 2018). When such an engineered macrophage binds to the tumour cell via its tumour target, an activation signal is transmitted triggering Myd88-dependent (myeloid differentiation primary response 88 dependent) and Myd88-independent signalling cascades, thus activating the macrophages and polarising them towards a proinflammatory phenotype. MOTO-CAR cells exhibit a consistent M1 phenotype expressing high levels of CD14, CD80, CD206 and low levels of CD163. Moreover, cell death and phagocytic activity were observed on contact of MOTO-CAR cells with their target malignant cells, thus highlighting the potential of human macrophages as a suitable vehicle for CAR therapy to treat solid tumours (Velazquez et al., 2018). Similarly, all cells, and particularly all cells of the immune system including all subtypes of T cells or NK cells, could be considered as vehicles for cancer therapy in attempts to leverage the intrinsic characteristics of each such cell subtype, including, for example, MSCs that have remarkably been engineered to deliver the apoptotic protein TRAIL (TNF-related apoptosis-inducing ligand) in glioblastomas or to release selected output molecules specifically on sensing contact with a target cell, as well as neutrophils or erythrocytes as enabled by robust ex vivo manufacturing techniques (Brunck and Nielsen, 2014; Kojima et al., 2018; Lipsitz et al., 2017; Redjal et al., 2015; Shah, 2012; Souza et al., 1988). Next generation CAR-T cells, either resistant to combination therapies or expressing one or more cytokine on contact with malignant cells or any other cytotoxic agent including TRAIL and oncolytic viruses, or synergistic with other immunooncology therapeutics including checkpoint inhibitors, dendritic cell vaccines for presenting an array of antigens such as derived from tumour lysates, all these therapeutic modalities could very well prove to be the key to achieving sustainable or curative tumour regression and high objective survival rates in most solid cancers.

As highlighted earlier, the nature of the TME plays a critical role in the outcome to treatment using adoptive engineered immune effector cells. As a result, sensitising to therapy tumours that have ceased to respond or failed to respond at treatment initiation, that is, turning 'cold' tumours into 'hot' (i.e., turning tumour from non-T cell-inflamed to T cell-inflamed), is a fundamental step for immunooncology drugs to deliver broad clinical benefits to patients suffering from solid tumours. Hot tumours are characterised by an IFN- γ signature and a TME that contains antigen-specific CD8 $^{+}$ TILs (tumour infiltrating lymphocytes). Four types of TMEs have been identified based on the presence of TILs and levels of PD-L1 expression: (1) type I is characterised by adaptive immune resistance; it is the TME type for which anti-PD1 and anti-PD-L1 therapies are the most efficacious as it is where the checkpoint blockade best promotes CD8 $^{+}$ T cells immune-mediated killing; (2) type II is characterised by immunologic tolerance; (3) type III by intrinsic induction and (4) type IV by immune tolerance (Smyth et al., 2016). T cell tumour homing is mediated by a network of chemokines, as was observed in melanoma where the presence of TILs is correlated with the expression of CCL2, CCL3, CCL4, CCL5, CXCL9, and

CXCL10, with the IFN- γ -inducible chemokines CXCL9 and CXCL10 (secreted by local myeloid and stromal cells) recruiting CXCR3 $^+$ memory CD8 $^+$ T cells and being strongly associated with a Th₁ immune response and with favourable chemotherapy and immunotherapy outcomes; importantly, CXCR3 signalling enhances the transendothelial migration of T cells into the tumour (Lanitis et al., 2017). As previously discussed, T cell homing can be enhanced using an adjuvant treatment performed on T cells before their adoptive transfer and based on their ex vivo treatment with the enzyme fucosyltransferase and its substrate, GDP-fucose (Wolpe et al., 2019). Similarly, T cell agonists, for example, agonistic antibodies of the tumour necrosis factor receptor superfamily (TNFRS) have been demonstrated to enhance T cell effector function, their proliferation and survival, as well as to enhance memory CD8 $^+$ T cell differentiation and to overcome Treg suppression (Lanitis et al., 2017). Remarkably, disruption of the thymocyte selection-associated HMG box protein TOX, which is a target of the transcription factor NFAT and is highly expressed in CAR-T cells in PD-1 $^+$ solid tumours, has been reported to overcome CAR-T cells dysfunction in solid tumours (Seo et al., 2019). This observation notably highlights that TOX is a master molecular transcriptional regulator of T cell exhaustion and that conventional pharmaceutical intervention against this target is likely to lead to novel treatments with the perspective to better offensively destroy the bulk of malignant tumours and defensively destroy treatment-resistant cells while ensuring treatment tolerability (Vertès, 2019). On the other hand, agonists of APCs, for example, agonists of CD40, also a member of the TNFR superfamily, have been shown to promote the maturation of dendritic cells and the efficiency of antigens cross-presentation to T cells on the one hand and to induce the apoptosis of tumour cells as well as TAM polarisation to an M1-like macrophage phenotype on the other. CD40 is expressed broadly on APCs including dendritic cells, B cells and monocytes, as well as by nonimmune cells and a wide range of tumours (Lanitis et al., 2017). Another approach to promote polarisation towards the M1 macrophage subtype is the use of toll-like receptor agonists such as Pam3CSK4, lipotechoic acid, poly(I:C), lipo-polysaccharide, flagellin, CL264 or CpG, which synergise with IFN- γ to induce macrophage tumouricidal activity including the production of both nitric oxide and proinflammatory cytokines comprising TNF- α , IL-12p40 and IL-12p70 (Müller et al., 2017). It is also worth noting that the adoptive transfer of autologous TILs that specifically target mutated versions of various proteins has been reported to lead to complete durable regression in a case of metastatic breast cancer, a path worth exploring further (Zacharakis et al., 2019).

