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ESSENTIALS Cancer is a genetic disease in which progressive accumulation of mutations in the genome of somatic cells induces abnormal bio- logical capabilities. Cancer-inducing mutations may originate from single base substitutions or large chromosomal rearrangements; but ultimately they disrupt normal cellular processes by altering protein function or disturbing the regulation of gene expression. Loss-of-function mutations in tumour suppressor genes inactivate physiological control of cell processes, whereas gain-of-function mutations directly affect physiological networks and, for example, induce pathological activation of signalling pathways. In oncology, so-called driver mutations alter key cellular processes and confer proliferative advantages to the cancer cell which thus expands preferentially as a clonal population; in contrast, mutations that also occur in cancer cells but do not confer selective advantage are termed ‘passenger’ mutations. Ten behavioural hallmarks of cancerous cells result from disruption of physiological processes and ultimately induce clonal expansion of the cancer cell, leading to clinical presentation of a frank tumour mass. These behaviours define abnormalities within the cancer cell as well as the changes in the tumour-cell environment and the host immune system. The principal aim of translational research in oncology is to utilize insights into the molecular basis of cancer rationally to develop treatments—a contemporary effort, frequently described as ‘preci- sion’ or ‘personalized’ medicine. As sequencing technologies are ap- plied increasingly to diagnostic assays carried out on tissue biopsies and body fluids, the impact of these initiatives is declaring itself in the improved selection of treatments that

are specifically targeted to driver mutations in particular tumours. For many common cancers, we are now close to defining unique sets of somatic alterations which confer a specific signa- ture of the cancer type and are also highly specific to the indi- vidual patient. The nature of DNA mutations DNA mutations are stable aberrations which give rise to cancer by altering protein function or dysregulating the control of cellular gene transcription. The classes of DNA mutation are: base substi- tution in which a single nucleotide is changed; small insertions and deletions (indels) and larger structural variants (SV), which include chromosomal rearrangements (in which there is breakage of DNA and abnormal re-joining); or changes in the copy number of DNA segments (Table 5.2.1). In addition, heritable epigenetic changes, particularly methylation of cytosine nucleotides at CpG sequences, also function as stable mutations by directly repressing gene expres- sion or binding of transcription factors to DNA. Other terms are frequently used to describe the functional effects of mutations: nonsynonymous mutations cause an alteration in the amino acid sequence of a protein, whereas silent or synonymous mutations do not change protein coding. Loss-of-function muta- tions are predicted to completely abrogate normal protein function, either by creating abnormal translational stop signals (typically sub- stitutions) or by introducing frameshift and splicing alterations. Loss-of-function mutations are found in tumour suppressor genes which have recessive effects that require mutation of both copies in a cancer cell. Gain-of-function mutations augment protein activity, for instance, by increasing enzymatic activity or changing the af- finity for the substrate of an enzyme. These are typical mutations in oncogenes which have dominant effects as only one altered allele is required. The rate of acquisition of all mutations is proportional to ex- ogenous and endogenous exposures that cause DNA damage. These exposures may include normal or physiological exposure, for ex- ample, to reactive oxygen species produced during chronic inflam- mation, as well as harmful exposures such as alcohol and cigarette smoke in the aerodigestive tract. Other important mutagenic pro- cesses include infrequent errors of DNA editing, replication, and DNA maintenance. 5.2 The nature and development of cancer: Cancer mutations and their implications James D. Brenton and Tim Eisen

 The hallmarks of cancer In a seminal paper in 2000, Hanahan and Weinberg classified the many abnormalities noted in cancer biology into six groups or hall- marks (Fig. 5.2.1). These original six hallmarks are: (1) sustaining proliferative signalling; (2) evading growth suppressors; (3) resisting cell death; (4) enabling replicative immortality; (5) inducing angio- genesis; and (6) activating invasion and metastasis. Reflecting a decade of progress in the understanding of cancer biology, Hanahan and Weinberg updated their classification in 2011 and added four further hallmarks: (7) genome instability and mutation; (8) tumour- promoting inflammation; (9) avoiding immune destruction; and (10) deregulating cellular energetics. Mutations may be described as driver events or driver mutations when they alter key cellular processes and confer clonal advantage. Many driver mutations alter genes involved in pathways implicated in the hallmarks of cancer (Fig. 5.2.1). It is important to realize that driver mutations do not neatly fit into one hallmark and may cause multiple hallmarks of cancer. The close regulation of the cell cycle is necessary to maintain normal growth and maintenance of tissues. Sustained prolifera- tive signalling may result from driver mutations in one or more of the constituents of growth and proliferation signalling pathways. These driver mutations may be found in cell surface growth re- ceptors, their ligands, or in the downstream cytosolic components of the signalling cascade. Mutations in the RAS-RAF-MEK-ERK pathway are some of the commonest in cancer and are present in approximately a third of malignancies. Some of these mutations have been successfully targeted with mutation-specific inhibitors, for example, BRAF

V600E in melanoma. However, the commonly mutated protein RAS has proved a very difficult therapeutic target to date. Successful antagonism of sustained proliferative signalling has the potential to revolutionize therapy. For example, epidermal Table 5.2.1 Types of mutations Small-scale mutations Silent mutation Silent mutation Missense mutation Nonsense mutation Change in a single nucleotide Amino acid sequence is not changed Amino acid sequence is changed Amino acid sequence is changed to a stop
codon, therefore truncating the protein Insertion Addition of one or more extra nucleotides Deletion Removal of one or more nucleotides Large-scale mutations Amplification Multiple copies of chromosomal region Deletion Loss of chromosomal region Translocation Interchange of regions from different chromosomes Inversion Reversal of the orientation of a chromosomal region Loss of heterozygosity Loss of one allele Reprinted from Ma CX and Ellis MJ (2013). The Cancer Genome Atlas: Clinical applications for breast cancer. Oncology, 27(12). EGFR inhibitors Sustaining proliferative signalling Deregulating cellular energetics Resisting cell death Genome instability & mutation Inducing angiogenesis Activating invasion & metastasis Tumor- promoting inflammation Enabling replicative immortality Avoiding immune destruction Evading growth suppressors Aerobic glycolysis inhibitors Proapoptotic BH3 mimetics PARP inhibitors Inhibitors of VEGF signalling Inhibitors of HGF/c-Met Selective anti- inflammatory drugs Telomerase Inhibitors Immune activating anti-CTLA4 mAb Cyclin-dependent kinase inhibitors Fig. 5.2.1 Hallmarks of cancer and potential therapeutic approaches. Reprinted from Cell, 144(5), Hanahan D and Weinberg RA, Hallmarks of Cancer: The Next Generation, 646–74, Copyright © 2011, with permission from Elsevier.

5.2 The nature and development of cancer 447 growth factor receptor (EGFR) inhibitors have greatly extended the life expectancy of patients with non-small cell lung cancer expressing EGFR with activating and sensitizing mutations. This example also illustrates the importance of understanding mech- anisms of resistance to therapy. After initial success with treat- ment with an EGFR inhibitor, a resistant clone arises and the disease progresses. The commonest cause of this resistance is a further mutation in the EGFR, T790M, which renders the pro- tein resistant to first-line EGFR inhibitors. It has proved possible to develop highly effective inhibitors of the T790M mutated EGFR which offer patients a substantial further period of disease control. Healthy cells possess several layers of regulatory control of pro- liferation, including control of the cell cycle at checkpoints. Evasion of growth suppressors allows cancer cells to divide but also leaves cancer cells vulnerable. Further disruption of cellular control by cyclin-dependent kinase inhibitors is effective in cancer cells by leading to mitotic catastrophe and cell death in sensitive cells. Normal cell death may be due to activation of extrinsic cellular death receptors or activation of an intrinsic pathway sensing cellular stress, such as irreparable DNA damage or lack of essential nutri- ents. These stresses are sensed by the regulatory protein p53 which alters the balance between pro-apoptotic and antiapoptotic proteins and results in cell death. Many cancer cells can resist cell death, often due to inactivating mutations in p53, which are present in up to half of all cancers. Other mechanisms of inducing apoptosis, for ex- ample, by mimicking the pro-apoptotic protein BH3, are finding an increasing role in oncology. If a cell survives a severe stress, it may irreversibly exit the cell cycle and become senescent. A characteristic feature of senescent cells, as well as of premalignant cells, is shortening of the telomeres. Cancer cells circumvent this telomere shortening by upregulating the enzyme telomerase which normally maintains the length of the telomere. This property allows cancer cells to continue proliferating and develop replicative immortality. Inhibitors of telomerase have shown activity in several tumour types. Without developing new blood vessels, tumours are limited to a volume of 3 mm3 because they are

dependent on diffusion of nu- trients and waste products. Tumour blood supply may form by a number of mechanisms including co-option of normal vascula- ture and induction of angiogenesis. Targeting of angiogenesis has proved particularly effective in tumours characterized by patho- logical activation of hypoxia inducible factor (HIF) and conse- quent upregulation of vascular endothelial growth factor (VEGF). Inhibitors of VEGF receptors have greatly improved the outlook for patients with clear cell renal carcinomas in which upregulation of VEGF is ubiquitous. Most deaths from cancer are due to metastatic spread of the tu- mour from its original or primary site to other distant sites. In order to invade and metastasize, a cancer cell must successfully complete a sequence of tasks: it must move through the extracellular matrix and basement membrane to reach a blood or lymph vessel; it must invade the vessel and then survive in the circulation in the absence of contact with other cells; it must lodge at a distant site, extravasate and invade the distant organ; it must survive and grow in its new environment and then develop a blood supply as described earlier. Key to the early steps in this sequence in carcinomas is the cell's transition from a polarized epithelial phenotype to a mesenchymal phenotype able to invade surrounding tissues. This is called the epithelial-mesenchymal transition (EMT). Upregulation of cell signals such as hepatocyte growth factor or c-Met is implicated in EMT. C-Met inhibitors show anticancer activity in several tumour types. Genome instability and mutation is a double-edged sword which serves the ability of the cancer cell to evolve, survive, and metas- tasize but also makes the cancer cell vulnerable to therapies which further limit the cancer cells ability to repair DNA damage. The gene breast cancer 2 (BRCA2) encodes one of several proteins in- volved in repair of DNA damage. When BRCA2 is nonfunctional, as is the case in some breast, prostate, and ovarian cancers, the cancer cell is particularly dependent on other DNA repair path- ways. Inhibition of such a rescue pathway would result in mas- sive DNA damage accumulating in the cancer cell and its eventual death. This is the concept of synthetic lethality. The use of poly ADP ribose polymerase (PARP) inhibitors has been highly ef- fective in BRCA2 tumours and is now being developed in a wider group of malignancies. Cells are particularly susceptible to DNA damage and mutation when dividing. States of chronic inflammation lead to increased cell turnover and proliferative stimulation. Environmental toxins such as tobacco smoke may lead to tumour-promoting inflammation and increased cellular turnover including in the stem cell compartment. Coupled with direct DNA damage, this substantially increases the risk of lung malignancy. Withdrawal of the environmental toxin by stopping smoking has a pronounced benefit even after many years of exposure. In other disease set- tings, trials of anti-inflammatory drugs have produced some encouraging results. As for other hallmarks of cancer, inflammation may both be a risk factor for cancer and present a therapeutic opportunity. It has long been recognized that a patient's immune system is able, in certain circumstances, to attack and destroy cancers. It is not uncommon for immune cells to account for up to half of tumour volume. However, cancer cells have become adept at avoiding immune destruction, for example, by downregulating cell surface antigens or by expressing molecules which inactivate or kill cytotoxic T cells, such as pro- grammed death-ligand 1 (PD-L1). Tumours differ in the extent to which they exhibit immune cell infiltration, from complete lack of immune cells (immune desert) to heavily infiltrated or inflamed. In the most exciting development in the systemic treatment of can- cers to date, drugs which inhibit the normal T-cell checkpoints have shown activity in a wide variety of tumours, including the ability to eliminate them completely. Examples include antibodies which in- hibit the T-cell checkpoints' programmed death-1 (PD-1) and cyto- toxic T lymphocyte antigen 4 (CTLA4). The likelihood of success varies between tumours and appears to be related to the degree of mutational load, the degree of inflammatory infiltrate, and the level of PD-L1 expression in the tumour. Deregulation of cellular energetics was first described

in 1927 by Warburg and colleagues. The ‘Warburg effect’ is the overuse of glu- cose by cancer cells as an energy source. The most important cur- rent application of this knowledge is the detection of even very small volumes of metabolically active tumour by [18F] fluorodeoxyglucose positron emission tomography (PET) scanners. Therapeutic ex- ploitation of deregulated cellular energetics is a field of very active research.

448 SECTION 5 Principles of clinical oncology Detection of cancer mutations Cytogenetic analysis Driver mutations are typically present in the earliest invasive le- sions but may also arise during subsequent divergent evolution of cells within the tumour mass (see later in this chapter). Passenger mutations do not confer a cellular advantage but also accumulate over time. The task of distinguishing new driver mutations from the background noise of passenger mutations is complex and combines bioinformatic, statistical, and functional analyses. The current tally of proven cancer genes is estimated to be approximately 400, which comprises less than 2% of all human protein coding genes. The earliest evidence for mutations in cancer cells was obtained from light microscopy studies on cancer chromosomes car- ried out at the end of the end of the nineteenth century by von Hansemann and followed by similar studies by Boveri. The Philadelphia chromosome was discovered by karyotyping studies on chronic myeloid leukaemia in 1960 and subsequent improve- ments in cytogenetic analysis led to the identification of recurrent translocations between chromosomes 9 and 22 in 1973. These dis- coveries moved cytogenetic analysis out of research laboratories into clinical practice, and over the next 20 years karyotyping was used to describe differences in chromosome number and chromosomal rearrangements (translocations) resulting in new molecular classifications, particularly in haematological and paediatric malignancies. Despite low spatial resolution and lack of molecular informa- tion, karyotyping arguably provided the first evidence for key differences in the type and number of mutations between mor- phologically similar neoplasms. These methods provided the first demonstration of numerical chromosomal instability, for instance, some colorectal cancers were shown to have normal chromosome number, in contrast to cases with prominent aneu- ploidy. Subsequent discoveries showed that this distinction was due to microsatellite instability in colorectal cancer which does not cause marked changes in chromosome number, but pro- duces a hypermutator phenotype with very large numbers of indel mutations. The development of fluorescent in situ hybridization (FISH) in the 1990s provided the first molecular evidence that specific chromosomal loci were rearranged or amplified in cancer, and was used to detect recurrent translocation between the BCR and ABL genes in chronic myeloid leukaemia (CML) and the later detec- tion of the BCR-ABL fusion oncogene. This discovery was followed by the identification of other recurrent translocations in haem- atological cancers and in some solid tumours, notably sarcomas and paediatric cancers. However, the application of karyotyping and FISH to solid tumour specimens is difficult and over the past 10 years, alternative methods using DNA microarrays or sensitive immunohistochemistry have been developed to detect important structural variants including MYCN amplification in neuro- blastoma and ERRB2 amplification in breast cancer (HER2 testing). Although karyotyping and FISH are still important laboratory assays, they will be progressively replaced by tests based on next- generation sequencing as this becomes the major technology for clinical genomics. Next-generation sequencing The main catalyst for the development of sequencing technology over the past two decades has been the international effort to cata- logue human genetic variation. DNA sequencing became feasible in 1977 with Sanger’s development of chain terminating inhibitors, which allowed the resolution of individual nucleotide positions using radioactive gel electrophoresis of synthesized DNA prod- ucts. Automated

sequencing machines first appeared in 1986 using fluorescent terminators, and were further improved a decade later by the development of capillary electrophoresis which could accommodate up to 96 individual sequencing reactions (Fig. 5.2.2). These DNA fragmentation In vivo cloning and amplification Cycle sequencing Electrophoresis (1 read/capillary) 3'-... GACTAGATACGAGCGTGA...-5' ...CTGATC ...CTGATCT ...CTGATCTA ...CTGATCTAT ...CTGATCTATG ...CTGATCTATGC ...CTGATCTATGCT ...CTGATCTATGCTC ...CTGATCTATGCTCG G C T C G T A T C 5'-... CTGAT Polymerase dNTPs Labelled ddNTPs (template) (primer) Fig. 5.2.2 Sanger sequencing. Reprinted by permission from Macmillan Publishers Ltd: Nature Biotechnology (Shendure J and Hanlee J, 2008, Next-generation DNA sequencing, Nature Biotechnology, 26, 1135–1145), copyright © 2008.