Again, the aim of combination therapy is dual: on the one hand to ensure the presence of a baseline immune response and on the other hand to unleash preexisting immunity, which is the underlying fundamental of trials around IDO1 inhibition or around attempts to promote T cell infiltration of solid tumours as this has the potential to significantly increase the response rates to immunotherapy and thus to increase overall survival (Galon and Bruni, 2019; Prendergast et al., 2018; van der Woude et al., 2017).

While it appears at first logical and reasonable to combine a targeted agent with the standard of care, clinical experience suggests that the parallel administration of several therapies may lead in certain cases to inferior outcomes as exemplified in breast cancer, where the simultaneous administration of a hormonal agent with chemotherapy results in a poorer outcome than when these are used in sequence (Albain et al., 2009). Simultaneous administration of relatively novel compounds also seems a logical approach when independent mechanisms of action are leveraged; however, even when the toxicity profiles of the elemental compounds of the combination exhibit nonoverlapping toxicities, unexpected severe toxicities can be observed as exemplified in a clinical trial in melanoma in which a combination of vemurafenib (Zelboraf), a B-Raf enzyme inhibitor and the anti-CTLA-4 ipilimumab resulted in hepatic toxicity and thus in the early termination of the trial (Ribas et al., 2013). Similarly, antibody agonists of the OX40 receptor (i.e., CD134 or TNFRS member 4 or TNFRSF4) have been tested in combination with anti-PD1 therapies; however, the combined administration of a PD-1 inhibitor to an OX40 antibody agonist was observed to negate the antitumour effects of the OX40 antibody, as the infiltration of the antigen-specific CD8⁺ T cell into the tumour was diminished due to T cell apoptosis both in the periphery and the tumour, expectedly resulting in a reduced antitumour response and survival (Shrimali et al., 2017). Furthermore, the sequential combination of an OX40 antibody agonist followed by anti-PD-1, but remarkably not the reverse order, was reported to result in a mouse model in significant increases in therapeutic efficacy (Messenheimer et al., 2017). In contrast, the ORR of salvage chemotherapy administered after anti-PD-1 immunotherapy was demonstrated in a 73-patient NSCLC trial to result in a 66.7% ORR compared with 39.5% for patients whose last chemotherapy was administered before immunotherapy, a result that is congruent to that of the KEYNOTE-024 study testing in randomised patients with advanced NSCLC with either pembrolizumab or pemetrexel (a folate antimetabolite that inhibits purine and pyrimidine synthesis) and the platinum anticancer drug carboplatin (which reacts with guanosine residues in the DNA to produce interstrand DNA cross-links) as first-line therapy; here again immunotherapy followed by chemotherapy was a significantly better treatment sequence than chemotherapy followed by immunotherapy (Park et al., 2018). In contrast, the Phase II TONIC trial in triple negative breast cancer (NCT02499367) suggested that short-term doxorubicin or cisplatin conditioning could induce a more favourable TME by preliminary killing of cancer cells and presentation of TAAs, and thus increase the response rate to PD-1 blockade by the upregulation of immune-related genes in the PD-1/PD-L1 and cytotoxic T cell pathways including JAK-STAT and TNF- α (Voorwerk et al., 2019). Perhaps, a short course of chemo- or radiotherapy conditioning followed by checkpoint blockade therapy and subsequently a full course chemotherapy regimen could be worth exploring further?