5.2 The nature and development of cancer 449 first-generation sequencing machines provided most of the data for the Human Genome Project, the first large international sequencing project. The goal of determining the entire nucleotide sequence of human DNA was a highly expensive effort as large banks of sequen- cers and significant infrastructure were needed to support semi-automated parallel processing of the huge number of DNA reads required. By contrast, next-generation (NGS) sequencing machines operate as massively parallel processing devices (Fig. 5.2.3). The Illumina sequencing method is able to array an entire size-selected library of DNA templates onto a glass slide called a flow cell. Each single DNA molecule in the library is amplified more than one million times, forming a discrete cluster, which is large enough to be imaged on the glass slide. Sequencing is performed by sequential synthesis, in which fluorescent bases are incorporated onto the DNA template in each cluster. Base calling is performed between each base in- corporation step by imaging the entire flow cell and analysing the nucleotide-specific colour at each cluster location. Next-generation sequencing provides shorter sequencing reads than the Sanger method with higher error rates and longer run times—but dramatically cheaper costs and more rapid throughput. The key determinant of cost and quality of this sequencing is the theoretical or expected coverage (also called depth of sequencing), defined as the average number of times that each nucleotide is ex- pected to be sequenced given a certain number of reads of a given length and random distribution. For comparison, the Human Genome Project achieved 6–8× average depth across the entire hap- loid human genome of 3.2 billion base pairs (3.2 giga-base pairs [Gb]). With increased error rates and shorter reads, to achieve similar whole genome sequencing fidelity with next-generation methods requires 30× depth. Cataloguing and classifying mutations The task of comprehensive sequencing of cancer genomes was finally made possible in 2000 when the Human Genome Project delivered a near-complete sequence for all human chromosomes. This identi- fied approximately 22 000 protein coding genes which covered only 1–2% of the entire genome. These data provided the essential map to enable targeted sequencing approaches to discover new candi- date cancer genes, for example, by sequencing genes from the same protein families or signalling pathways. Early pioneering studies to identify new cancer genes carried out Sanger sequencing of all pro- tein coding genes using large cancer cell line collections and small cohorts of breast and colorectal cancer specimens. This was fol- lowed by rapid development of next-generation sequencing surveys of individual cancers, including acute myeloid leukaemia and breast cancer, which have been followed by studies including essentially all tumour types and large patient cohorts. These studies use whole genome sequencing (WGS), in which all noncoding and coding regions of the genome are sequenced, or whole exome sequencing (WES) in which the protein coding gene sequences are first puri- fied away from the huge excess of noncoding sequencing by various Fragments Add adaptors Attach to flowcell Dissociation PCR

extension Bind to primer Cluster formation Sequencing Signal scanning C G A A T C G. . . T
Fig. 5.2.3 Next-generation sequencing using sequencing by synthesis method. Reprinted from Lu Y, et al. (2016) Next Generation Sequencing in Aquatic Models, Next Generation Sequencing—Advances, Applications and Challenges, Dr. Jerzy Kulski (Ed.), InTech, DOI: 10.5772/61657. Available from: https:// www.intechopen.com/books/next-generation-sequencing-advances-applications-and-challenges/ next-generation-sequencing-in-aquatic-models.

450 SECTION 5 Principles of clinical oncology purification methods. The advantage of WGS is that it provides a comprehensive analysis of all possible mutations (including SV and changes in intergenic regions) and is technically easier to perform. A key aim of the Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) projects has been to identify all cancer mutations while recognizing that these may be present at low prevalence (c.5%). To provide enough statistical power re- quires more than 500 cases using WES and WGS, and the current analysis suggests that in the majority of cancer genomes there are typically less than 10 mutated driver genes. The main achievement of the international sequencing projects has been to create detailed catalogues for over 50 cancer types and to make them publicly avail- able, which enables clinical translation of mutations as diagnostic and predictive biomarkers. Clinical sequencing The goal of providing a whole genome sequence for less than US$1000 was achieved in 2014 and this has provoked significant debate as to whether this technology should now replace less com- prehensive sequencing methods in the clinic. However, the principal barrier to clinical use is the complexity of the computational ana- lysis required for whole genome data and this is allied to the need to handle the large volume of data produced by the sequencing runs. The possibility of using whole genome sequencing at scale in health services is currently being addressed by many countries. In the United Kingdom, the Genomics England 100 000 genome project in patients with genetic disorders and cancer has been initiated and uses a centralized model for sequencing and analysis. Worldwide, the most frequent application of next-generation sequencing for cancer management is the use of targeted panels covering several hundred cancer genes. A clinically accredited benchtop sequencing device can generate over 5 gigabases of sequence data in nine hours. This sequencing coverage can be used simultaneously to analyse up to 200 patient DNA samples using multiplexed analysis with barcoded DNA primers, making it feasible to sequence 250 or more cancer genes at a price point of £100–200 per patient in 2016. Mutational analysis and its implications Mutational signatures reveal cancer aetiology The availability of numerous cancer genomes has facilitated com- parative analyses: these have revealed new integrative molecular classifications based on detection of mutations. The number and type of mutations show wide variation between and within cancer types (Fig. 5.2.4) and these differences correlate with grade and aetiology of the cancer. For example, the number of DNA substitu- tions and indels is very low in low-grade cancers such as carcinoid tumours and paediatric medulloblastoma, whereas in melanoma and lung cancer very high numbers of mutations are observed, reflecting the powerful mutagenic exposures of UV light and to- bacco carcinogens. Some cancer types show hypermutation with extremely large numbers of mutations, exemplified by colorectal cancers with microsatellite instability and uterine cancers with mutations in DNA polymerase epsilon exonuclease (POLE). The number and type of structural variants also differs between cancer types. A comparative analysis of over 3000 cancer genomes shows a hyperbolic relationship between the frequency of nucleotide substi- tution and structural variants across common cancers. High-grade serous ovarian and triple negative breast cancers have the greatest number of SVs, have very few oncogenic mutations, and show fre- quent loss of tumour suppressor genes including TP53, RB1,

NF1, and BRCA1/2. 1000 n = 22 20 52 134 26 23 81 227 91 57 121 13 63 214 11 394 219 20 49 181 231 76 88 35 335 179 121 100 10 Somatic mutation frequency (/Mb) 1 0.1 0.01 Rhabdoid tumour Ewing sarcoma Thyroid AML Medulloblastoma Carcinoid Neuroblastoma Prostate CLL Low-grade glioma Breast Pancreas Multiple myeloma Kidney clear cell Kidney papillary cell Ovarian Glioblastoma multiforme Cervical DLBCL Head and neck Colorectal Oesophageal adenocarcinoma Stomach Bladder Lung adeno- carcinoma Lung squamous cell carcinoma Melanoma C T C A C G T C T A T G Fig. 5.2.4 The frequency of mutation in common cancers. Dots show individual cancer cases and tumour types are ordered by median frequency shown on a log scale. Lower panel shows relative proportions of the six possible nucleotide substitutions. Reprinted by permission from Macmillan Publishers Ltd: Nature (Lawrence MS, et al., 2013, Mutational heterogeneity in cancer and the search for new cancer-associated genes, Nature, 499, 214–18), copyright © 2013.

5.2 The nature and development of cancer 451 These differences suggest that the mutational signature (also called the mutational spectrum) of a cancer can reveal the im- print of mutagenic processes accumulated over the lifetime of a cancer cell. This notion was first developed after the demon- stration that the mutagenic effect of UV light induced highly characteristic C to T mutations (C>T) in DNA, particularly at dipyrimidine sites when two cytosines are adjacent (CC>TT). This specific base-change was confirmed by the demonstration of a huge predominance of C>T mutations in squamous cell carcinomas of the skin. Other DNA mutational signatures were discovered by aggregating patterns of transition and transversion mutations (Fig. 5.2.5) discovered in the TP53 gene across many cancer types. Importantly, these signatures could also be shown to be induced in experimental systems using relevant chemical exposures. For example, the TP53 mutational spectrum in lung cancer is dominated by C>A substitutions, which were shown to be the result of misrepair of bulky DNA adducts formed by carcinogenic components of tobacco smoke. In hepatocellular carcinoma, C>A transversions were shown to be induced by en- vironmental aflatoxin exposure. 20% 10% 10% 10% 10% 10% 10% 10% 10% 0% 0% 0% 0% 0% 0% 0% 0% 20% 20% 20% 20% 20% 20% 20% 10% 0% 20% 10% 0% 0% 20% C > A C > G C > T T > A T > C T > G C > A C > G C > T T > A T > C T > G C > A C > G C > T T > A T > C T > G C > A Signature 1 Signature 4 Signature 7 Signature 10 Signature 13 Signature 16 Signature 19 Signature 22 Signature 25 Signature 28 Signature 2 Signature 5 Signature 8 Signature 11 Signature 14 Signature 17 Signature 20 Signature 23 Signature 26 Signature 29 Signature 3 Signature 6 Signature 9 Signature 12 Signature 15 Signature 18 Signature 21 Signature 24 Signature 27 Signature 30 C > G C > T T > A T > C T > G C > A C > G C > T T > A T > C T > G C > A C > G C > T T > A T > C T > G Fig. 5.2.5 Patterns of mutational signatures. Bar plots show proportions of each substitution in each trinucleotide context (labelling not shown). Courtesy of the Catalogue of Somatic Mutations in Cancer (COSMIC).

452 SECTION 5 Principles of clinical oncology These early discoveries of the links between exposures and somatic mutation were extremely important but had several limitations. First, they were only able to elucidate the dominant mutational sig- nature in a cancer and could not distinguish between multiple sig- nals from different mutagenic processes. Secondly, the use of a single gene (usually TP53) to catalogue mutational signatures can bias the statistical analysis, owing to strong selection for particular driver mutations in cancer development. For example, the commonest mu- tations in BRAF in melanoma are T to A transversions, which are not characteristic of UV crosslinking, but nevertheless are likely to have arisen from UV induced mutation. The availability of detailed catalogues of whole exome- and whole genome sequencing from multiple

cancer types has re- sulted in the discovery of many other mutational signatures, by providing thousands of passenger mutations for analysis instead of a limited number of mutations found in driver genes. Whole genome sequencing is superior to whole exome sequencing for analysing signatures because it includes noncoding sequences that comprises 99% of the normal genome, offering many more mutations for analysis. In addition, the application of signal ana- lysis methods to whole genome data can identify many signatures in a single cancer by deconvoluting the imprints of multiple mu- tational processes. Although there are six possible DNA substi- tutions based on Watson–Crick changes in DNA (Fig. 5.2.5), mutational signatures are described as trinucleotide sequences by including the 5′- and 3′-base at each site of mutation. By including the sequence context for the mutation, different mutational pro- cesses can be unambiguously discriminated. For example, a UV light-induced C>T transversion at a dipyrimidine site (NpCpC) can be distinguished from the same C>T change arising from de- amination of 5-methyl cytosine at a NpCpG site (where N indi- cates any nucleotide). Currently, detailed analysis of 7000 genomes from 30 cancer types has identified 30 distinct mutational signatures (Figs. 5.2.5 and 5.2.6). This number is likely to increase as other mutational data, including deletions and large structural variants, are inte- grated into pattern-recognition methods. Several of these signa- tures already provide strong candidate biomarkers for therapy, and it is to be expected that the rapid uptake of whole exome and whole genome methods in clinical genomics will soon lead to pre- cise characterization of tumours as they occur in routine onco- logical practice. The two most common signatures found across all cancer types arise from deamination of 5-methyl-cytosine and are related to ageing and carcinogenesis. Other signatures reveal specific expos- ures and mutational processes. Liver, uterine, and stomach cancer can have up to six distinct signatures, suggesting complex DNA damage and repair phenotypes in their aetiology. Mutational signatures can now provide strong diagnostic and aetiological information for cancer research. Whole genome Signature 1 Adrenocortical carcinoma ALL AML Bladder Breast Cervix Chondrosarcoma CLL Colorectum Glioblastoma Glioma low grade Head and neck Kidney chromophobe Kidney clear cell Kidney papillary Liver Lung adeno Lung small cell Lung squamous Lymphoma B-cell Lymphoma Hodgkin Medulloblastoma Melanoma Myeloma Nasopharyngeal carcinoma Neuroblastoma Oesophagus Oral gingivo-buccal squamous Osteosarcoma Ovary Pancreas Paraganglioma Pilocytic astrocytoma Prostate Stomach Thyroid Urothelial carcinoma Uterine carcinoma Uterine carcinosarcoma Uveal melanoma 40 7+ 4 2 5 13+ 7+ 2 5 4+ 3+ 4 6 3 4 4 10+ 7 4 5+ 6 3 3 5+ 4 5 3 7 6 6 3 6+ 2 3 3 11+ 4 5 8 6 3 22 3 7 40 17 3 2 2 6 2 1 22 2 2 1 6 3 1 2 1 2 1 1 1 4 1 1 1 2 Signature 2 Signature 3 Signature 4 Signature 5 Signature 6 Signature 7 Signature 8 Signature 9 Signature 10 Signature 11 Signature 12 Signature 13 Signature 14 Signature 15 Validated mutational signatures Signature 16 Signature 17 Signature 18 Signature 19 Signature 20 Signature 21 Signature 22 Signature 23 Signature 24 Signature 25 Signature 26 Signature 27 Signature 28 Signature 29 Signature 30 Other signatures Mutational signature present Total validated mutational signatures in a cancer type Total cancer types in which a signature is operative
Fig. 5.2.6 Occurrence of mutational signatures by cancer type. Courtesy of the Catalogue of Somatic Mutations in Cancer (COSMIC).

5.2 The nature and development of cancer 453 sequencing studies have revealed distinct patterns of smoking- related signatures by analysing 5000 tobacco-associated cancers from smokers and nonsmokers. Cancers from smokers had sig- nificantly higher numbers of base substitutions and lung cancers in smokers had higher numbers of copy number aberrations. Signature 4, which represents exposure to the tobacco carcinogen benzo[a] pyrene, was most prominent in lung and

laryngeal cancers, where epithelia are directly exposed to tobacco smoke but was also found in lung cancer from nonsmokers, suggesting contributions from sec- ondary smoke inhalation. Studies on radiation-associated cancers show that the additional induced burden of mutations is relatively low, but in contrast to other mutational signatures, the distribution of mutations is evenly distributed across the genome, and does not show bias with replication timing, sequence complexity, or GC base content. Radiation-induced tumours also show a very rare aberra- tion of balanced inversions which may uniquely identify these can- cers. These data show that the stochastic nature of radiation is not affected by the chromatin context of DNA but induces relatively in- frequent additional mutations, which may explain the low absolute risk of radiation-induced cancer. Cancers with microsatellite instability can be identified by a strong preponderance of signature 6 (Fig. 5.2.6), which reflects very high numbers of substitutions and 1-base pair indels in nucleotide repeats. This signature is most commonly seen in colorectal, uterine, and stomach cancer and is strongly associated with loss of mis- match repair genes. Detection of this signature has important thera- peutic implications as it indicates patients who are likely to benefit from with immune checkpoint inhibitors, owing to increased fre- quency of expressed neoantigens in the tumour microenvironment. Signature 3 is frequent in breast, ovarian, and pancreatic cancers and is strongly correlated with homologous recombination deficiency (HRD) and is commonly caused by mutation in BRCA1 and BRCA2. Cancers with HRD can also be identified by different signatures based on the pattern of structural variants as they characteristic- ally have large indels (≤50 bp) with overlapping microhomology at breakpoint junctions. Patients with HRD show increased sensitivity to platinum-based chemotherapy and poly (adenosine diphosphate [ADP]) ribose polymerase inhibitor (PARPi) therapy. Sequencing for germline and somatic mutations in BRCA1 or BRCA2 (and other less frequent mutations in homologous recombination genes) cannot identify all patients with HRD, whereas the finding of a dominant signature 3 is strongly suggestive of possible benefit from PARPi therapy. The analysis of signature 3 across 32 cancer types has shown that 7–12% of cases of gastric cancer have features of HRD. As gastric cancer is the second commonest cancer worldwide, there may be significant benefits from routine profiling for mutational sig- natures of HRD or MSI. Mutations offer targets for personalized therapies Most targeted therapies currently in the clinic are designed to dis- rupt gain-of-function properties in cancer cells and these com- monly overlap with the hallmarks of cancer. These effects are often mediated by oncogenic mutations that induce constituent activity of the respective protein. They are often driver mutations, and the term ‘oncogene addiction’ is used to describe the dependency of the cancer cell on the induced gain of function, hence targeting of these mutations may have dramatic effects on cancer growth (Table 5.2.2). This is exemplified by the development of therapeutics against BRAF mutations in malignant melanoma. The BRAF gene encodes a serine threonine kinase and is a member of the Raf family of growth Table 5.2.2 Overview of relationships between cancer genotypes and their predicted responses to targeted therapy Cancer type Genotype Therapy Colorectal cancer Mutant KRAS Cetuximab/Panitumimab (no response) Chronic eosinophilic leukaemia (CEL) PDGFR translocations Imatinib Chronic myeloid leukaemia (CML) BCR-ABL translocation Imatinib Resistant CML Mutant BCR-ABL translocation Dasatinib Ponatinib Bosutinib Gastrointestinal stromal tumour (GIST) Mutant KIT Imatinib Breast cancer HER2 amplification Trastuzumaba Pertuzumaba ado-trastuzumab emtansinea Melanoma Mutant BRAF Vemurafenib Dabrafenib Trametinib Dabrafenib/Trametinib Myelofibrosis Mutant JAK2 Ruxolitinib Non-small cell lung cancer Mutant EGFR Erlotinib Gefitinib Afatinib Non-small cell lung cancer ALK translocation Crizotinib Ceritinib Non-small cell lung cancer ROS1 translocation Crizotinib a Eligibility is not strictly on genomic amplification of HER2, as strong HER2-positivity by immunohistochemistry is

also an eligibility criterion for treatment. Reprinted from Tursz T and Bernards R (2015). Hurdles on the road to personalized medicine. Molecular Oncology, 9(5), 935–9. Published under a Creative Commons Attribution (CC BY) License.