All these observations demonstrate that the design of combination immunotherapy for cancer needs to be prudently tested taking into account not only the composition of the combination therapy but also the timing of the different administration events and whether the multiple treatments should be delivered in parallel or in sequence as well as what are the

optimal doses and schedules and if treatment holidays are required. Retrospective cohort studies, particularly in metastatic renal cancers, have suggested a benefit for sequencing systemic therapies (Albiges et al., 2015). Nevertheless, combination therapies have a tremendous remission and curative potential and ought to become, even more than they are today, a key part of the oncology treatments of the future, as critically illustrated by the robust preclinical efficacy observed with the combination of an anti-PDL-1, an anti-CTLA-4 and an IDO1 inhibitor in a mouse model of GBM, an indication that has vexed efforts of the pharmaceutical and biotechnology industry to this date (Wainwright et al., 2014). Notably, the anti-VEGF bevacizumab is of controversial use in GBM as vascular remodelling induced by the anti-VEGF treatment was shown to lead to a more hypoxic TME, which promotes a change in the malignant cell metabolism towards glycolysis, thereby promoting enhanced tumour cell invasion into the normal brain. Nonetheless, a further combination of VEGF inhibitors with drugs that target specific signalling pathways or metabolic pathways linked to the glycolytic phenotype could perhaps open novel therapeutic avenues, eventually in complex combinations with checkpoint and IDO/TDO inhibitors as highlighted above (Keunen et al., 2011). What is more, it could be worth exploring whether blocking TGF- β or ablating CD80 might constitute a useful part of the therapeutic cocktail to prevent relapses (Miao et al., 2019). Another important consideration is that the concept of combination therapy needs to be envisaged not solely from the point of view of increasing efficacy but also from the point of view of maintaining and if possible increasing treatment tolerability, as exemplified by the use of a prophylactic TNF blockade that was demonstrated in a mouse model to uncouple efficacy from toxicity in dual anti-PD-1/anti-CTLA-4 immunotherapy, a strategy that could be instrumental in enabling combination immunotherapies, as the combination of the anti-PD-1 and anti-CTLA-4-targeted agents nivolumab and ipilimumab is effective against melanoma, RCC and NSCLC as discussed previously, but this treatment regimen is plagued with frequent and severe immune-related adverse events (Hellmann et al., 2018a; Perez-Ruiz et al., 2019). The uncertainties that remain regarding whether to use sequential or simultaneous combination therapies need to be carefully evaluated on a case-by-case basis, including reverse sequences, to assess otherwise unpredictable tolerability and efficacy issues.

PERSPECTIVES

Considering cancers via the prism of ‘wounds that do not heal’, a periphrase coined by Harold Dvorak in 1986 and borne from the discovery of VEGF, which results in tumours co-opting the wound-healing response to induce the stroma they require for maintenance and growth, this prism constitutes a milestone in the cognition by immunologists and oncologists of the molecular pathobiology of cancer and constituted one of the triggers of the deeper understanding of the intricate overlap that exists between the therapy areas of inflammation and cancer (Dvorak, 1986, 2015).

Similar sequences of events take place in a variety of cancers and in a variety of important inflammatory diseases that are both deeply rooted in cellular immunity dysfunction. The process involves (1) increased vascular permeability, (2) plasma extravasation as well as

of fibrinogen and various plasma proteins, (3) activation of the clotting system outside the vascular system, (4) deposition of an extravascular fibrin gel that serves as a provisional stroma and a favourable matrix for cell migration, (5) induction of angiogenesis and arteriovenogenesis, (6) subsequent degradation of fibrin and its replacement by ‘granulation tissue’ that is a highly vascular connective tissue and (7) vascular resorption and collagen synthesis that lead to the formation of a dense fibrous connective tissue, which are referred to as ‘scar tissue’ in wounds and ‘desmoplasia’ in cancer (Dvorak, 2015).

The pathobiology of cancer involves numerous complex and interrelated sensing, signalling and responding molecular pathways. The concept of hallmarks of cancer is useful in that it enables to create fundamental knowledge and interventional cognitive chunks. Again, these hallmarks include (1) sustaining proliferative signalling; (2) evading growth suppressors; (3) avoiding immune destruction; (4) enabling replicative immortality; (5) tumour-promoting inflammation; (6) activating invasion and metastasis; (7) inducing angiogenesis; (8) genome instability and mutation; (9) resisting cell death and (10) deregulating cellular energetics. The corresponding therapeutic classes include, respectively, (1) EGFR inhibitors; (2) cyclin-dependent kinase inhibitors; (3) immune-activating checkpoint blockade such as anti-CTLA-4 or anti-PD-1 monoclonal antibodies; (4) telomerase inhibitors; (5) selective antiinflammatory drugs; (6) HGF/c-met inhibitors; (7) VEGF signalling inhibitors; (8) PARP inhibitors; (9) proapoptotic BH3 mimetics and (10) inhibitors of aerobic glycolysis (Hanahan and Weinberg, 2011).

Conventional malignant tumour treatments have relied on the mechanical removal of tumour whenever resection is feasible and of course on radio- or chemotherapy to induce the apoptosis of cancer cells. Targeted therapies, based on the molecular knowledge of actors of oncogene addiction, have also relied on promoting the apoptosis of malignant cells or the arrest of their cellular cycles. An interesting parallel can be made here in bacteriology and notably biofilms that can increase bacterial antibiotic resistance, as remarkable synergies can be achieved between a cocktail of antibiotics to tackle treatment-recalcitrant pathogens when using agents that promote mechanically the penetration of antibiotics on the one hand by disaggregating the extracellular matrix and on the other achieving the disruption of the stringent stress response and inhibiting the signalling pathways involved in biofilm formation that promotes resistance (Grassi et al., 2017; Pletzer et al., 2018). Remarkably, treatment order matters to destroy pathogens in biofilms, as suggested by the significant augmentation of antibiotic activity on *Staphylococcus aureus* when preceded by lytic bacteriophage treatment, but not the reverse sequence (Kumaran et al., 2018). The proposed model for this observation is that exposure of the biofilm first to the bacteriophage triggers the disruption of the matrix as well as the phage-mediated lysis of biofilm-associated cells including antibiotic-resistant cells, differentially regulated cells, metabolically inactive cells and persister cells. Adding the antibiotics to such a system results in enhanced antibacterial effects due to higher local concentrations from deeper penetration of these agents, with the sublethal concentrations experienced by bacteriophage-infected cells in

deeper layers of the biofilm eliciting the activation of phage-mediated lysis (Kumaran et al., 2018). Another relevant learning from the field of microbiology is that sublethal stresses can lead to changes in the nature and scale of the bacterial antibiotic resistance, suggesting that bactericidal rather than bacteriostatic agents should be used, as population dynamic studies suggest that sublethal methods may contribute to the dissemination and development of antibiotic resistance (McMahon et al., 2007). Bacteriostatic–bacteriocidal antibiotic combinations typically are antagonistic interactions and, as flagged in a preceding section, exhibit a general trend of slower resistance evolution than synergistic ones, albeit synergistic combinations can reduce side effects and increase the potency of drugs that are much less effective when used a monotherapy (Bollenbach, 2015).

Putting all these perspectives together, a line of oncology therapy remains tumour resection when feasible and ‘shotgun’ cytotoxic chemotherapeutic agents or radiotherapy as well as targeted therapies. However, this regimen is in many cases insufficient due to the emergence of treatment-resistant cells in typical population dynamics patterns. It is thus critical in most cases to address the complexity of cancer biology by a cocktail of interventions addressing more than one of the biological fundamentals, or hallmarks, of cancer, with the view to limit toxicity side effects while enhancing efficacy. Theoretically, one could envisage developing a platform of therapeutic modalities for creating the basis of combination therapeutic regimens, for example, targeting some or even all of the pathways discussed above, with the view to further tailor the treatment to the specific oncology indication of interest or to the patient-dependent disease pathobiology. A foundation for combination therapy would be combining the shotgun approach of a cytotoxic chemotherapy and the sniper approach permitted by targeted therapeutics, enhanced by various ‘adjuvant’ therapies to address the various challenges posed by solid tumours that include a hypoxic and immunosuppressive TME. In principle, what would be worth exploring are all the various combinations of (1) checkpoint inhibitors (e.g., PD-1 and CTLA-4 inhibitors); (2) engineered immune effector cells (e.g., CAR-T or CAR-NK cells); (3) neovascularisation inhibitors (e.g., VEGF inhibitor); (4) tSC sensitizers to prevent relapses (e.g., a TGF- β blocker); (5) targeted therapies and (6) radiotherapy or chemotherapies to promote ICD and killing of rapidly dividing (malignant) cells both as treating regimens and conditioning or induction regimens, for example, before checkpoint blockade therapy with the objective to leverage ICD. In addition, akin to the biofilms observation, it could be worth exploring prior treatment with oncolytic viruses. Oncolytic viruses could for example be delivered using a nonimmune effector cell that expresses a tumour-targeting moiety at its surface and a docking moiety that triggers the local expression of the oncolytic virus. This concept can be exemplified by MSCs. Notably, MSCs naturally home to tumours, are generally well tolerated and their industrial-scale manufacturing is already in place; in addition, they have been engineered to deliver an oncolytic virus for example in GBM or pancreatic cancers (Kaczorowski et al., 2016; Parker Kerrigan et al., 2017; Zendedel et al., 2019). Remarkably, MSCs have