 promoting proteins. Activating mutations in BRAF were first iden- tified by the Cancer Genome Anatomy Project from melanoma cell lines. Sequencing studies on clinical specimens showed that 50–70% of malignant melanoma cases have the V600E BRAF mutation, and this is also found in 8–12% of colorectal cancers and less commonly in other cancers. The V600E mutation induces constitutive activa- tion of the BRAF kinase, while successful pharmacological targeting of the kinase CP-pocket was achieved with the clinical develop- ment of the drug vemurafenib. Use of vemurafenib in patients with metastatic melanoma has shown dramatic responses in over 70% of patients treated, but resistance to single agent therapy develops by activation or mutation of other pathways that circumvent BRAF in- hibition, the implications of which are discussed later. Mutational analysis measures cancer evolution Genomic studies carried out over the past 20 years have revealed considerable intratumoural genetic heterogeneity and strong evi- dence of evolutionary selection of subclonal tumour populations during treatment, as well as during metastasis. These studies were initially carried out in paediatric acute lymphoblastic leukaemia, but similar processes of clonal evolution and selection have now been confirmed in many epithelial tumours. This has led to the clinical realization that profiling a single sample of an individual's cancer may not represent a comprehensive depiction of all driver genes. In addition, reliance on the original diagnostic cancer sample cannot provide accurate information about subsequent somatic changes contributing to therapy resistance. Optimal decision-making for personalized medicine now re- quires contemporaneous genomic information from repeat or se- quential biopsies. The use of image-guided biopsies, particularly using ultrasound with 14 G or 16 G cutting needles, can provide high-quality samples for next-generation sequencing. However, biopsy may be difficult at metastatic sites such as bone or abdom- inal lymph nodes, and the expense and discomfort for the patient may limit repeatability over their treatment. Mutational profiling of cell-free DNA in blood offers an alternative 'liquid biopsy' and is being rapidly developed for clinical use in many cancer types and for different applications, from diagnostic information to detection of emerging resistance mechanisms. Detecting cancer mutations in cell-free DNA Cell-free DNA was first demonstrated in blood in 1948 and shown to be increased in the serum of cancer patients in 1977. The possi- bility that it could be used as a cancer biomarker was established in the early 1990s by the detection of cancer mutations in plasma, stool, and sputum as well as other body fluids. Most cell-free DNA in plasma originates from normal haemopoietic cells and in healthy individuals the concentration of plasma DNA concentrations range between 1 and 10 ng/ml. However, these levels are dynamic and may be altered by disease processes causing tissue injury such as trauma or stroke, as well as physiological changes during pregnancy and physical exercise. Cell-free DNA in blood is fragmented into short fragments of approximately 166 base pairs, which likely reflect the length of DNA strands in close contact with the nucleosome pro- teins in chromatin. Deep sequencing of cell-free DNA can also pre- dict likely gene expression in the parental cells by predicting sites of DNA localization to nucleosomes. Cancer circulating tumour DNA (ctDNA) molecules are typically shorter than normal DNA at around 140 base pairs, although the reasons for this are unknown. The mechanisms determining how DNA enters the bloodstream as ctDNA have not been fully defined, but are most likely caused by apoptosis of tumour cells. The half-life of ctDNA is between 16 min- utes and 2.5 hours and this, together with the wide dynamic range and relatively low cost of ctDNA

assays, make cell-free DNA a highly attractive diagnostic and tumour response indicator. By contrast, the detection of circulating tumour cells is more difficult and has higher expense. Critically, often only low numbers of circulating tumour cells can be detected from a blood sample which limits potential diagnostic and prognostic information. Clinical evidence that ctDNA can indeed be used as a ‘liquid’ biopsy that can replace tissue biopsy has emerged from improvements in the diagnostic pathway for lung cancer (Fig. 5.2.7). Lung Size of clone Time Clone 1 Clone 2 Clone 3 Surgery (or other) Cancer detection: screening or earlier diagnosis Molecular profiling or prognostication Detection of residual disease Monitoring response Monitoring clonal evolution Quantitative analysis • Disease staging • Response monitoring • Prognostication Genomic analysis • Mutation profiling • Treatment selection • Monitoring clonal evolution Serial liquid biopsies Treatment selection Treatment 1 Treatment 2 (a) (b) Fig. 5.2.7 Applications of circulating tumour DNA for cancer diagnosis, prognostication, detection of minimal residual disease and monitoring clonal evolution. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Cancer (Wan JCM, et al., 2017, Liquid biopsies come of age: towards implementation of circulating tumour DNA, Nature Reviews Cancer, 17, 223–38), copyright © 2017.

5.2 The nature and development of cancer 455 cancer is an important exemplar for development of ctDNA as- says owing to the need to identify patients who can benefit from targeted therapy and the relative difficulty of obtaining tissue bi- opsies. Existing CT-guided and bronchoscopic biopsy have a comparatively high failure rate and costs. The first FDA and EMA approvals for the use of ctDNA in lung cancer are for assays based on real-time polymerase chain reaction (rtPCR) to detect common oncogenic mutations in EGFR. These assays depend upon allele- specific primers that generate different fluorescent PCR products that discriminate between mutations (deletion of exon 19, L858R, or T790M) from wild-type sequences and can be used for tumour specimens or ctDNA. These assays are now in clinical use for the selection of gefitinib, erlotinib, and osimertinib therapy and regu- latory approval now allows a therapeutic decision to be made based solely on a ctDNA result if a tumour sample is not available. These advances widen the availability of therapy to lung cancer patients who may be too frail for biopsy or who have a tumour that is not easily accessible. The rapid uptake of ctDNA assays in lung cancer trials has also provided strong evidence for utility in the clinic that will be relevant to other cancer types. Over 4000 patients have had ctDNA results in lung cancer trials, and meta-analysis of these data show an overall sensitivity of 60% and specificity of 94% for detection of EGFR mu- tation. Direct comparison between plasma ctDNA and lung cancer biopsies in over 650 patients showed a sensitivity of 66% and a spe- cificity of 100%. A key question now is how effective ctDNA assays will be for the detection of secondary resistance mutations during therapy and on follow-up. Perhaps not surprisingly, the sensitivity of detecting the classical EGFR resistance mutation T790M is less good, reflecting lower abundance of revertant mutations and the effects of tumour heterogeneity. In colorectal cancer the presence of KRAS mutation is a strong predictor of resistance to the EGFR inhibitor cetuximab. Use of sequential ctDNA assays for KRAS has shown the emer- gence of clonal populations marked by KRAS mutations that are resistant to cetuximab therapy. In addition, these low-frequency populations can be demonstrated at diagnosis in some patients, which are selected to become the predominant population with therapy. There are now intensive efforts to improve the sensitivity of next-generation sequencing methods for ctDNA and to widen the number of genes included in these assays. These advances are likely to overcome current sensitivity limitations by better discriminating between early driver mutations (sometimes called ‘stem mutations’), which have higher abundance in plasma and the rarer revertant mutations. Clinical trials are now needed to

address whether outcomes and response rates are different for patients treated on the basis of ctDNA and tumour monitoring. These impacts may further change therapeutic approaches. A key challenge will be the ability to detect ctDNA in patients with early stage disease as ctDNA strongly correlates with tumour volume and can vary by 100–640-fold between stage I and stage IV disease. See Chapter 3.10 for broader discussion of the development and uses of circulating DNA for molecular diagnostics. Conclusion The development of precision medicine using DNA sequencing has had several dramatic successes in the past decade, notably with the targeting of BRAF and EGFR. There are grounds for considerable opti- mism about the wider applicability of sequencing technology in clinical practice. An absolute requirement for the wider development of clin- ical sequencing will be the need to use fresh or frozen specimens from biopsy and surgical procedures because—although current technology can provide high-quality sequencing data for multiple genes using DNA extracted from formalin-fixed paraffin-embedded tissues—the DNA from these tissues is degraded and not suitable for whole genome sequencing. As whole genome sequencing becomes cheaper and the utility of integrative genomic signatures becomes established, the rou- tine pathological processing of fresh or frozen material will be essential. The second main challenge will require clear recognition that re- sistance to conventional and targeted therapies often reflects evolu- tionary or selective pressure, which induces expansion of new clonal populations of tumour cells. This observation immediately prompts investment in suitable infrastructure for sequential or repeat biopsy of patients undergoing routine standard of care treatment, and the wider use of noninvasive methods such as circulating tumour DNA. Finally, despite the impending arrival of thousands of cancer gen- omes, we are faced with major challenges for data interpretation, sharing of the data with patients, and more widely for increased knowledge and the training of healthcare staff to interpret the clin- ical meaning of genomic change. These challenges will, in part, be addressed by an increasing focus on clinical trials based on the molecular targeting of patients har- bouring particular mutations in genes implicated in cancer develop- ment, and also the rapid dissemination of positive or negative results of such trials. It is difficult, however, to conceive how even dedicated oncology specialists will be able to keep abreast of all potential muta- tions, functional changes, and therapy. One solution to this challenge may be wider data sharing and the use of computerized decision sup- port tools. At the same time, it will be critically important to have high-level clinicians working in molecular pathology, as these indi- viduals with specialized knowledge will be at the nodal point of most clinical decisions based on tumour mutation analysis. FURTHER READING Alexandrov LB, et al. (2013). Signatures of mutational processes in human cancer. Nature, 500, 415–21. Diaz LA, Jr., et al. (2012). The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature, 486, 537–40. Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next gen- eration. Cell, 144, 646–74. Helleday T, Eshtad S, Nik-Zainal S (2014). Mechanisms underlying mutational signatures in human cancers. Nat Rev Genet, 15, 585–98. Kucab JE, et al. (2019). A compendium of mutational signatures of en- vironmental agents. Cell, 177, 821–36. Misale S, et al. (2014). Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. Cancer Discov, 4, 1269–80.

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ESSENTIALS All cancer can be termed ‘genetic’ as the disease is caused by somatic cell mutations (alterations in the DNA code), which result in abnormal cellular growth and/or proliferation. Most of these mu- tations are sporadic (only occurring in the cancer cell), but some are due to the inheritance of a germline mutation in a cancer predisposition gene. Cancer predisposition genes can be rare and confer a high cancer risk (about 10-fold lifetime relative risk), or common and confer a low to moderately increased risk (from just over onefold, up to two- to threefold). They have been shown to be involved in causing some of the most common cancers, as well as some rare cancers. Mechanisms of inherited cancers Cancer predisposition genes are usually (1) tumour suppressor genes—for example, retinoblastoma caused by mutations in RB1— when, although the mutations are recessively inherited at the cel- lular level, they tend to manifest with a dominant inheritance pattern because the chance of a mutation being inherited by the offspring is 50%, and a sporadic mutation of the remaining normal allele oc- curs in a somatic cell during the lifetime of the germline mutation carrier to lead to cancer development; (2) oncogenes—for example, the RET oncogene in the multiple endocrine neoplasia type 2A syndrome—when gain-of-function mutations act in a dominant manner; (3) mismatch repair genes—for example, causing genetic instability leading to the hereditary nonpolyposis colorectal cancer (Lynch) syndrome. Clinical features Genetic predisposition to cancer should be suspected when can- cers: (1) occur at a younger age than is seen in the general popula- tion; (2) occur in more than one site or at multiple times at the same site in an individual (multiple primary tumours); or when (3) rare cancers are seen in clusters in a family; or (4) common cancers are seen in clusters in a family, often at a young age or with multiple primaries. Genetic predisposition to common cancers—this includes (1) breast—BRCA1 and BRCA2 mutations confer 80–85% lifetime risk of breast cancer by 80 years (and also a significantly increased risk of ovarian cancer); TP53 (Li–Fraumeni syndrome) mutations confer 90% risk of breast cancer by 60 years; (2) colon—mutations in the APC gene cause familial adenomatous polyposis and a virtually 100% risk of colon cancer by the age of 40 years; hereditary nonpolyposis colorectal cancer, which is also associated with other cancers in addition to colon cancer, particularly endometrial cancer (15–60% lifetime risk) and ovarian cancer (9–12% lifetime risk). Rare inherited cancer syndromes—there are many of these,

including hereditary retinoblastoma, neurofibromatosis type 1 (optic nerve glioma, sarcoma, phaeochromocytoma), neurofibromatosis type 2 (acoustic neuroma and other tumours of the central nervous system), multiple endocrine neoplasia type 1 (parathyroid adenomas, pancre- atic islet tumours and anterior pituitary tumours), multiple endocrine neoplasia, type 2A and 2B (medullary thyroid cancer, phaeochromo- cytoma, parathyroid adenomas), Cowden's syndrome (breast and other cancers), tuberous sclerosis (childhood brain tumours, car- diac rhabdomyomas), Gorlin's syndrome (multiple basal cell naevi/ carcinomas), Von Hippel–Lindau syndrome (cerebellar and spinal haemangioblastomata, renal cell carcinoma, phaeochromocytoma, pancreatic tumours). Clinical management Patients and/or families known or suspected to carry cancer pre- disposition gene mutations require genetic counselling and risk as- sessment, which may lead on to (1) cancer screening—for example, colonoscopy for some individuals at increased risk of colon cancer; (2) lifestyle changes—for example, avoidance of known cancer- causing factors such as sunlight in Gorlin's syndrome; (3) preven- tion strategies—for example, prophylactic total colectomy in the familial adenomatous polyposis syndrome; (4) cancer treatment considerations—for example, tumours with a particular genetic ab- normality may respond to particular treatments; and (5) genetic testing—which may either be diagnostic (the detection of a mutation in an individual affected by cancer) or predictive (the detection of a mutation in a clinically unaffected individual). Future prospects—gene alterations that predispose to cancer affect prognosis and treatment, hence genetic information is increasingly recognized as important in oncological practice. Cancer genetics will become part of mainstream clinical pathways for cancer care in the next decade and is likely to contribute to healthcare that is tailored to individual patients. 5.3 The genetics of inherited cancers Rosalind A. Eeles

5.3 The genetics of inherited cancers 457 Introduction Cancer is a common disease; it affects up to one-half of the popula- tion during their lifetime. All cancer can be termed 'genetic' as cancer is caused by somatic cell mutations (alterations in the DNA code), which result in abnormal cellular growth and/or proliferation. Most of these mutations are sporadic (occurring only in the cancer cell) and only a proportion of these cases is due to the inheritance of a germline mutation in a cancer predisposition gene. In these latter cases, the genetic alteration is in all cells of the body with the excep- tion of the gametes where, on average, the genetic alterations are in one-half of the gametes. It used to be thought that such alterations were rare, but each conferred a high cancer risk (about 10-fold life- time). However, recent studies have shown that there are also more frequent alterations in cancer predisposition genes with each of such mutations conferring a slightly increased risk (with just over a one- fold, up to a two- to threefold relative risk). This has implications for the role of genetic predisposition to cancer in general medical and oncological practice, as a larger proportion of cancer cases may harbour these latter alterations in the genetic code. Identification of such alterations will become important in the genetic profiling of the population to aid targeted cancer screening and prevention. There is emerging evidence that gene alterations that predispose to cancer af- fect prognosis and treatment and thus their significance is becoming incorporated into the clinical pathway for cancer care. Cancer gen- etics will become part of mainstream cancer care in the next decade and is likely to contribute to healthcare that is tailored to individual patients. Historical perspective Since Roman times, cancer has been known to run in families. In some families, the pattern of cancer incidence among family mem- bers is consistent with the inheritance of a mutated gene and car- riers of this mutated gene have a high risk of cancer. The chance that cancer will develop if an individual has a mutation in a cancer predisposition gene is called the penetrance. Most cancer predis- position genes have incomplete

penetrance (i.e. the cancer risk is <100%). There are several types of evidence that inherited susceptibility plays a role in the development of cancer (also see Table 5.3.1): • In some inherited syndromes, which are rare in the general popu- lation, there is an increased risk of cancer in carriers of genetic mutation(s) which give rise to the syndrome, for example, neuro- fibromatosis type 1 (an autosomal dominant genetic syndrome— see later) which confers an increased risk (of a few per cent) of sarcoma and phaechromocytoma. Such syndromes can be ac- cessed using the database initiated in the early 1960s by Dr Victor McKusick as a catalogue of mendelian traits and disorders, first entitled Mendelian Inheritance in Man, now the website Online Mendelian Inheritance in Man (OMIM). • Some rare cancers cluster in families and form a 'cancer family syndrome', for example, the association of tumours in multiple endocrine neoplasia type 2 (MEN2: the association of medul- lary thyroid cancer, phaeochromocytoma, and hyperparathyr- oidism). Such cancers are rare in the general population and so the occurrence of such rare cases either in relatives or in one indi- vidual is highly indicative of a genetic predisposition. • The observation that families exist which have several cases of 'common' cancers. Even though these cancers are prevalent in the general population, the number of such cases in these families far exceeds the number predicted by population rates. Often these cancers occur at ages earlier than seen in the general population (see Fig. 5.3.1) and family members have an increased occurrence of synchronous and metachronous lesions. • Epidemiological studies in the general population which show that there is an increased risk of cancer to relatives of cases and this risk markedly increases as the proband or index case with cancer is affected at a younger age or with bilateral cancers. • Genes have now been identified which, when mutated, are associ- ated with an increased risk of cancer. These may be rare mutations which confer a high (about tenfold) or moderate (just over two- to threefold) cancer risk, or common lower-penetrance genes (which confer an increased risk of just over onefold up to about twofold). Historically, it was thought that genetic predisposition to cancer was a rare phenomenon and was predominantly observed in rare syn- dromes, such as multiple endocrine neoplasia, or was a rare compo- nent of other genetic diseases (such as neurofibromatosis). However, the advances in the Human Genome and HapMap projects (see next) have challenged this view and have shown that in fact genetic vari- ants which are common in populations form an important contri- bution to cancer risk. Inheritance, mechanisms of cancer predisposition, and the retinoblastoma story Inheritance of germline mutations in cancer predisposition genes may be either dominant, recessive, or X-linked. We all carry two copies (alleles) of every gene, one copy from each parent, and as only one allele can be passed down to the next generation, there is a 50:50 chance as to which allele we inherit. In dominant inheritance the presence of a single mutated allele is usually sufficient to cause the associated disease and approximately 50% of all offspring develop the disease. In recessive inheritance the presence of a single mutated allele is insufficient for disease expres- sion and two mutated alleles are required. Usually both parents have to carry the mutated allele for the creation of an offspring affected by disease, but they themselves are unaffected, as their 'normal' allele overrides the effects of the mutated one. Two parents with a reces- sive mutated allele therefore have a 25% chance of having an affected child. An example of such a condition predisposing to colon cancer is the MutYH syndrome where adenomas occur in the colon. In such families colon cancer tends to cluster in siblings, as it is recessive (the colon cancer risk in mutation carrier parents is not thought to be raised above the general population). Most cancer predisposition genes are recessively inherited at the cellular level, but dominantly inherited in families (i.e. there is a 50:50 chance that the mutated allele will be inherited, but in the cancer cell both copies of the allele have to be altered for cancer to occur). In X-linked inheritance the mutated gene is carried on the X chromosome. Females have

two X chromosomes, and can therefore be carriers of the condition but are not usually affected. Males have