also been used to encapsulate and efficiently deliver to tumour sites a variety of small molecules either naked or encapsulated in a PLGA (poly-DL-lactide-co-glycolide) nanoparticle to shield the MSCs from the cytotoxicity of their cargo (Layek et al., 2018; Pacioni et al., 2015; Sadhukha et al., 2014). This concept was successfully tested to deliver paclitaxel to induce cytotoxic damage in GBM xenografts in a rat model (Pacioni et al., 2015). Furthermore, this approach might be particularly useful in metastatic cancers and notably in metastatic lung cancers, as MSCs delivered systemically tend to biodistribute primarily in the lungs, as demonstrated *in vivo* by the delivery of PLGA-encapsulated doxorubicin, whereby the active migration and the native penetration potential of the engineered MSCs improved drug concentrations in the lungs and in sites of metastasis, which resulted in enhanced antitumour efficacy in mice bearing lung melanoma metastases (Layek et al., 2018; Pacioni et al., 2015; Zhao et al., 2017). What is more, the replicative immortality of malignant cells could perhaps be tackled in parallel and early in the treatment, for example, with human telomerase reverse transcriptase (hTERT) inhibitors, as the enzyme hTERT, a marker of stemness, has been reported in adult tissues to be almost exclusively tumour-associated on the one hand and on the other to be expressed by approximately 85% (80%–95%) of solid tumours, with the maintenance of telomere length being a hallmark of cancer and critical to the survival of malignant cells (Gomez et al., 2012; Hannen and Bartsch, 2018; Mengual Gomez et al., 2016). This is an avenue particularly interesting to explore as cancer cells resistant to erlotinib, paclitaxel/ carboplatin or gemcitabine/cisplatin have been reported to be highly sensitive to the hTERT inhibitor, and nucleoside analogue 6-thio-2'-deoxyguanosine (6-thio-dG) in cell culture as well as in mouse models and was thus proposed as a therapeutic option characterised by minimal toxicities to prolong disease control in therapy-resistant NSCLC patients (Mender et al., 2018). Similarly, azidothymidine (AZT), which is used in AIDS therapy, has been reported to increase the radiosensitivity of cancer cells and to inhibit their growth (Wang et al., 2017b). Zidovudine (AZT) in combination with 5-fluorouracil and leucovorin was notably reported to be well tolerated in metastatic colorectal cancer patients who exhibited uncommon WHO grades III–IV toxicities related to previous treatments (Falcone et al., 1997). Remarkably, rapamycin, an mTOR inhibitor, was shown to reduce telomerase activity but without modifying hTERT mRNA activity, suggesting that mTOR inhibitors, or PI3K/AKT inhibitors that target the same signalling pathway, and hTERT inhibitors could be used in combination to achieve a multiplied effect on the telomere length of malignant cells and hence leading to the disruption of the replicative immortality of these cells, their senescence and apoptosis (Mengual Gomez et al., 2016; Dogan and Avci, 2018; Lee et al., 2018).

Remarkably, not only is the composition of the cocktail of drugs critical but also its directionality is critical too, with some drugs being safer or more efficacious if delivered after or before another. Given the multiple targets in each of the 10 biological hallmarks, as well as wide variations in possible doses and schedules, the number of possible therapeutic cocktails and corresponding treatment algorithms represents an extremely vast

combinatorial space. An oncology road map to apprehend this combinatorial explosion is presented in [Fig. 20.11](#), which recapitulates in ontologic chunks key knowledge and hypotheses as a guide to generate treatment hypotheses (here, an ontology refers to the relationships that exist between various entities of cancer as a system) ([Vertès, 2015b](#)).

As emphasised throughout this Chapter, there are numerous options with a sound rational basis for combination therapies. The first difficulty is to determine among this vast combinatorial space the pecking order of cocktails of drugs to explore in vitro and in vivo and subsequently in the clinic and then define the appropriate doses, regimens, eventual necessary treatment holidays and either concomitant delivery or sequential ones and with which directionality. In bacteriology, large-scale screens for potent drug combinations have been complemented by molecular approaches to characterise in detail the effects of drug combinations on cells and particularly the mechanisms of drug interactions; it is worth noting that drug interactions can be particularly difficult to unravel when a drug in the cocktail perturbs the cellular physiology and causes cellular responses that subsequently impact the activity of another ([Bollenbach, 2015](#)). Three-dimensional models based on serially propagated and molecularly characterised cell slurries directly derived from human biopsies representing a variety of solid tumour oncology indications and numerous patients are particularly useful here in that they replicate the human TME and enable the practitioner to conduct multiplex experiments for drug validation or unbiased approaches in drug combinations with the potential to unravel unexpected but promising combinations of approved drugs and repurposed drugs or drugs in development or indication discovery on the one hand, and to generate informed clinical strategy options on the other ([Nair et al., 2017](#); [Nowak-Sliwinska et al., 2019](#)). These assays notably enable to measure proliferation and viability end points over a three-log dose range as well as resistance and sensitivity profiles ([Nair et al., 2017](#)). Another critical element is the ability to generate predictive biomarkers for developmental purposes as well as for precision oncology purposes ([Domenyuk et al., 2018](#)). For example, gene expression signatures can be useful in that they can reflect the activation status of oncogenic pathways and thus they enable to distinguish between specific cancers and tumour subtypes. Furthermore, clustering tumours based on such pathway signatures has been proposed to enable prognosis in patient subsets and thus to enable linking pathway deregulation with responsiveness to therapeutics, that is, to use these oncogenic pathway signatures for guiding therapeutic options and for better targeting the molecular actors of the deregulated pathway(s) ([Bild et al., 2006](#)). Furthermore, tolerability and treatment-related toxicities will increasingly become predictable in the laboratory as body-on-a-chip systems come of age ([Dehne et al., 2019](#)). Moreover, leveraging the experience in treating the cancers of companion animals such as dogs can be particularly informative, as these animals develop numerous cancers that very well recapitulate many human ones, and importantly, they are kept throughout their natural life spans, thus enabling to recapitulate cancers that develop in parallel with immunosenescence ([Kaeberlein et al., 2016](#); [Kol et al., 2015](#); [Vertès et al., 2015](#)).