458 SECTION 5 Principles of clinical oncology Table 5.3.1 Some of the ‘rare’ syndromes associated with an increased risk of malignancy and their mode of inheritance (for further details, see text) Neoplasia or syndrome Malignancy Riska Modeb Gene Location NF1 Plexiform Neurofibroma Optic glioma Sarcoma <4% <15% <5% AD NF1 17q11 NF2 Bilateral acoustic Neuroma Meningioma Spinal tumours Other brain tumours 85% 45% 26% <10% AD NF2 22q12 Gorlin’s syndrome Basal cell carcinoma Medulloblastoma Meningioma 90% 5% 1% AD PTCH 9q22 Tuberous sclerosis Renal cancer Subependymal giant cell astrocytoma 4% 14% AD TSC1 TSC2 9q34 16p13 Cowden’s syndrome Breast cancer Endometrial cancer Renal cancer Thyroid cancer Colon cancer Melanoma 30% by age 50 28% 33% 15% 9% 6% AD PTEN 10q23 Li–Fraumeni syndrome Brain tumours Breast cancer Sarcomas Leukaemia Adrenocortical cancer Other cancers Childhood cancer All adult tumours: 90% by
60 in women, 74% in men 24% by 20 AD TP53 17p13 MEN1 Pituitary tumour Pancreas Parathyroid Carcinoid 95% AD MEN1 11q13 MEN2A Medullary carcinoma of thyroid Phaeochromocytoma 70% 50% AD RET 10q11 Retinoblastoma Retinoblastoma Osteosarcoma Soft tissue sarcomas Melanoma Lung cancer Lymphoma Bladder cancer Uterine cancer Breast cancer Brain tumours Cancers in the mouth or nose 90% <10% AD RB1 13q14 Ataxia telangiectasia Lymphoma Leukaemia 60% 27% AR ATM 11q22 Bloom’s syndrome Leukaemia Solid tumours Rarely live to >40 AR BLM 15q26 Werner’s syndrome Various—thyroid cancer, melanoma, soft tissue sarcoma, and osteosarcoma are reported Increased but not quantified AR RECQL2 8p12 Rothmund–Thomson syndrome Various including osteosarcoma Increased but not quantified AR RECQ4 8q24 Fanconi anaemia Leukaemia Head and neck cancer Oesophagus/cervix/anus 22% at the age of 36 28% at the age of 49 AR FANC genes Xeroderma pigmentosum Skin cancer 90% often <20 yrs AR XP genes NF, neurofibromatosis type; MEN, multiple endocrine neoplasia; NA, not available. a Lifetime risk of cancer. b Mode of inheritance is classified as autosomal dominant (AD) or autosomal recessive (AR).

5.3 The genetics of inherited cancers 459 only one copy of the X chromosome, so if they inherit a mutated gene on the X from their mother, they will inherit the condition. A carrier female therefore has a 50% chance of passing the condition on to each of her sons, and a 50% chance that her daughters will be carriers. X-linked familial prostate cancer has been observed in a few families, although the causal locus has not yet been refined. Mechanisms of action Cancer predisposition genes are usually tumour suppressor genes, oncogenes, or mismatch repair genes. Very rare instances have been described where alterations in the germ line have a downstream effect which is called an ‘epigenetic’ effect; in most such cases the germline change results in alteration of methylation of genes, which alters their expression and this results in increased cancer risk. Tumour suppressor genes are normal genes in which mutation tends to cause a ‘loss of function’ effect in the control mechanisms of growth and/or cellular proliferation pathways. The first example was retinoblastoma, a tumour of the eye, usually in children, caused by mutations in the tumour suppressor gene RB1. Most cancer pre- disposition genes are tumour suppressor genes and are recessively inherited at the cellular level. However, they tend to manifest dom- inant inheritance (the chance of a mutation being inherited by the offspring is 50%). A sporadic mutation of the remaining normal al- lele occurs in a somatic cell during the lifetime of the germline mu- tation carrier to lead to cancer development. This two-stage process in the development of cancers (where one stage is germline and the other is somatic) is known as Knudson’s two-hit hypothesis.

Oncogenes or proto-oncogenes are mutated normal genes in which a mutation in only one allele tends to cause a ‘gain of function’ effect, resulting in increased growth or proliferation of the affected cells. They act in a dominant manner. They rarely cause predispos- ition to cancer, but examples of those causing cancer include the RET oncogene in the multiple endocrine neoplasia 2A syndrome and the MET oncogene in familial papillary renal cancer. Mismatch repair genes maintain the integrity of the genome and mutations in them allow acquired genetic damage to accumulate, re- sulting in the creation of a cancer cell. They classically predispose to a colorectal cancer syndrome called Lynch syndrome (named after a famous cancer geneticist, Dr Henry Lynch, who was one of the first to recognize that predisposition to common cancers could be in- herited), also known as hereditary nonpolyposis colorectal cancer (HNPCC; see next). Hereditary cancer predisposition genes have also been classified into ‘gatekeeper genes’ and ‘caretaker genes’. Gatekeeper genes are those that regulate progression through the cell cycle. Disturbance of their function leads to an imbalance of cell division over cell death. This cellular proliferation is followed by the accumulation of mul- tiple somatic genetic events causing tumour development. Examples of gatekeeper genes include TP53 and RB1. Caretaker genes main- tain the integrity of the genome. Mutations occurring in these genes result in genetic instability, and it is this that results in mutation in other genes, including gatekeeper genes. The DNA mismatch repair genes in HNPCC are examples of caretaker genes. The multistep pathway of carcinogenesis The development of cancer is thought to be due to a multistep pathway involving several genetic changes. This is likely to be the ex- planation for incomplete penetrance, as not all genetic changes will occur in every individual who inherits the first genetic change. In in- herited predisposition to cancer, the first change is inherited in most cases. Less commonly, the first change is still in the germline but the mutation has occurred de novo in the germ cells (i.e. the carrier of the germline genetic mutation is the first individual in the family to har- bour the mutation; the rate at which this occurs is termed the ‘new mutation rate’). This is more common in some types of inherited predisposition to cancer than others. For example, the new mutation rate in HNPCC is about 50%, but in familial breast/ovarian cancer due to BRCA1 or BRCA2 it is extremely rare. In the latter example, analyses of genetic variation flanking the region of the BRCA1 gene and knowledge of the genetic recombination rate have enabled the occurrence of mutations in this gene in the Ashkenazi Jewish popu- lation to be dated to pre-Roman times. The most classic multistep model which has been published is that of the progression from colorectal adenomatous polyp to invasive colo- rectal carcinoma; a multistep pathway which involves at least five gen- etic changes (the so-called colorectal adenoma–carcinoma sequence, proposed by Vogelstein). This is shown diagrammatically in Fig. 5.3.2. See Chapter 5.2 for further discussion of the nature and develop- ment of cancer. Research approaches for the identification of cancer predisposition genes There are several approaches to locate a cancer predisposition gene. Once it is located and characterized, genetic testing can then be offered in the clinical setting. Cytogenetic alterations Gross chromosomal changes can be analysed by a karyotype or cyto- genetic analysis from a blood sample. Rarely, karyotypic abnormal- ities in an individual who has an unusually early onset of cancer and other unusual phenotypic features have indicated the location of a cancer predisposition gene. The chromosomal study of a man with mental retardation and polyposis led to the finding of a loss of part of chromosome 5, subsequently found to be the location of the polyp- osis gene APC, which predisposes to familial polyposis. 0 0 10 20 30 40 50 60 70 80 90 100 10 20 30 40 Age at diagnosis (years) Proportion of cases due to a breast cancer predisposition gene 50 60 70 80 90 Single first degree relative affected Two first degree relative affected Fig. 5.3.1 Graph showing the probability that breast cancer is due to a predisposition gene by age at diagnosis of breast cancer. Source

data from Claus EB, Risch N, Thompson WD (1991). Genetic analysis of breast cancer in the cancer and steroid hormone study. Am J Hum Genet, 48, 232–42.

460 SECTION 5 Principles of clinical oncology Linkage analysis The concept of genetic linkage was first recognized by William Bateson, who noted that certain characteristics of his experi- mental plants tended to be coinherited, a phenomenon that had been described by the monk Gregor Mendel, 34 years previously. The explanation for this became clear once Morgan recognized that chromosomes contain the genetic material and two traits are coinherited (linked) only if the corresponding genes for them reside close together on the same chromosome. The search for cancer pre- disposition genes using linkage relies on collections of families with numerous cancer cases of the same cancer type. Coinheritance of specific genetic markers with the disease is said to show evidence of linkage if the coinheritance is greater than would be expected by chance. This is expressed as a 'LOD score' which is similar to a P value in clinical trials. A LOD score of more than 3 is considered statistic- ally significant and equivalent to odds of linkage of 1000 to 1. Phenotypic features A physical characteristic associated with a cancer predisposition syn- drome may give a clue as to its location. An example of this is the coexistence of aniridia and genitourinary abnormalities with Wilms' tumour in the Wilms tumour-aniridia syndrome (WAGR) syndrome. This is caused by a contiguous gene deletion on chromosome 11. Association studies Over 2000 disease susceptibility loci have been identified using genome-wide association studies (http://www.ebi.ac.uk/gwas). In these case–control studies, allele frequencies are compared be- tween affected individuals and controls. It is important in such studies to have controls from the same ethnic/racial group to avoid false as- sociations. Advances in the knowledge of the structure of the human genome have identified single nucleotide polymorphisms (SNPs or single base changes) throughout the genome (the HapMap pro- ject) and large-scale genomic analysis using chip array technology (e.g. the Illumina or Affymetrix systems) has enabled millions of such SNPs to be analysed in each DNA sample at once. Such studies have identified common variants associated with disease risk, which are present in at least 5% of the population: in some cases they are found in over one-half of the population. Although each SNP is as- sociated with a small increased risk (usually less than twofold), the risks can be multiplicative and so a combination of SNPs can give different 'risk profiles' in different individuals. This therefore opens up the possibility of SNP profiling to determine the risk of cancer in defined populations. Direct sequencing and the potential role of whole genome sequencing As the Human Genome Project has identified a large proportion of the genetic code, parts or all of this can be subjected to direct analysis by sequencing of genes to look for base pair changes (point mutations or nonsense mutations), or insertions or deletions of bases (frameshift mutations). Most genetic tests for cancer predis- position now involve such sequencing technology of specific genes from blood DNA (e.g. the current breast cancer predisposition genetic tests sequence the BRCA1 and BRCA2 genes). One of the main current research activities is the sequencing of whole genomes (e.g. the '1000 genomes project' which has completed the sequence of 100 000 human genomes to find genetic variation associated with phenotypes in the United Kingdom). Although at present a research tool, it is envisaged that this could become a routine investigation. The difficulty will arise in the interpretation of all the genetic vari- ants, their potential interaction effects, and the accurate prediction of disease risk. Detection of other mechanisms of gene alteration Not all genetic changes involve alterations of DNA bases, as already mentioned here. Some mutations can be due to larger deletions or gene rearrangements and these can easily be overlooked by conven- tional sequencing methods. Most are detected by multiple probe li- gation analysis (MLPA) or dosage analyses of sequence reads.

For example, the standard BRCA1/2 genetic test for breast cancer pre- disposition also includes MLPA as well as sequencing, as up to 3% of BRCA1/2 mutations in some populations can be caused by alter- ations which are only detected by MLPA. Epigenetic changes are often due to methylation of genes. Rarely, alterations in the germ line can cause downstream methylation of cancer predisposition genes; such changes have recently been described in Wilms tumour and some cases of HNPCC. Cancer risks associated with cancer predisposition genes Cancer risks depend on the presence of mutations in a cancer pre- disposition gene and its penetrance (Table 5.3.2). Penetrance may be affected by external factors, such as lifestyle, and other environ- mental effects; it may also depend on the ethnic origin of an indi- vidual due to population-specific mutation risks. For example, for BRCA1 or BRCA2, which predispose to breast and ovarian cancer, using data from the Breast Cancer Linkage Consortium based on breast and ovarian cancer families identified from a worldwide population of high-risk families with breast cancer, the risk of breast cancer is estimated to be 85% by 80 years, but the risk associated with the Icelandic founder mutation in the BRCA2 gene is estimated Normal epithelium Genetic alteration APC loss Genetic instability ?TP53 loss Activation of KRAS by point mutation SMAD4 loss ?TP53 loss Further genetic mutations Hyperproliferative epithelium Early adenoma Intermediate adenoma Late adenoma Carcinoma Metastasis Fig. 5.3.2 The colorectal adenoma–carcinoma sequence.

 to be as low as 37% by this age. The ethnic population differences may be due to a founder mutation dependent risk, the effect of other modifying genes in a population, or the added effect of environ- mental influences, which may be shared within specific populations. Therefore it is important to ascertain the ancestry of the patient be- fore genetic counselling is initiated. The estimate of penetrance can be confounded by the presence of phenocopies when research into the identification of a cancer predisposition gene is undertaken. Table 5.3.2 Genes with high penetrance for the common cancers Neoplasia or syndrome Malignancies Risk of cancera Location Gene Breast/ovary Breast/ovary cancer syndrome Breast Ovary/Fallopian tube Other cancers, e.g. pancreas, prostate Breast Ovary/Fallopian tube Prostate, pancreas Other cancers, e.g. melanoma/bile duct 80–85% 40–60% <10% 80–85% 27% <10% 10–14% by 74 years 17q21 13q12 BRCA1 BRCA2 Colon Familial adenomatous polyposis Bowel cancer Duodenum/periampullary Hepatoblastoma/ thyroid/brain Desmoid c.100% <5% 5q21 APC HNPCC Colon Endometrium Ovary Colon Endometrium Ovary Colon Endometrium Ovary Colon Endometrium Ovary Other cancers, e.g. renal tract, pancreas, bile duct, gastric, brain By age 70 45–75% 30–40% 10% 35–65% 20–25% 10–15% 20–70% 25–70% 1% 15–20% 15% 1% <5% 2p21 3p21 2p16 7p22 MSH2 MLH1 MSH6 PMS2 MSH2; MLH1; PMS2 Muir–Torre syndrome As HNPCC (see above) with skin lesions 2p21 hMSH2 Peutz–Jeghers Ovarian cancer (sex cord) Gastrointestinal <10% 19p13 STK11 Juvenile polyposis Colon 70% 18q21 10q23 10q22 SMAD4 PTEN BMPR1A MutYH Colon 70% 1p34 MUTYH (homozygotes) Turcot's Colon 70% can be <20 APC/HNPCC (biallelic) Gastric cancer Diffuse gastric cancer Stomach 90% 16q22 CHD1 Melanoma Melanoma Melanoma 65% 9p21 CDKN2A Renal Renal cancer (papillary) Papillary renal (type 1) 70% 7q31 MET Von Hippel–Lindau Cerebellar Haemangioblastoma Retinal angioma Renal cell carcinoma 60–80% 85% 40% 3p25 VHL WAGR Wilms' tumour is part of syndrome 11p13 WT1 Birt–Hogg–Dube Renal carcinoma 15% 17p11 FOLLICULIN Hereditary leiomyomatosis Renal carcinoma 10–16% 1q42 FH a The risk is either the 'lifetime risk' quoted to age 80 years unless otherwise stated. Note: The common cancers—colon, breast, ovarian, prostate, lung, pancreas, testicular, melanoma, and lymphoma—have had numerous lower-risk variants identified by genome-wide association, and rare more moderately penetrant genes have been found by panel

candidate genetic mutation analysis in some of these cancers (see text).

462 SECTION 5 Principles of clinical oncology Phenocopies are people who have developed the disease of interest but are found not to carry mutations in the disease predisposition gene, so that the disease has occurred by chance alone or may have been due to environmental influences. Phenocopies are a particular problem in the analysis of syndromes associated with common cancers such as breast, prostate, ovarian, or colon cancer. When quoting cancer risks it is important to quote the risk by a specific age as the profile of risk may alter over time; for example, the risk of breast cancer from a deleterious mutation in BRCA1 is a sigmoid curve, starting to rise from the age of 30; the steepest part of the curve is in the 40s and there is still some risk until the age of 80 years. An unaffected woman with a BRCA1 mutation will there- fore have a lower residual cancer risk if she is aged 70 than she will at the age of 40 years. Genetic predisposition to the common cancers Breast cancer Breast cancer is the most common noncutaneous cancer in women in the Western world. Several genes predispose to high risks of breast cancer, most notably BRCA1 and BRCA2 (breast cancer 1 and 2 genes) which were isolated in 1994 and 1995. These genes, when mutated, also predispose to ovarian cancer, and also have a small (<10%) risk of causing other cancers (e.g. pancreas, bile duct, melanoma, male breast cancer, prostate cancer). They are highly penetrant for female breast cancer (80–85% risk by 80 years) and ovarian cancer (40–60% risk for BRCA1 and 27% risk for BRCA2). The profiles of the penetrance curves are slightly different (in general, those for BRCA2 start to rise at an older age for both breast and ovarian cancer) and this is taken into consideration when consid- ering the timing of preventative surgery (see next). A rarer breast cancer predisposition gene is TP53 which usually predisposes to the Li–Fraumeni syndrome, the association of early onset sarcoma with cancer at less than 45 years in at least two close relatives. Often this syndrome is associated with childhood cancer. The penetrance of breast cancer is 90% by age 60 and this gene can cause breast cancer at particularly young ages (in the 20s). Other rarer cancer predispos- ition genes in the DNA repair pathway have been shown to be asso- ciated with increased breast cancer risk (PALB2, ATM, CHEK2), but these risks (about two-four fold) are not as high as those from muta- tions in BRCA1/2 or TP53. Cowden's multiple hamartomatous syn- drome has an increased risk of female and male breast cancer and also thyroid and uterine cancer. It is associated with gynaecological and brain abnormalities and bowel polyps, but there is debate as to whether there is also an increased risk of bowel cancer. The pathology of the breast cancer is characteristic in some of these condi- tions; in BRCA1 mutation carriers, it is often hormone receptor and HER2 negative (so-called 'triple negative') and has cellular features of the basal type. There is an increased risk of lobular breast cancer in association with diffuse gastric cancer, which is due to mutations in the CDH1 E-Cadherin gene. Colon cancer It is thought that at least a proportion of colon cancers arises from polyps in the bowel. The colon cancer syndrome with the highest bowel cancer risk is associated with the presence of thousands of such polyps in the large bowel (familial adenomatous polyposis or FAP; Fig. 5.3.3). This is due to mutations in the APC gene. The APC protein is a negative regulator of β-catenin, a critical component of a signal transduction pathway that regulates cell–cell adhesion, cel- lular polarity, and tissue architecture. The penetrance is high, with a virtually 100% risk of colon cancer by the age of 40. There is also a risk of other cancers, such as hepatoblastoma, periampullary, thy- roid and brain cancer, sarcoma, and desmoid tumours. Polyps can also occur in the upper gastrointestinal tract and pigment can be present in the retina. The polyps are so extensive that the mainstay of prevention is a colectomy once the polyps appear on sigmoido- scopic monitoring, of individuals who have APC mutations, from the age of 11 years. Lynch's syndrome or HNPCC has

fewer polyps (usually <100) and classical HNPCC conforms to a definition also known as the Amsterdam criteria, so-called because Vasen in Amsterdam found that if these criteria were used then over half of families had mu- tations in the mismatch repair genes hMLH1 (chromosome 3p21), hMSH2 (2p21), hMSH6 (2p16), and PMS2 (7p22). There is con- troversy as to whether PMS1 (2q31) increases colon cancer risk. These criteria are colon cancer in at least three individuals in two generations, with at least two being first-degree relatives of each other and at least one of the colon cancers occurring at less than 50 years of age. Classically the cancers tend to occur more often in the right side of the colon whereas sporadic colon cancer is more often left-sided. This condition is also associated with other cancers, particularly endometrial cancer, ovarian cancer, and smaller risks of biliary and pancreatic cancer, cancer of the renal tract (particularly transitional cell carcinoma), and upper gastrointestinal tract cancer (Table 5.3.2). Extracolonic cancers are more common in hMSH2 mutation carriers, and hMSH6 is particularly associated with uterine cancer. There are less stringent clusters which are also due to mutations in these genes and so more loose criteria have been developed (the so-called Bethesda criteria, but as the criteria get less stringent, the mutation frequency decreases). The mismatch repair genes are involved in DNA base excision repair and, if mutated, give rise to genetic instability, particularly of repeats in the DNA (micro- satellite instability). This can be analysed in tumour specimens to Adenomatous polyp Fig. 5.3.3 Large bowel with numerous adenomatous polyps (arrowed) due to familial adenomatous polyposis.

 determine if there is likely to be an underlying mismatch repair gene defect. Protein products of these genes can also be detected by immunohistochemical staining and so lack of staining in colonic tumours is used as a triage to determine which gene may be mu- tated. More rarely, brain tumours can occur in association with very early onset (often <20 years) colorectal cancer and these cases have been found to harbour homozygous (both gene copies are altered) mutations in mismatch repair genes (Turcot’s syndrome). The pres- ence of sebaceous adenomas or keratoacanthoma should raise the possibility of Muir–Torre syndrome, which is HNPCC with these additional skin features and is also due to mutations in mismatch repair genes. Genes predisposing to other rarer colorectal cancer syndromes have also been described. STK11 predisposes to Peutz–Jeghers syn- drome (autosomal dominant) where hamartomatous polyps are associated with pigmentation of the lips and buccal mucosa. This syndrome also has an increased risk of breast, ovarian, uterine, pan- creatic, and testicular cancer. Juvenile polyposis is an autosomal dominant condition and causes diffuse hamartomatous polyps of the colon, small bowel, and stomach which develop at an early age (<10 years) or older (c.55 years). About half of patients have mutations in the SMAD4 gene (which, interestingly, is mutated in the multistep pathway of colorectal cancer; see Fig. 5.3.2, within the cancer cells) or PTEN or BMPR1A. Rarely families with mutations in the TGFBRII gene have been described; this is in the SMAD4 pathway. If a family has a recessive pattern of inheritance (i.e. disease in siblings but not the parents) of multiple colonic polyps, then MutYH should be considered. This gene usually has mutations at specific sites and so the genetic test can examine these regions specifically, at least in the first instance. Upper gastrointestinal cancer and pancreatic cancer There are rare reports of familial gastric cancer where the cancer can occur at a very young age (<20 years) and is of diffuse type. These are associated with mutations in the CDH1 gene. The treatment is prophylactic gastrectomy. Duodenal cancers can occur as part of FAP. Familial pancreatic cancer is described but the genetic basis is still being researched; rare instances are due to mutations in the BRCA2 and other genes. Ovarian cancer The main genes to consider are BRCA1 (on chromosome 17q21)

and BRCA2 (on chromosome 13q12). These account for the ma- jority of families with two or more cases of ovarian cancer and are predominantly associated with the serous type of ovarian adenocar- cinoma. Peutz–Jeghers syndrome can cause unusual ovarian lesions such as mucinous tumours or sex cord tumour with annular tubules. Ovarian cancer can also be part of HNPCC but the penetrance is lower (about 12%) than with BRCA1/2. Mutations in BRIP1, RAD51C, and RAD51D have recently been reported and confer ovarian cancer risks high enough to warrant offering preventative removal of the ovaries and fallopian tubes. Melanoma Mutations in the multicancer tumour suppressor gene CDKN2A (P16) have been associated with melanoma. Mutations in this gene should only be considered if there are multiple melanomas in an individual, and/or at least three cases of melanoma in a family, often with early onset disease (≤40 years). Prostate cancer Prostate cancer is the most common noncutaneous cancer in men in the Western world. Over 80% of cases occur at more than 65 years of age. Rare cases (<5%), particularly at young age (<60 years), are due to mutations in the DNA repair genes, particularly BRCA2 which predisposes to more aggressive disease with a poorer prognosis. Sequencing of a linkage region on 17q showed a recurring mutation in HOXB13, G84E which is more common in younger onset cases or those with a family history. Most of the prostate cancer loci (>100) have been found by genome-wide association studies. Variation at these loci resulting in slightly increased risk is common. A man in the highest 1% of the risk profile from SNP combinations will have nearly six times the average risk of the general population. As fur- ther SNPs are found, genetic profiling will be possible in the popu- lation. It is uncertain at present if these variants will help to identify more aggressive disease and further research is needed in this area. Common lower-risk variants Genome-wide association studies are revealing genetic changes (SNPs) associated with disease. These are more common than most of the genetic changes mentioned earlier, and often the SNPs are not in genes and so are presumably exerting their effect by altering gene function in another part of the genome. These types of alter- ations are currently the predominant types of variants predisposing to prostate and lung cancer. In the latter disease, one of the SNPs is on chromosome 15q25 which contains the nicotinic acetylcholine receptor subunit genes CHRNA3 and CHRNA5, suggesting that sus- ceptibility may be mediated through smoking behaviour. Rare inherited cancer syndromes Hereditary retinoblastoma: a classical example of a cancer predisposition syndrome The first cancer predisposition genes were identified by studying rare but striking clusters of conditions that occur as part of recog- nized clinical syndromes (Box 5.3.1). Hereditary retinoblastoma is a classical example of this phenomenon. Retinoblastoma is a cancer of the retinal cells and mainly occurs at in children less than 5 years of age (most occur at <2 years). One in 13 500–25 000 children are affected, with an equal sex distribution. About 10% of patients have a family history of the disease, with an autosomal dominant pattern of inheritance. Inherited forms of the disease are due to mutations Box 5.3.1 Features of inherited cancer predisposition syndromes • Earlier onset than sporadic cancer • Bilateral or multifocal cancer • Rare cancers either alone or more often in clusters in families than is expected by chance • Phenotypic abnormalities indicating a disorder of tissue formation/ regulation (e.g. overgrowth syndromes or skin manifestations) • New germline mutations may account for new cases where there is no family history

 in the RB1 gene on chromosome 13q14; the new mutation rate is also high. The other cases are apparently sporadic but many of these have a germline mutation which has occurred de novo (see earlier). Knudson calculated that only one additional mutation is necessary for tumour development, leading to the two-hit hypothesis. All bi- lateral cases where retinoblastoma occurs in both eyes should there- fore be considered to be gene

mutation carriers. Eighty-five per cent (85%) of retinoblastomas presenting at less than 6 months of age af- fect both eyes and are likely to be the inherited form. The proportion of bilateral cases declines to 6% by 24 months, when most cases are of the sporadic type, with a much lower risk of genetic transmis- sion (<5% of cases will be mutation carriers). The penetrance of RB1 mutations is 90%. Individuals with hereditary retinoblastoma are at an increased risk of developing a variety of other cancers (espe- cially osteosarcoma and bladder cancer). Retinoblastoma illustrates the cardinal clinical features of inherited cancer predisposition syn- dromes: early onset, bilateral cancer, familial clustering, and pre- disposition to multiple tumours both within the eye and also at multiple sites. Many genetic alterations in RB1 are gene deletions and insertions. Of interest, point mutations may be associated with a lower penetrance, suggesting a genotype–phenotype effect (the gen- etic and physical effects, respectively). Neurofibromatosis type 1 This is one of the most frequent single gene disorders with a fre- quency of 1 in 2500–3300 individuals. The diagnosis is clinical and the clinical features of neurofibromatosis type 1 (NF1, von Recklinghausen's disease) require at least two of the following: • At least six café-au-lait spots larger than 5 mm (if prepubertal) or 15 mm (if postpubertal) • At least two neurofibromas or one plexiform neurofibroma • Axillary or inguinal freckling • Optic nerve glioma • At least two iris hamartomas (Lisch nodules) • An osseous lesion such as sphenoid dysplasia or thickened long bone cortex ± pseudarthrosis • A first-degree relative with NF1 Learning difficulties can also occur in about 30%. About 3–5% of cases have a malignancy which is usually an optic nerve glioma, sar- coma, or phaeochromocytoma. Recent analyses of other tumour risks have also reported a moderately increased breast cancer risk. The NF1 gene (on chromosome 17q11) is very large (60 exons) and it encodes a guanosine triphosphatase activating protein known as the NF1-GAP-related protein, or neurofibromin. GAP proteins negatively regulate RAS to control cell proliferation. Mutations are numerous and varied in type, probably because the gene is so large. There is no genotype–phenotype correlation of the mutation with the NF1 features. Some patients have numerous neurofibromas while others have very few. The phenotype is thought to be controlled by other genes. The new germline mutation rate is high (30–50%), again probably because of the extremely large size of target locus. Neurofibromatosis type 2 Neurofibromatosis type 2 (NF2) is less common than NF1 (1 in 33 000 births) and has an autosomal dominant pattern of inherit- ance. The neurological effects of neurofibromas predominate in NF2. There is a predisposition to development of tumours of the central nervous system, particularly schwannoma of the eighth cranial nerve ('acoustic neuroma', which occurs bilaterally in 85% of cases), men- ingioma, spinal cord schwannoma, and malignant gliomas. Deafness and tinnitus due to acoustic neuromas, as well as muscle weak- ness and wasting due to spinal cord compression are not unusual. Criteria for diagnosis are bilateral acoustic neuromas or a family his- tory of NF2, plus unilateral acoustic neuroma at less than 30 years or any two of the following: meningioma, glioma, schwannoma, and juvenile posterior subcapsular lenticular opacities. There is a milder and a more severe type with presentations at under and over 20 years of age, respectively. The NF2 gene, located at chromo- some 22q12, encodes a protein named schwannomin (or Merlin, for Moesin Ezrin Radixin-like protein) which communicates between the extracellular matrix and cytoskeleton. About half the cases are de novo. There seems to be a genotype–phenotype correlation with milder phenotypes associated with point mutations and more severe phenotypes associated with nonsense and frameshift mutations. It is noteworthy that about 20% of apparently de novo cases will not have germline mutations, and the NF2 mutation is only at the tumour site as a result of mosaicism (only some cells in the body have the mu- tation). Such patients have a lower risk of transmission to offspring as the chance of their gametes being involved in the mosaicism is less. Patients should be

managed in specialist centres as complex screening and interdisciplinary management is needed. Multiple endocrine neoplasia type 1 This syndrome (MEN1) is associated with parathyroid adenomas, pancreatic islet tumours, and anterior pituitary tumours (for ease of recall, the 3Ps) and is autosomal dominant. Carcinoid can also occur. The MEN1 gene on 11q13 codes for menin, which acts as a growth suppressor protein. Mutations can occur throughout the gene and there is no genotype–phenotype correlation. Only about 10% of cases are de novo. Multicentric pancreatic tumours and hyperparathyroidism at young age (<30 years), with or without pi- tuitary tumour, should raise the possibility of MEN1 and genetic testing can be undertaken using gene sequencing, although in 20% of classical cases no mutation is found. The penetrance is high (95% by 70 years). Multiple endocrine neoplasia types 2A and 2B and familial medullary thyroid cancer Three disorders are due to activating mutations in the RET tyro- sine kinase-linked cell surface receptor encoded by the RET gene at 10q11.2, which is an oncogene (only one mutated copy is necessary for disease development). Medullary thyroid cancer is common to all three conditions and is histologically associated with C-cell hyperplasia which should be looked for in the pathology report. When no other features are present, the condition is termed familial medullary thyroid cancer. In multiple endocrine neoplasia (MEN) types 2A and 2B, additional unusual tumours arise including phaeo- chromocytoma (in 50%) and parathyroid adenomas in 20–30% (particularly in MEN2A). MEN2B is associated with marfanoid habitus, intestinal ganglioneuromas (causing Hirschsprung's dis- ease), and mucosal neuromas, often in the lips, causing them to be prominent. Particularly in MEN2B, medullary thyroid cancer can occur at less than 10 years of age. In 95% of cases of MEN2A, muta- tions affect cysteine residues in the extracellular binding domain of