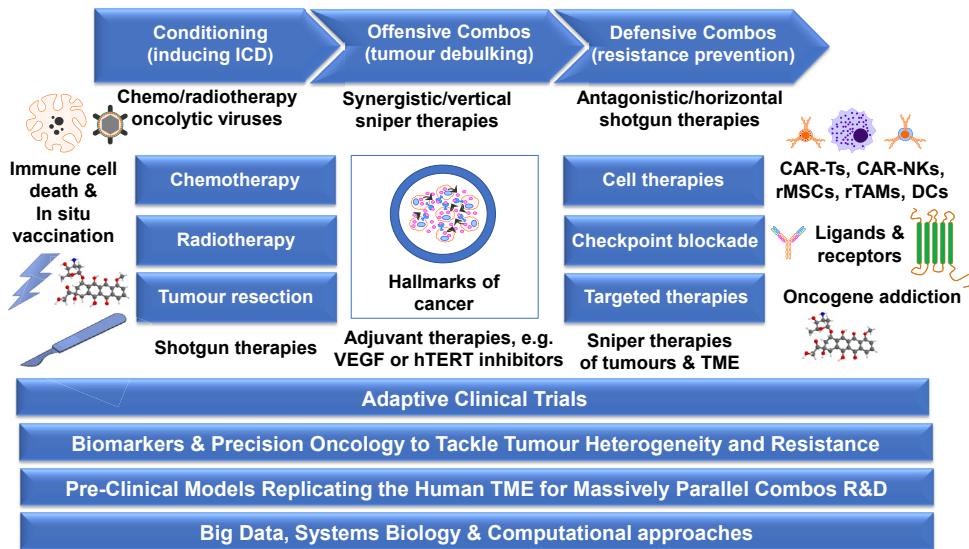


Figure 20.11 Oncology therapeutic combinations road map. Conventional cancer treatments have relied on tumour removal whenever possible and shotgun radio- or chemotherapies that preferentially destroy rapidly dividing cells. Deeper understanding of the triggers and development of cancer, as well as advances in sequencing techniques, to better analyse patient-to-patient and intratumour heterogeneity have enabled targeted therapies that tackle oncogene addiction. The understanding of the fundamental mechanisms of cancer, the hallmarks of cancer, and the recognition of the intricate molecular relationships between inflammation and cancer have further expanded the therapeutic armamentarium with molecules to modify the human tumour microenvironment and address the immunosuppressive environment of solid tumours notably using a checkpoint blockade strategy. This progress was notably enabled by advances in complex antibody architectures, albeit small molecules having also been developed to achieve the blockade of immune checkpoints such as PD-1. Chimeric antigen receptor T (CAR-T) cells and CAR-NK (natural killer) cells represent another approach to harness the immune system by delivering large quantities of engineered immune effector cells, an advance that takes its root in the deep understanding of the mechanisms of T cell costimulation and coinhibition and in progress in gene therapy as well as in industrial-scale T cell- and NK cell-manufacturing technologies. The complexity of the neoplastic process and resistance to therapy needs to be addressed by combination therapies. Ideally, the treatment algorithm would firstly include an offensive arm, for example, using vertical attack by at least drugging two targets in the same signalling pathway and to search for synergies between drugs from the cocktail and secondly a defensive arm, for example, using horizontal attack by drugging targets from different signalling pathways or shotgun therapies and to search for antagonistic interactions as an approach to prevent the expansion of resistant tumour cell clones. In concept, a conditioning regimen could be introduced, for example, by way of oncolytic virus therapy or chemo- or radiotherapy as a means to attack the tumour stroma and induce an immunogenic cell death (ICD) response for in situ vaccination. Adjuvant therapies, which target secondary hallmarks of cancer, could be added to enhance the therapeutic effect, such as inhibitors of the human reverse transcriptase or of vascular endothelial growth factor (VEGF). However, the complexity of translating such an approach through the clinic is phenomenal, as the plethora of available compounds, architectural construct building blocks and possible treatment regimens leads to a combinatorial explosion that can only be addressed by rational design. As a result, implementing systems biology, harnessing body-on-a-chip systems to assess tolerability, crunching big data sourced from a very large number of patients, having predictive preclinical models to substantiate a clinical