 RET, resulting in inappropriate disulphide bond formation, dimer- ization, and activation of the RET tyrosine kinase. Familial medul- lary thyroid cancer results from mutations which similarly involve cysteine residues in most cases, but at different sites. The mutation found in MEN2B is distinct and involves a methionine to threonine substitution in the adenosine triphosphate (ATP) binding site of the receptor tyrosine kinase, leading to excessive receptor activity. There is a strong genotype–phenotype correlation with certain mu- tations particularly associated with phaeochromocytoma. Rarely, other mutations may occur in other parts of RET and some of these are associated with a lower penetrance. As medullary thyroid cancer can occur in early childhood, current optimal management is gen- etic testing and prophylactic total thyroidectomy in gene mutation carriers. Many geneticists will undertake predictive tests from birth in families where there is a known RET mutation. Screening for phaeochromocytoma and monitoring of parathyroid hormone and calcium levels should be undertaken; the disease is penetrant by age 70. All cases of medullary thyroid cancer should be referred to a cancer geneticist to offer genetic testing for RET mutations; if no mutation is found in such a case then the chance that it is inherited is less than 5%. Cowden's syndrome This autosomal dominant condition is a multiple hamartomatous syndrome. It has characteristic skin and tongue hamartomatous lesions, gynaecological abnormalities, and intestinal hamartomas. Craniomegaly and mental subnormality occur in about 50% of af- fected individuals. The pathognomonic mucocutaneous lesions include trichilemmomas, acral keratoses, papillomatous papules, hyperkeratosis, and oral fibromas. Breast cancer occurs in 30% of female gene carriers by age 50 and multiple painful fibroadenomas of the breast are common, often necessitating prophylactic mastec- tomy. Thyroid cancer, male breast cancer, and endometrial cancer can also occur. Glial masses may present as cerebellar ataxia and seizures (Lhermitte–Duclos disease). The gene concerned, 'phos- phatase tensin homologue deleted in chromosome 10' or PTEN, is located on

10q23. The PTEN phosphatase, by operating in oppos- ition to the phosphoinositol-3-kinase pathway, inhibits cell survival and growth. Tuberous sclerosis This is a disease of variable severity characterized by the develop- ment of multiple hamartomas involving many organs; it is auto- somal dominant. Characteristic lesions—facial angiofibromas (adenoma sebaceum), shagreen patches, and ungual fibromas— along with epilepsy and learning difficulties often suggest the diagnosis. There is an association with cardiac rhabdomyomas. Often there is no family history since as many as 60% of cases are due to a de novo mutation. There is a 5–15% incidence of child- hood brain tumours in affected individuals, mostly subependymal giant cell astrocytomas. In addition, a weak association with renal cell cancer has been reported. A wide variety of benign tumours, including hamartomas, angiofibromas, and renal lesions occur. The renal tumours are characteristically angiomyolipomas, which can cause renal haemorrhage or compress the normal kidney leading to renal failure. Linkage studies have identified two genes, TSC1 at 9q34 and TSC2 at 16p13. TSC1 encodes a protein called hamartin. Most mutations described within this gene result in a truncated protein. TSC2 encodes tuberin, a protein showing some homology to GTPase activating proteins. Li–Fraumeni syndrome This is a rare but important autosomal dominant syndrome. It is named after the two epidemiologists who noticed an increased cancer risk in first- degree relatives of patients with rhabdomyo- sarcoma. The key feature is sarcoma, particularly at young age (<45 years). The definition of classical Li–Fraumeni syndrome is sarcoma in the proband before age 45 years and cancer before age 45 years in two close relatives, one of whom is a first- degree rela- tive. The tumour spectrum is wide and multiple tumours can occur in one individual; there is also a high (24% by age 20) risk of tu- mours in childhood. The lifetime penetrance by age 60 years is 90% in women and 74% in men; penetrance is increased by exposure to carcinogens, particularly smoking. The spectrum of early onset tu- mours particularly includes bone and soft tissue sarcoma (excluding Ewing’s sarcoma), breast cancer, brain tumour, leukaemia, and adrenocortical carcinoma. Approximately 75% of Li–Fraumeni syn- drome families have germline mutations within the TP53 gene lo- cated at 17p13. TP53 has been referred to as the ‘guardian of the genome’ because of a critical role in arresting the cell cycle in the presence of DNA damage. It can act as a tumour suppressor and also as a dominant oncogene. Radiation may increase the risk of second tumours (57% rate of second tumours over 30 years) and so should be avoided if possible. Basal cell naevus syndrome (Gorlin’s syndrome) This condition should be considered in any patient presenting with a basal cell carcinoma before the age of 30 years, or with a per- sonal or family history of multiple basal cell naevi/carcinomas. It is associated with abnormalities of skin, bone, and tooth forma- tion, including polyostotic bone cysts, odontogenic keratocysts (jaw cysts), bifid ribs, ectopic calcification (lamellar calcification as seen on a posteroanterior skull radiograph is pathognomonic), and palmar or plantar pits. An increased incidence of other cancers, including medulloblastoma, ovarian carcinoma, and sarcomas, may also occur. The incidence has been estimated at 1 in 31 000. The gene responsible for the majority of cases, PTCH on 9q22.3, is a homo- logue of the Drosophila patched gene that encodes a transmembrane receptor for an extracellular ligand (Hedgehog). This pathway con- trols the fate of cells, body patterning, and growth by forming gradi- ents in embryonic tissues. The most important management feature to note is that such patients should avoid therapeutic radiation as this induces further tumours. Renal cancer and syndromes Mutations in several genes have been demonstrated to predis- pose to renal cell carcinoma. These include the VHL gene (as- sociated with von Hippel–Lindau syndrome), FOLLICULIN (associated with Birt–Hogg–Dubbe syndrome), FH (associ- ated with leiomyomas), the succinate dehydrogenase genes, and rare reports of disruption of the TRC8 gene (by a translocation). Translocations involving chromosome 3 have also been found con- stitutionally in

renal cancer cases. Hereditary papillary renal cell carcinoma has been related to mutations in several genes, particu- larly the oncogene MET.

466 SECTION 5 Principles of clinical oncology Von Hippel–Lindau disease Von Hippel–Lindau syndrome (VHL) is a dominantly inherited fa- milial cancer syndrome predisposing to a variety of malignant and benign neoplasms, most frequently retinal angioid streaks, cere- bellar and spinal hemangioblastomas, renal cell carcinoma, phaeo- chromocytoma, and pancreatic tumours. There are numerous liver, pancreatic, and renal cysts which can be seen on imaging. The in- cidence in the United Kingdom is 1 in 36 000, with near complete penetrance by 70 years. There are two types, VHL type 1 and type 2 (without and with phaeochromocytoma respectively; type 2 is div- ided into three further types A–C depending on the combinations of associated features). The VHL gene at 3p25–p26 contains three exons that encode a 213-amino-acid protein. There is a genotype– phenotype correlation with missense mutations more often associ- ated with phaeochromocytoma. The VHL protein plays a role in the transduction of growth signals generated by changes in oxygen ten- sion, promoting the translation of target genes that include vascular endothelial growth factor. VHL is a classical tumour suppressor gene, with a second, somatic mutation required for the development of cancer. Mutations in VHL are common in sporadic renal clear cell carcinoma within the tumour cells only. Management of VHL pa- tients should be in a multidisciplinary clinic as several systems need monitoring (eye, neurological, and urological). Specialist urological management is necessary as renal tumours are often multifocal, so nephron-sparing surgery is used. Familial papillary renal cell carcinoma Any case of the less common renal cancer type, papillary renal cell carcinoma, should be considered for genetic analysis of the MET oncogene (on chromosome 7q31) which predisposes to familial papillary renal cancer. It codes for a transmembrane tyrosine kinase receptor for hepatocyte growth factor or scatter factor, a peptide with essential roles in embryogenesis, cell motility, and tumour in- vasion. Germline MET missense mutations in cysteine residues, homologous to those involved in aberrant dimerization and activa- tion of the RET receptor, are associated with familial papillary renal cell carcinoma. Monoallelic activating mutations in MET are also found in 15% of cases of the sporadic form of the disease. The spec- trum of mutations found in sporadic papillary renal cell carcinoma is wider and includes activating mutations in the MET tyrosine kinase domain. Rarely other loci may be associated with papillary renal cancer, but MET is the gene to be most considered. Birt–Hogg–Dubbe syndrome This syndrome is a rare inherited genodermatosis character- ized by hair follicle hamartomas, kidney tumours (usually a renal oncocytoma or chromophobe renal cancer), and spontaneous pneumothorax; fibrofolliculomas on the face are its hallmark and trichodiscomas (tumour of the hair disc) and acrochordons (‘warts with a thin neck’; skin tags) are associated features. Onset is in adulthood. It is due to mutations in the FOLLICULIN gene on chromosome 17p11. Hereditary leiomyomatosis and renal cell cancer Multiple skin and uterine leiomyomas (fibroids) associated with renal cancer are associated with mutations in FH (on 1q42) causing fumarate hydratase deficiency. Wilms tumour (nephroblastoma) Wilms tumour is a poorly differentiated tumour of the kidney, usually in childhood. It occurs in 1 in 10 000 children and accounts for 8% of childhood cancers. It is associated with aniridia, hemihypertrophy, and developmental abnormalities of the genitourinary tract (the WAGR syndrome). Males and females are equally affected and usu- ally present early in childhood, most often with an abdominal mass. Two sites of loss of heterozygosity have been identified in Wilms tumours, WT1 at 11p13, and WT2 at 11p15.5. There are also rare familial cases in which linkage to neither 11p locus has been es- tablished (referred to as the WT3 group). In 10–30% of patients, the disease is bilateral or multifocal, but less than 1% of all cases

are truly familial. Most cases of bilateral nephroblastoma are due to new germline mutations in WT1. The protein encoded by the WT1 gene is a ‘zinc finger’ DNA-binding transcription factor. WT1 interacts with another tumour suppressor, TP53, to bind and sup- press transcription from the epidermal growth factor receptor and insulin-like growth factor 2 gene promoters. When WT1 function is compromised, transcription from these growth- and survival- promoting proteins is increased, initiating tumour development. WT1 is not, however, a strictly Knudson-type tumour suppressor. Statistical analysis of age at diagnosis and proportion of bilateral and unilateral tumours does not follow the pattern described for retinoblastoma. Furthermore, the children of patients who sur- vive Wilms tumour are at lower risk of the disease than would be expected from a dominant-acting tumour suppressor gene. There is evidence that ‘genomic imprinting’ may explain some of these anomalies. Imprinting is a process of gene inactivation through DNA methylation that preferentially favours expression from genes inherited from one or other parental lineage. There is a report in some Wilms tumour cases of genetic alteration of a methylation centre in the genome, which is an example of a germline change that has epigenetic effects. Chromosome fragility syndromes All these syndromes are autosomal recessive and they cause other abnormalities of phenotype, such as short stature, autoimmune and immunodeficiency disease, and other features. Although they are very rare, they are important as such patients are sensitive to DNA-damaging agents, which is an important consideration when treating the associated cancers with such agents. Ataxia telangiectasia This is a rare recessive condition (1 in 30 000–100 000). Ataxia tel- angiectasia patients who are homozygous for ATM mutations have telangiectases in the eye, progressive ataxia due to cerebellar de- generation, general neuromotor dysfunction, and immune defects. There is a 30–40% lifetime risk of malignancy including epithelial tumours, chronic T-cell leukaemia, and lymphoma. ATM heterozy- gotes do not exhibit any of these defects, but have a two- to threefold increase in the risk of cancer, particularly female breast cancer. The ATM gene (11q22) encodes a 350-kDa protein which contains a do- main sharing homology with members of the phosphatidylinositol- 3-kinase family and which is a signal transduction protein that regulates cell cycle checkpoints.

5.3 The genetics of inherited cancers 467 Bloom’s syndrome This a rare autosomal recessive disease of unknown incidence, more common in Ashkenazi Jews. Features include short stature, sensi- tivity to the sun, skeletal abnormalities (a bird-like face), and suscep- tibility to infection. An increased frequency of malignant neoplasms occurs throughout life, with dramatically reduced life expectancy; it is very rare to live into the 30s. Lymphoma and leukaemia predom- inate before the age of 25 years; those that survive into their 20s and 30s are prone to a variety of common solid tumours, particularly squamous cell carcinoma of the head and neck, breast cancer, and gastrointestinal tumours. The age at diagnosis for these carcinomas is usually 20 years earlier than in the general population. The gene responsible, BLM (15q26), is a RecQ DNA helicase, and mutations result in genetic instability with spontaneous chromosomal abnor- malities and increased sensitivity to radio- and/or chemotherapeutic agents. Treatments therefore have to be appropriately tailored. Males are infertile because of a defect in meiosis. Werner syndrome Werner syndrome is recessive and is characterized by a scleroderma- like, multisystem premature ageing phenotype, which is also due to a RecQ helicase defect. It is associated with atherosclerosis and diabetes mellitus and short stature. The incidence is 1 in 50 000– 100 000. Affected individuals have an excess of neoplasms (espe- cially osteosarcoma, meningioma, and thyroid cancer). Mutation in the RECQL2 gene (8p12) leads to genetic instability. Rothmund–Thomson syndrome This is a hereditary dermatosis characterized by atrophy, poikiloderma (marbleized pigmentation),

and telangiectasia, and is frequently accompanied by juvenile cataract, saddle nose, con- genital bone defects, disturbances of hair growth, short stature, and hypogonadism. Classical features are absent radii and a rudimen- tary/absent thumb. Survival is fairly good and can be into the 40s. This is a recessive cancer predisposition syndrome, due a defect in a different helicase (gene RECQ4 at 8q24). There is a predisposition to malignancy, especially osteosarcomas and skin tumours.
Fanconi anaemia Fanconi anaemia is a collection of recessive diseases characterized by a complex variety of developmental abnormalities, progressive marrow failure, and predisposition to acute myeloid leukaemia (15 000 times that of the general population). Fanconi anaemia commonly presents in early to middle childhood with anaemia and bruising. Progressive pancytopenia and chromosome breakage, worsened by exposure to alkylating agents, is characteristic. Fanconi anaemia homozygotes may develop a wide variety of common can- cers occurring at an early age. Squamous cell carcinomas, especially of the head and neck, oesophagus, cervix, vulva, and anus, occur with increased frequency, as do liver adenomas. Life expectancy is poor, around 12 years, with most deaths resulting from marrow failure and cancer. Approximately one-fifth of childhood aplastic anaemia is associated with Fanconi anaemia and treatment is bone marrow transplantation. Treatment using radiation and chemo- therapy for the transplant has to be carefully given (reduced doses of conditioning are used as the cells are sensitive to DNA-damaging agents). The heterozygote frequency is estimated to be 1 in 300 to 600; the frequency is greater in Ashkenazi Jews. Fanconi anaemia homozygous cells form abnormal chromosomes when exposed to cross-linking agents such as mitomycin C, which is one of the diagnostic tests for the condition. Spontaneous chromosome ab- errations are seen in a variety of cell types. There are several com- plementation groups and two of them are due to genes which also predispose to breast cancer in heterozygotes (BRCA2 and BRIP1). The other groups do not appear to have an increased malignancy in heterozygotes.
Xeroderma pigmentosum This is a group of rare autosomal recessive disorders, with an in- cidence of 1 in 1 000 000. The classical feature is photosensitivity, which starts in childhood, and freckling and telangiectasia leading to progressive degenerative skin changes and early development of cancers of the skin (squamous, basal cell, and melanoma) and eye. Fifty per cent (50%) of these children have a skin cancer by age 14 years. About 20% have concurrent neurological abnormalities and some have impaired immune systems. There is also an increased risk of solid and haematological tumours. Benign neoplasms in- clude conjunctival papillomas, actinic keratoses, lid epitheliomas, keratoacanthomas, angiomas, and fibromas. Defects of several enzymes involved in excision repair of ultraviolet-induced pyr- imidine dimers are responsible for this syndrome. There are sev- eral complementation groups which differ in their action in this process (damage recognition, nuclease function, DNA polymerase function).

Identification and management of known or suspected cancer predisposition gene mutation carriers

Cancer genetic counselling This involves assessment of cancer risk, discussion of screening and management options, and the offer of genetic testing if appropriate.
Risk assessment This can be complex; it involves a risk estimate and this information has to be communicated to the patient in the manner most appro- priate to the individual concerned for optimum understanding and retention, but so they are not made inappropriately anxious about the associated risks. The first risk estimation is the chance that a familial cluster is due to genetic predisposition (the ‘prior probability’ of a genetic predis- position gene being present in a family). An extensive family history is important to determine this, often out to third-degree relatives. Confirmation of diagnoses is important for some sites (e.g. abdom- inal tumours) as these can be misreported in families in about 17% of cases. The risk estimation is based upon published data or

clin- ical experiences when published data are lacking, which unfortu- nately is often the case with rare genetic conditions. For example, for breast cancer clusters estimates can be made using data such as those shown in Fig. 5.3.1. There are also now computerized models for some common cancers which can aid prediction of the presence of a genetic mutation, such as the BOADICEA model for the chance of a BRCA1/2 mutation.