To efficiently tackle the biological and cognitive complexity of cancer, computational approaches, big data and artificial intelligence, as alluded to in Chapter 7, constitute critical tools to identify and rationally design potentially useful and well-tolerated therapeutic combinations. Made possible by exquisitely precise and potent genetic engineering techniques, systems biology enabled by global systems data including genomics, transcriptomics, proteomics, metabolomics and fluxomics, which collectively have defined various networks and molecular spaces of intervention, have revolutionised industrial microbiology, thereby boosting process yields and economics (Vertès et al., 2012). Similarly, genome-scale genetic interaction maps as exemplified in the yeast *Saccharomyces cerevisiae* help identify functional cross-connections between all cellular processes, thereby establishing a cellular wiring diagram of pleiotropy and providing a key to unlock chemical–genetic interactions and facilitate drug target identification as well as establishing genotype-to-phenotype relationships (Costanzo et al., 2010, 2016). Similarly, in medicine, powerful computational methods backed up by powerful genome editing tools such as the CRISPR/Cas9 system, reviewed in Chapter 8, are coming of age to generate genetic interaction maps of the human cells and interaction landscapes of the metabolome with immune gene networks and genetic regulation networks to predict potentially beneficial drug combinations (Al-Lazikani et al., 2012; Horbeck et al., 2018; Nath et al., 2017). A first step, which prolongs the concept of ‘virtual patients’ reviewed in Chapter 7, is the establishment not only of the detailed ‘social’ architecture of the human immune cells but also of a detailed nosotaxonomy to clarify disease maps with the view to facilitate indication discovery and a general taxonomy of cytokine networks as well as of the human kinome and the intertwined human phosphorylation signal transduction systems that can be established by mining existing data, for example, by harnessing a large database such as PubMed (Vertès, 2015b; Kveler et al., 2018; Lun et al., 2019; Mazein et al., 2018; Rieckmann et al., 2017). Such tools that permit systems pharmacology include evolutionary modelling, network modelling for both drug combinations and cellular connectivity in cancer and ‘drugome’ approaches where big data analyses collate knowledge of drug features, molecular targets, pharmacological data, patient data and safety profiles (Al-Lazikani et al., 2012; Xie et al.,

strategy that would need to be adaptive and stratifying the patient population before and during treatment, all these advances appear critical. Nonetheless, however challenging, solutions to this task are nowadays within sight as demonstrated firstly by the availability of all these complementary therapeutic modalities and secondly by the availability of already highly informative cytokine networks and of an increasing focus on revisiting nosotaxonomy in oncology, as well as the availability of genetic interaction maps of the human cells and interaction landscapes of the metabolome with immune gene networks and genetic regulation networks to predict potentially beneficial drug combinations. The key attributes of the solid cancer treatments of the future to deliver long-term remission and even cure to patients in need are guided by the three pillars of combination therapies: efficacy, safety and prophylaxis. *CAR*, chimeric antigen receptor; *DCs*, dendritic cells; *rMSCs*, recombinant mesenchymal stem cells; *rTAMs*, recombinant tumour associated macrophages; *TME*, tumour microenvironment.

2017). Here, data commons and more advanced computational methods are still necessary for this vision to become routine (Fröhlich et al., 2018; Krempel et al., 2018). However, as suggested in the I-PREDICT study (NCT02534675) that explores the use of molecular profile-related evidence to define a personalised combination therapy treatment, it appears likely that precision oncology approaches will be necessary to attain long-term remissions in most cancers and that treatment paradigm revolution will be enabled by combination therapies, with the choice of the said combination therapies being driven by a deep profiling of patient parameters including of their metabolomes, with frequent reassessments to stay ahead of the heterogeneity and adaptive responses exhibited by malignant cells in any given patient (Li et al., 2019; Sicklick et al., 2019). A first example of the applicability of precision oncology is the observation in lung cancers that high tumour mutational burden is linked to increased clinical benefits of anti-PD1/anti-CTLA-4 (nivolumab/ipilimumab) dual checkpoint blockade therapy (Hellmann et al., 2018b).