 The second risk estimation is the chance that the individual has inherited a particular gene based upon their cancer status (affected or unaffected), their position in the family tree, and their age. This is termed the ‘posterior probability’. The final calculation is the chance that cancer will develop, which is the posterior probability multiplied by the penetrance. The ex- pression of this risk can be delivered in several formats: the optimal format is unknown. Currently, risk estimates tend to be given as a percentage risk or a ‘1 in X’ value and followed up with a written summary, incorporating this risk estimate, to the individual at- tending the genetics consultation. There are data which suggest that individuals prefer not to have, or do not remember, numerical infor- mation, but are able to report the qualitative category of their risk (low, medium, high) with reasonable accuracy. Identification of an at-risk family A family at genetic risk of cancer must first be identified. This is usually via the general practitioner (family doctor) or a hospital oncology clinic; it is now becoming more common for family his- tory to be requested by cancer geneticists working as part of the multidisciplinary team coordinating the patient’s care. Because of the limited time available during most consultations, it is not ap- propriate to obtain a detailed family history from the patient. As a quick guideline, taking a history of all first-degree relatives only (parents, siblings, and children) and then asking if there are any other cancers in the family will detect 95% of familial syndromes. From this quick family history it should, however, be possible to make an assessment of whether the family history warrants fur- ther investigation. Referral guidelines have been developed; for example, in the United Kingdom, there are national guidelines for familial breast cancer (http://www.nice.org.uk). These guide- lines aim to delineate the management and referral pathway ac- cording to the Kenilworth model (http://www.macmillan.org.uk) whereby individuals whose risk does not exceed that of the general population are managed in the primary care setting, individuals at moderate risk are managed in secondary care, and individuals at high risk are managed in tertiary care in cancer genetics centres. In the cancer genetics clinic, after a full family history, initial clin- ical examination involves looking for any dysmorphic features and congenital anomalies. The skin should be carefully examined, as many cancer syndromes are associated with dermatological fea- tures, as noted earlier. Genetic testing is increasingly being offered as part of the cancer care pathway (the mainstreaming programmes; e.g. https:// mcgprogramme.com). The results of testing at diagnosis, which does not increase psychological distress, can be used to guide chemo- therapy and surgical management decisions (e.g. in breast cancer for the addition of platinum to the chemotherapy regimen and bilateral mastectomy for management of the primary lesion). Throughout the consultation, it is important to be sensitive to any issues relating to bereavement due to the premature death of close relatives, particularly a parent or child. Unresolved bereave- ment may make it difficult for people to accept their own risks and make decisions about their own management. Some individuals are particularly worried when they are approaching the age at which their relatives were diagnosed. Others erroneously assume that they are more likely to have inherited the cancer predisposition gene because they resemble their affected relative, either physic- ally or in temperament. Patients are sometimes unable to cope with their concerns, and referral for formal psychological counselling may be needed. Of particular concern are those

individuals who have prophylactic surgery because of excess anxiety but who, while being temporarily relieved, could return at a later date with further cancer-phobic symptoms. A psychological assessment and coun- selling should be part of the referral process before prophylactic mastectomy. Clinical management The subsequent management of an individual and their family will depend upon the final risk estimates regarding the inheritance of a cancer predisposition gene and the potential cancer risks. In general, management strategies fall into five categories: cancer screening, lifestyle changes, prevention strategies, cancer treatment consider- ations, and genetic testing. Cancer screening Not all of the screening schedules have been proven to reduce mor- tality from the relevant cancer, but these schedules represent a prag- matic approach to the management of individuals at increased risk. There is, however, some evidence that screening individuals with HNPCC by colonoscopy reduces mortality due to colorectal cancer, as any suspicious polyps observed on colonoscopy may be removed at an early stage. The guidelines promulgated by the United Kingdom National Institute for Health and Clinical Excellence (NICE) men- tioned earlier have made recommendations for mammographic and MRI screening in certain groups at risk of familial breast cancer. Prostate and ovarian cancer screening are contentious and are cur- rently subjects of research. Lifestyle changes Lifestyle changes may involve avoidance of known cancer-causing factors such as sunlight in Gorlin’s syndrome and X-ray exposure in the Li–Fraumeni syndrome. Other lifestyle changes are less well es- tablished in the prevention of cancer and are being assessed in trials. Prevention strategies Primary prevention strategies include prophylactic surgery and chemoprevention. The evidence in support of the efficacy of these measures is variable, mainly due to the rarity of the genetic muta- tions, making clinical trials difficult to perform. Established meas- ures include total colectomy in the familial adenomatous polyposis syndrome, total thyroidectomy in the MEN2 syndrome, and bilat- eral salpingo-oophorectomy in women with BRCA1/2 mutations. Limited retrospective data suggest that the risk of breast cancer is re- duced by 90% following prophylactic mastectomy, although there is still a small residual risk (about 1.5%) due to the inability to remove all breast epithelial tissues at mastectomy. The role of chemoprevention is much less certain. In meta-analyses tamoxifen reduces breast cancer risk by at least 33%, but it should be noted that the type of tumour prevented is hormone receptor- positive, and this has a better prognosis. The oral contraceptive pill reduces ovarian cancer risk by about one-third in those on the pill for 2 years. Recent data report a reduction in colon cancer risk in HNPCC families in individuals who have taken aspirin after 5 years.

5.3 The genetics of inherited cancers 469 Cancer treatment Data are emerging which report a difference in prognosis when cer- tain genetic changes are present in the germ line (e.g. ovarian cancer due to mutations in BRCA1/2 has a better prognosis and a higher response to platinum-based chemotherapeutic agents). Certain syndromes are associated with altered response to treatment (e.g. colonic tumours which have microsatellite instability are less re- sponsive to 5-fluorouracil, and recent data show dramatic responses to immunological agents). In VHL and PTEN mutation carriers, agents can be offered which target the hypoxia-inducible factor (HIF) and mammalian target of rapamycin (mTOR) pathways, re- spectively. Agents are now being developed which specifically target tumours with certain genetic defects (e.g. the poly-ADP ribose poly-merase (PARP) inhibitors), which enforce the cancer cell to use the homologous recombinant DNA repair pathway which is deficient in BRCA1/2-null cells, have resulted in promising early response rates in tumours in patients with germline BRCA1/2 mutations. Olaparib is now licensed for use in ovarian cancer in women with germline mutations in BRCA1/2 genes. Genetic testing Genetic testing is possible for most cancer predisposition genes and is performed on DNA from venous

blood or saliva after gen- etic counselling. Genetic testing may either be diagnostic (the detec- tion of a mutation in an individual affected by cancer) or predictive (the detection of a mutation in a clinically unaffected individual). Mutations in cancer predisposition genes often occur throughout the gene and the vast majority of mutations so far have only been observed in limited numbers of families, except in specific ethnic groups with known founder mutations such as the Icelandic and Ashkenazi populations with BRCA1/2 mutations. Hence, unless an individual is a member of such a group, the specific mutation for that family must first be identified. An affected family member is usually tested first because they are the family member most likely to have the cancer-predisposing mutation. Once a mutation is identified, it is important to check that the 'mutation' is likely to be cancer- causing and not a normal variant of the gene (polymorphism). When a pathogenic mutation is identified, predictive testing may be offered to unaffected family members for the identified mutation. Misleading results may occur if an unaffected individual has a genetic test in order to identify a mutation without first identifying it in an affected relative. A negative result (i.e. no mutation is identified in the cancer predisposition gene tested) may not be a true negative for several reasons: • The family history is caused by a gene other than the one being tested or may not be genetic at all. • The alteration may be regulatory, which means that it controls how the gene is expressed but the gene itself (and therefore the test which looks at the gene code) is normal. • The genetic test sensitivity is not 100% for the genetic coding mu- tations and may therefore have missed mutations. With the advent of next-generation sequencing the risk of this is very low as the sensitivity is about 98%. When the specific mutation has been identified in an affected indi- vidual, if it is not found in an unaffected relative, this is then a truly negative result. The personal and wider social implications of posi- tive and negative results are issues discussed during genetic counsel- ling sessions. A positive result could have psychological implications as well as widespread repercussions involving the rest of the family. A negative test result may have psychological consequences due to the recognized 'survivor guilt syndrome', which has been docu- mented in the setting of Huntington's disease. There is a moratorium on the use of some genetic information in the United Kingdom, as detailed on the Association of British Insurers' website http://www.abi.org.uk. The code was reiterated in Oct. 2018 and now has no renewal date. For genes predisposing to adult-onset cancers, testing of young children is not advised as the age of cancer onset permits the in- dividual to make their own decision to have genetic testing once they have reached adulthood, following full genetic counselling. Children are offered genetic testing when it may alter manage- ment, for example, in the MEN2A syndrome when thyroidectomy is offered before age 5, in retinoblastoma to avoid unnecessary eye examinations, or in FAP where regular colonoscopies or colectomy may be avoided. Recently genetic testing has been licensed for preimplantation genetic diagnosis for certain cases of hereditary cancer in some countries. The future The number of low penetrance, common variants discovered to be associated with common cancers is increasing, and this will result in genetic profiles being identified which can be used to stratify popu- lations into risk categories. This is being studied to identify groups at higher risk for targeted screening programmes. Cancer is a common disease and only a proportion of cases will be due to the inherit- ance of rarer higher risk mutations in specific genes that predispose to cancer. However, because cancer occurs with high frequency in the population, this represents a large number of individuals. Recent advances have led to the development of 'panel' genetic tests where more than one cancer predisposition gene is tested e.g. breast cancer predisposition panels; colon cancer predisposition panels etc. The developments of more rapid genome sequencing will enable cancer genetics to become part of cancer care, as more targeted treatments are developed for such individuals and targeted

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

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

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

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

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

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

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

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

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

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

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

has not proceeded. Peptide-based vaccines have also been evaluated in fairly large trials in kidney cancer (RCC). One noteworthy approach focused on targeting a series multiple antigens in the context of a less complex adjuvant. To select relevant antigens, kidney tumours from a series of 32 patients expressing the common class I MHC molecule HLA- A2 were isolated, and the cell surface peptides bound to class I MHC molecules were eluted and analysed. This work led to the identification of a set of nine tumour-associated peptides (TUMAPs), which were tested in a vaccine using granulocyte-macrophage colony stimulating factor (GM-CSF) as an adjuvant. GM-CSF is a strong inducer of DC migration, but is probably not as potent as the PAMPs discussed earlier in terms of DC activation. In two early phase trials, the vaccine IMA901 was shown to be safe, well-tolerated, and to in- duce T-cell responses when the vaccine was given with a low dose of the chemotherapy agent cyclophosphamide to deplete regulatory T cells (Treg). Notably though, no objective responses were reported in the early trials. A randomized phase III trial was initiated in which IMA901 was added to first-line tyrosine kinase inhibition (sunitinib) in patients with metastatic RCC. Enrolment was completed in 2012 (330 patients total), and results were recently reported in abstract form. Like the MAGRIT trial in NSCLC, this randomized phase III trial was negative, with no benefit on overall or progression-free survival. Peptide-based vaccines have also been evaluated in large, ran- domized controlled trials in patients with glioblastoma multiforme. In this disease, approximately 30% of patients express a common mutant form of the epithelial growth factor receptor known as epi- dermal growth factor receptor (EGFR) variant III (EGFRvIII). In glioblastoma multiforme, data suggest that mutated EGFR may drive malignant cell proliferation, differentiation, and survival, so EGFRvIII represents both a tumour-specific antigen as well as a po- tential driver mutation. To target EGFRvIII a peptide-based vaccine was developed, the vaccine couples a mutant EGFRvIII peptide to the non-self-protein keyhole limpet hemocyanin which serves as an adjuvant. A phase III randomized double-blind trial comparing vac- cination to placebo in 745 newly diagnosed EGFRvIII-positive glio- blastoma multiforme patients was recently discontinued based on the recommendation by the Data Safety and Monitoring Committee that the trial was unlikely to meet its overall survival (primary) end point. Taken together, these phase III experiences (and several others) suggest that peptide-based vaccines are unlikely to have a significant impact in cancer as a monotherapy. It is worth noting, however, that multiple preclinical models suggest that cancer vaccines may prove synergistic with immune checkpoint blockade, and several early phase trials are currently exploring such combinations. Cell-based vaccines Perhaps an ideal source of antigens for cancer vaccination would come from a patient’s individual tumour cells, such vaccines could theoretically include multiple mutation-associated neoantigens, as well as a panoply of overexpressed shared antigens. However, harvesting viable tumour cells and converting them to an individu- alized vaccine has proved challenging, so several approaches have focused on using immortal allogeneic tumour cells engineered to increase immunogenicity. One noteworthy approach transduced tumour cells with GM-CSF, which (as already described) attracts dendritic cells to the vaccine site; this approach is known as GVAX. Phase III trials of GVAX were launched in prostate cancer, and as was the case for the vaccines listed here were negative in terms of their primary overall survival end points. A similar approach was developed for pancreatic cancer; here cells were modified based on the observation that in humans, a large fraction of pre-existing immunity is directed against a sugar moiety (α-gal) present in all mammalian species other than humans. So, tumour cells were modified to express α-gal, when injected intradermally the alpha-gal expressing vaccine induces a hyperacute immune response, me- diated by pre-existing antibodies and characterized by the rapid, immunogenic destruction of the vaccine cells. Although early trials were promising, a recently reported phase III trial in 700 patients with fully resected

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

5.4 Cancer immunity and immunotherapy 477 if positive, could prove potentially pivotal. In this trial, men with later stage prostate cancer (metastatic, castration-resistant dis- ease) were randomized to either ProstVac VF alone, the combin- ation of ProstVac VF and GM-CSF, or to placebo. The trial's primary end point is overall survival, and although accrual has been com-pleted, survival data were not available at the time this chapter was completed. Chimeric antigen receptor (CAR) T cells Introduction and mechanism of action Monoclonal antibodies are high-affinity molecules which can be reliably generated against cell surface proteins. Indeed, tumour- directed monoclonal antibodies have shown efficacy in several tu- mour types including anti-Her-2 in breast cancer (trastuzumab) and anti-CD20 (rituximab) in B-cell malignancies. These agents func- tion through several mechanisms, including antibody-dependent cellular toxicity and by blocking pro-survival and pro-proliferation signals. Despite their high affinity and exquisite specificity, mono-clonal antibodies lack intrinsic lytic function, instead relying on the host immune system for this facet of their function. The T-cell re- ceptor, by contrast, is of fairly low (micromolar) affinity, but exists in nature on the cell surface of T cells, incredibly efficient engines of cellular destruction which employ multiple nonoverlapping mechanisms to drive facilitate lysis of their specific targets. A clever approach to cancer immunotherapy involves the fusion of these two biological mechanisms via the generation of chimeric molecules which fuse the portions of an antibody involved in specific recogni- tion of a target antigen (the variable heavy (VH) and variable light (VH) chains) to the transmembrane and intracellular domains of the T-cell receptor (zeta) chain. This short antigen recognition re- gion (a single-chain fragment variable or ScFv) provides for a high specificity of recognition, while the remainder of the construct func- tions to fully activate the associated T cells, driving proliferation, effector function, and persistence. Together, the fusion of the ScFv with the T-cell apparatus is known as a chimeric antigen receptor (CAR), and T cells modified to express CAR are known as CAR T cells (see Fig. 5.4.3). CAR T-cell technology has

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

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

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

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

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

specificity. Cross-linking of CD3 leads to T-cell activation and acquisition of effector (killing) function. The short distance between the directing antibody and the anti-CD3 region serves to mimic a natural T-cell synapse and may facilitate target cell lysis. Shorter constructs are hampered by a short in vivo half- life: many additional constructs have been engineered in an attempt to overcome that limitation. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Clinical Oncology (Batlevi CL, et al., 2015, Novel immunotherapies in lymphoid malignancies. Nat Rev Clin Oncol, 13(1), 25–40), copyright © 2015.