The field of oncology is very dynamic, and major advances will continue to be made with the emergence of more potent and safer medicines to better tackle a variety of oncology indications. The enhancement of the molecular toolbox of functional moieties will be one of the key drivers, and particularly multivalent antibodies will be further developed as well as miniaturised antibody designs that provide smaller elements for novel architectures, an approach that is already announced by progress in the design and validation of nanobodies that are 15 kDa in size as compared with 150 kDa for mAbs (Kijanka et al., 2015). In addition, significant advances have already been made to design cell function by building genetic circuits mimicking electronic ones to programme and control cellular functions with high spatiotemporal precisions (Xie and Fussenegger, 2018). Regarding CAR-T cells, the current primary objectives of engineering efforts are focused on (1) safety, (2) manufacturing, (3) tumour targeting, (4) T cell potency both at the level of functionality and persistence (intrinsic attributes) and at the level of action on the TME (extrinsic attributes) and (5) achieving broader applicability (Sadelain et al., 2017; Chanier and Chames, 2019). Notably, the dimension of broader applicability can be simply addressed not only by developing allogeneic HLA-unrestricted CARs but also a ‘lego-like’ approach where CARs designed to target the CD19 antigen, for example, can be redirected to other specificities using antibody-based engagers (Rennert et al., 2017). As discussed in a previous section, safety issues can be addressed by using tumour-restricted targeting, controlled *in vivo* expansion, optimal persistence and safety ON/OFF switches. Efficacious targeting can be achieved notably by combinatorial targeting. Regarding intrinsic potency, several paths to explore include using chimeric costimulatory receptors enabled by synthetic biology approaches or using a T cell subset as is being performed by developing $\gamma\delta$ -T cells (reviewed in Chapter 3), autocostimulation, effective trafficking, metabolic adaptability, Treg resistance and delayed exhaustion. Extrinsic potency parameters can notably be enhanced by engineering the secretion of various cytokines to arm the engineered immune effector cells, transcostimulation and scFv secretion (Sadelain et al., 2017).

Notwithstanding the myriad of exciting novel possibilities that make use of immune checkpoint blockade as treatment backbone, or of adoptive engineered immune effector cell therapies, and in spite of solid underlying scientific rationales to guide combination designs, given the typical costs of large randomised Phase III trials in the US\$50–\$100 million range, rational design is essential, and early clinical study strategies might need to implement flexible designs to permit creative dose escalation or deescalation or to determine the optimal timing and sequencing of treatment regimens (Al-Lazikani et al., 2012; Mahoney et al., 2015). Again, rational design backed by robust patient population stratification or personalised oncology approaches is critical as some combinations may reveal unexpected severe toxicities, while others may fail to create synergies or to generate additive effects, whereas some others might even decrease the antitumour effect (Mahoney et al., 2015). Exploring the case of NSCLC where 83.4% of the patients who received the combination of ipilimumab and nivolumab required immunosuppressive agents to treat treatment-related toxicity events, many nonetheless responded to the combination therapy, therefore suggesting the promises of conducting a series of clinical studies to further clarify the role and tolerability of immune checkpoint blockade before, in combination, or following the current standard of care (Mahoney et al., 2015).

Ideally, one would leverage multiple pathways in parallel without significantly increasing side effects. Perhaps, treatments of the future will be of the form: (step 1) induction to trigger ICD, e.g., via chemotherapy, radiotherapy or oncolytic virus therapy; (step 2) offensive/synergistic treatment by combining checkpoint blockades, adoptive immune effector cell therapy and targeted therapy and (step 3) chemotherapy (Fig. 20.11). However, the complexity to optimise different therapeutic combinations and different treatment regimens cannot be underestimated as the clinical challenge to validate such complex treatment algorithms would be phenomenal including for recruiting patients. An open question remains if appropriate synergies and additive effects could be attained by implementing the lower range of the therapeutic window of each of the components of a complex cocktail of therapeutic modalities, while maintaining tolerability and enhancing their collective efficacy.

Drug combinations appear to be the best option to efficiently tackle the complexity of the biology of cancer, captured in its different hallmarks. Integrating various therapeutic modalities to maximise efficacy and minimise long-term side effects is the next frontier in oncology. This frontier is enabled both by computational tools that are more powerful than ever and by supporting databases that are deeper than ever. The convergence of all the different technological domains alluded to in this overview is required to achieve such a radical transformation in cancer care. Despite this next iteration in cancer management being still at the bottom of a new innovation S-curve, conventional small molecules and biologics already combine with the sensing and responding capabilities of living drugs to deliver novel and patient-centric treatment algorithms. It is this convergence of fundamental knowledge, technologies, biomarkers and therapeutic modalities that opens exciting new therapeutic paths and routine precision oncology for better clinical outcomes and better patient journeys.

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