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

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

 of chemotherapy alone. If patients had stable or responding dis- ease, they were permitted to continue on maintenance ipilimumab (once every 12 weeks) until disease progression. The primary end point of this randomized phase II study was progression-free survival. A total of 204 patients were enrolled, and the study met its primary end point of improved progression-free survival for the phased versus the control arm. In addition, overall survival dif- fered between arms, with a median overall survival of 12.2 months, 9.7 months, and 8.3 months in the phased, concurrent, and control arms, respectively, but these were not significantly different. In a preplanned subset analysis, patients with squamous cell histology showed a significantly improved progression-free survival as well as overall survival with the phased schedule versus control (chemo- therapy alone), although the patient numbers were relatively small. Taken together, these phase II data suggested that a phased treat- ment schedule could potentially provide clinical benefit in patients with lung cancer compared to chemotherapy alone. A phase III trial testing that hypothesis was initiated in 2011; it compares the phased schedule of ipilimumab with paclitaxel and carboplatin versus paclitaxel and carboplatin alone in patients with stage IV squamous- cell carcinoma. The primary end point of the trial is overall survival and, although enrolment has been completed, final clinical data have not yet been reported. Early phase I studies suggested that CTLA-4 blockade might have some activity in

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

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

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

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

 on data from a single-armed phase II study as well as preliminary data comparing nivolumab versus docetaxel in second-line disease. The randomized second-line study was stopped early because an in- terim analysis determined that it met its

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

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

 a higher (20%) rate of this adverse event, which is by contrast quite common with CTLA-4 blockade. Perhaps the most worrisome ad- verse event mediated by PD-1 blockade is inflammation of the lung (pneumonitis) which was responsible for three deaths in the phase Ib study of nivolumab. Subsequent studies showed that this was a true side effect, but with a real rate that is likely less than 3–5%. Still, distinguishing worrisome pneumonitis from disease progression can be challenging—particularly in patients with lung cancer or with other cancers metastatic to the lungs. Management of immune- related toxicities is facilitated by several widely available treatment algorithms which mostly involve a stepwise progression from fre- quent monitoring, followed by increasing doses of corticosteroids and hospitalization if the patient fails to respond adequately after the adverse event. Immune agonist approaches Introduction In addition to immune checkpoint molecules, whose engagement downregulates T-cell function, there are several molecules that pro- vide an activating signal to T cells. So, the ultimate outcome of a T cell's interaction with a dendritic cell involves an integration of both the positive and negative signals present during that interaction. While still in early stages of development, agonist antibodies that activate immune cells have tremendous potential in cancer immun- ology, and are briefly discussed here to provide a context for future developments. OX40 on T cells OX40 (CD134) is a member of the tumour necrosis factor (TNF) receptor superfamily, and is a cell surface molecule expressed after either CD4 or CD8 T cells recognize

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

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

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

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

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

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ESSENTIALS Patients with cancer may present after detection of an abnormality in a screening programme, with vague symptoms, or with advanced disease. The commonest symptoms are local, directly related to the anatomical position and size of the cancer. Systemic features are classically those of anorexia and weight loss, but there can be many other manifestations including fever, hypercalcaemia, and a range of paraneoplastic syndromes. Early diagnosis of cancer is rightly seen as an important route to improving the survival of patients. Imaging is the basis for all solid tumour diagnosis and staging, and biopsy remains the gold standard diagnostic tool. Molecular pathology has revolutionized our under- standing of the biology of cancer, enabled more accurate prognosis and prediction of response to therapy, and led the way in relation to the development of targeted therapies. Cancer comprises many different conditions with very different requirements for the basic elements of a population-based cancer care service. The biggest change in the delivery of cancer manage- ment in the last 20 years has been the recognition that cancer man- agement is a multiprofessional and multidisciplinary effort. There is a hierarchy of aims for cancer treatment which stretch from cure to palliation. Four key measures to reduce the burden of cancer on the patient, their family, and on the healthcare system are: (1) early diag- nosis to maximize the chances of cure and minimize the morbidity of treatment; (2) access to best treatment irrespective of chronological age or home address of the patient; (3) increased physical activity; and (4) a ‘recovery package’ of care and support. Introduction Cancer is the commonest cause of death in the United Kingdom and by 2030 it is estimated that half the population will have had a diagnosis of cancer. The high prevalence of the disease explains why government and health systems pay so much attention to pre- vention, early diagnosis, treatment, and survivorship programmes. The complexity of cancer provision is not due to the numbers of patients who have (or potentially will have) cancer, but more to the simple fact that cancer is not a single disease: it is made up of many different conditions with, in some cases, very different requirements for the basic elements of a population-based cancer care service. There is also the added complication that cancer often presents to doctors who only rarely encounter malignant disease. This problem paradoxically applies to primary care physicians, who see huge numbers of patients, only a small percentage of whom have cancer. The issue of a lack of familiarity with the diagnosis of a particular cancer and its management in primary care is further confounded by the diversity of malignant conditions.

Presentation of cancer The diagnosis of cancer is arrived at in a variety of clinical scenarios. For example, patients may present after detection of an abnormality in a screening programme, or they may present with vague symp- toms to their primary care physician. A particular concern in the United Kingdom is that a high proportion of patients present in emergency departments (about 25%), and these patients often have advanced disease. Halfway along the continuum of screened and ad- vanced cases due to neglect of early symptoms, is a group of patients who have an incidental diagnosis. The incidental diagnosis of cancer is becoming increasingly fre- quent as the use of diagnostics such as CT and ultrasound is becoming routine in primary care and other specialties. Many cancers are now typically diagnosed as an incidental finding (e.g. kidney cancer). Although such diagnoses are to be welcomed as they often represent early stage and thus curable disease, they present the challenge of what might be appropriate treatment in the context of the concomitant dis- ease that was being investigated. For instance, an incidental small renal mass in the context of a patient with an aortic aneurism and cardiovascular comorbidities is a different therapeutic challenge to the presentation of a small renal mass in a young fit patient that has been diagnosed incidentally as a result of a CT being performed following a sporting injury. There is also the issue of whether incidental cancers have the same biology as those discovered as the result of the inves- tigation of a symptom. The treatment plan for an incidentally diag- nosed cancer requires knowledge of the natural history of the disease and the significance of the early stage of the cancer in question. 5.5 Clinical features and management Tim Eisen and Martin Gore† † It is with great regret that we report that Martin Gore died on 10 January, 2019.

 Localized symptoms of cancer The commonest symptoms of cancer often relate to its position and resulting local symptoms, with the consequences of a mass being directly related to its anatomical position and size. Local symptoms require a careful evaluation of their chronicity and associated symp- toms and signs, such as rectal bleeding, weight loss, or the finding of an abdominal mass in the case of intra-abdominal malignancy. Pain Pain is classically due to compression by a mass on surrounding structures and can either result in a dull ache over the site or se- vere pain on movement. Some cancer pains are very difficult to deal with, such as the neuropathic pain due to nerve involvement, while others such as pain due to the involvement of a vertebra can often be palliated by a single fraction of radiotherapy. Long bone involve- ment must be diagnosed promptly to prevent pathological fracture, which is a serious complication because the morbidity of treatment and outcomes are significantly worse compared to intervention for a lytic metastasis with an intact cortex. Referred pain can cause confusion and requires the physician to be very aware of the diagnostic requirements when ruling out the presence of a referred pain rather than pain due to a local cause. Classic examples include shoulder tip pain from disease in the right upper quadrant of the abdomen, and pain and paraesthesia in limbs due to compression of nerve routes. Intra-abdominal discomfort is perhaps the most difficult pain diagnostically, particularly in the context of primary care. Many pa- tients have symptoms of discomfort and bloating, which can be a classic symptom of intra-abdominal malignancies such as ovarian cancer, but equally is common among the population because of the prevalence of conditions such as irritable bowel syndrome and diverticulitis. Headache and other symptoms Headache is a common presentation of many benign conditions and primary brain cancer is a rare condition: nevertheless, it is a very common presenting feature of tumour within the cranium and the ability to recognize which patients require a brain scan is important. Mild symptoms may be sinister, and filtering of symptoms is as- sisted by considering their context. For example, a hoarse voice or chronic worsening cough is particularly

concerning in a smoker. Systemic features of cancer Systemic features of cancer are classically those of anorexia and weight loss, particularly in the case of upper gastrointestinal (GI) malignancies such as stomach and pancreatic cancer. There are, however, many other systemic features of cancer, some of which need to be carefully evaluated (e.g. fever and night sweats, or the concomitant symptoms associated with hypercalcaemia that can be difficult to recognize clinically unless the patient already has a malignant diagnosis). Hypercalcaemia has more than one genesis because it can occur as the result of both direct tumour destruction of bone, or result from ectopic secretion of parathyroid hormone or (perhaps more commonly) parathyroid hormone-related protein. The presence of fatigue is common in cancer patients and can re- late to anaemia, but not invariably so, and measuring the haemo- globin level is not a screening test for malignancy. There is a very wide variety of so-called paraneoplastic syndromes associated with cancer. These are usually associated with advanced disease and can be very challenging diagnostically outside of the better-known syndromes such as dermatomyositis and clubbing. Endocrine, neurological, dermatological,
and haematological syndromes Endocrine disorders such as hypercalcaemia, hypoglycaemia, in- appropriate secretion of anti-diuretic hormone and Cushing's syn- drome are all associated with paraneoplasia. A variety of neurological presentations including cerebellar de- generation and peripheral neuropathy can also be associated with malignancy. Many of the paraneoplastic neurological syndromes are uncommon (e.g. dementia, transverse myelitis, limbic encephal- itis, optic neuritis, amyotrophic lateral sclerosis, and the myasthenic syndrome known as Eaton–Lambert). There is often diagnostic delay, and such paraneoplastic phenomena often require very spe- cialist knowledge and management. An interesting feature of cere- bellar degeneration in some conditions such as ovarian cancer is that its onset can be associated with response to treatment rather than as an initial diagnostic presentation. There are many dermatological syndromes associated with malig- nancy, such as acanthosis nigricans, the syndrome of Leser–Trélat (multiple seborrheic keratosis), erythema migrans, exfoliative dermatitis, panniculitis, porphyria cutanea tarda, and ichthyosis. Haematological manifestations other than anaemia include autoimmune haemolytic anaemia, microangiopathic haemolytic anaemia, thrombocytosis, granulocytosis, and erythrocytosis. However, perhaps the most important haematological paraneoplastic syndrome, because of its frequency, is that of thrombosis, which can be due to a combination of a hypercoagulative state and compression of veins, particularly in the case of intra-abdominal cancers. Diagnosing and staging cancer Early diagnosis There is much attention paid to the early diagnosis of cancer as this is rightly seen as an important route to improving the survival of patients. The reason there is such attention paid to early diagnosis is that there is always a chance that early stage disease (of all tumour types) can be cured, whereas with advanced solid tumours cure rates are much lower. Early diagnosis is not simply an issue of diagnostic capacity re-lating to imaging and endoscopy, but rather one of public and healthcare worker education. Early symptoms of cancer need to be better understood by the population at large, and there is much dis- cussion as to how this might be best achieved. Many programmes tend to concentrate on one particular tumour type, such as cam- paigns relating to the early symptoms of lung cancer or colorectal cancer, but there also needs to be more attention paid to more gen- eral messages about health. Early diagnosis campaigns must be broad, including not simply high-risk patients, but also programmes in schools and places of education.

 Patients living in deprived areas and who have poor socio-economic circumstance are particularly vulnerable to lower survival rates from cancer, and

although there are many reasons for this, early diagnosis is one of the contributing factors, including poor health education and less access to healthcare and diagnostic provision. There is a tension between the cost-effectiveness of screening pro- grammes and early diagnosis. The essential element, however, is that there should be ease of access to diagnostics in the primary care set- ting, so that the primary care physician is not faced with prioritiza- tion decisions relating to individual patients but can easily access those tests that they feel are required for a particular patient at a specific time. The biggest challenges relating to early diagnosis are the avail- ability of CT scanning and, perhaps most challengingly of all, en- doscopy. There are several ways in which CT and other imaging procedures can be expanded and made more available through technological advances, including remote access reporting, ex- tended working hours, and the provision through mobile units of scanning in work places and sites near to patients' homes. However, the endoscopy challenge is much greater because the need is for very specific endoscopy suites and many highly skilled individ- uals to perform the procedure, both of which are space, time, and labour intensive. Investigation Imaging Imaging is the basis for all solid tumour diagnosis and staging. The ability to recognize the precise location of a local tumour and any metastases is central to prognosis as well as decisions relating to treatment. CT remains the most used and useful imaging technique in solid tumour oncology, and allows a fast and reliable way of obtaining an overall view of a patient's cancer. The use of MRI has increased rapidly over the last 10 years, and as well as giving better definition (mainly for the treatment of individual disease sites, particularly in the primary setting), has given particular insights into the patterns of spread of disease. The use of diffusion weighted imaging has be- come a very useful tool in assessing peritoneal and bowel serosal involvement. Continuing developments in central nervous system (CNS) MRI have proven extremely useful when defining specific treatments for primary or secondary CNS tumours. Positron emission tomography (PET) scanning has opened up a new field of molecular imaging, which is allowing real time and in vivo characterization of tumours, particularly in relation to their response to novel therapeutics such as targeted agents. The ability of PET scanning to help define which masses contain active tu- mour and which do not has had very important beneficial conse- quences, particularly for malignancies such as germ cell tumours and lymphomas, where this information is now routinely required to plan ongoing management. It is in the area of molecular imaging that there is the most re- cent excitement. For instance, with novel immunotherapeutic ap- proaches it is becoming feasible by the use of specific probes to define T-cell populations that are relevant to tumour response, ra- ther than having to biopsy patients. This work is still in its infancy and is likely to play an important role in the future design of novel targeted immunotherapies. Biopsy Biopsy remains the gold standard diagnostic tool, and advances in image-guided biopsy (both in terms of the imaging hardware and bi- opsy instruments) have meant that diagnosis has routinely become an outpatient procedure with minimal discomfort to patients. Biopsy has also taken centre stage in terms of research due to the recognition that the knowledge of serial changes within the tumour may have particular prognostic and/or predictive importance. There is thus a drive for ever less morbid biopsy techniques to allow for individual patients to have sequential samples taken. The knowledge of intrapatient changes, particularly related to a therapy, is of huge research value. So- called liquid biopsies are also starting to come out of the research arena and enter into the realm of standard of care (see Chapter 3.10 for further discussion). We are on the cusp of a con- siderable change of direction in relation to biopsy material as we de- velop an increasing understanding of the significance of circulating tumour cells, DNA, and ribonucleic acid (RNA), and how this relates to the genomic heterogeneity known to exist within a single cancer in an individual person. The significance of the results obtained from liquid biopsy is variable. Our knowledge is more advanced

in certain tumour types; for instance, work in prostate cancer has led the way with data suggesting that circulating tumour cells might be used to follow patients and their response to therapy. Serum tumour markers Serum tumour markers play an important role in diagnosis, assess- ment of treatment, and follow-up of patients after initial therapy. There are few examples of tumour-specificity relating to tumour markers, but there is no doubt that their measurement can play an important role in guiding diagnosis and helping evaluate the efficacy of subsequent treatment. Germ cell markers are the paradigm for the use of serum tumour markers, both in respect of specificity and following patients during and after treatment. However, even when tumour markers are not absolutely specific, the pattern of tumour markers can be useful (i.e. a comparison of the different levels of more than one marker in an individual patient at a single point in time). This is particularly true of intra-abdominal malignancies, where the relative rise of CA125, CA19.9, and carcinoembryonic antigen (CEA) can guide diagnosis depending on the values of the different tumour markers in relation to one another. The tumour markers associated with solid tumours, however, have some important pitfalls, particularly in relation to follow-up. If patients are followed-up with tumour markers that often rise before there is any evidence of relapse on imaging, this can cause the pa- tient enormous anxiety. This is particularly problematic when there is no evidence that the early treatment of relapse is of benefit to the patient. The use of tumour markers in follow-up for some cancers is therefore controversial, and it will remain so until new treatments are available that will benefit survival if instituted early at the first sign of relapse. There is little doubt that a serum tumour marker that appears very early in the disease and is specific would be an ideal tool for improving the mortality from cancer in a population, but it does not appear that such a simple solution is likely to exist in terms of a specific protein. However, perhaps the development circulating DNA and RNA technology may one day produce such tests.

 Molecular characterization of tumours Molecular pathology has revolutionized our understanding of the biology of cancer, enabled more accurate prognosis and prediction of response to therapy, and led the way in relation to the develop- ment of targeted therapies. The earliest example of molecular pathology impacting thera- peutics is in breast cancer, with recognition of the relationship be- tween the expression of oestrogen and progesterone receptors and response to hormone therapy. Breast cancer was also the first solid malignant disease to use molecular targeting, with the identification of HER2 and the development of Herceptin and other anti-HER2 strategies. In solid tumour oncology the ability to define molecular drivers of disease, such as mutations in c-kit in GIST, EGF receptor in lung cancer, and BRAF in melanoma, have had an enormous impact on the therapeutic landscape and outcome for many patients. Classic examples of the power of molecular pathology include the predictive value of NRAS mutations and the use of chemotherapy in colorectal cancer. In haemato-oncology, the use of monoclonal antibodies such as rituximab and imatinib in lymphoma and chronic myeloid leu-kaemia, respectively. This ever-expanding molecular knowledge and precision is not, however, without its difficulties. Definition of ever smaller patient population groups leads to problems in terms of evidence for the licensing of products, and equally (or perhaps even more import- antly) how those who pay for healthcare approach the cost effective- ness calculations of different targeted agents for cancer. Molecular pathology is moving us to a position where almost every patient within a tumour type may become a ‘molecular minority’ and that group may simply not be large enough for the traditional calcula- tions of treatment ‘worth’ to be made by the payers of healthcare. Thus, while molecular pathology has brought untold benefits to pa- tients, it is challenging our traditional ways of categorizing cancers, making therapeutic decisions and defining

cost effectiveness. Staging cancer All patients diagnosed with cancer require ‘staging’. Staging is a con- vention whose definition and methodology of process is tumour-type dependent. For instance, in some malignancies staging is defined at surgery, whereas for others stage is defined by imaging and other tests. Most cancers are now staged using the universal T (tumour), N (nodes), M (metastases) system, although some tumour types are still staged according to their own classification frequently denoted as Stage 1, 2, 3, and 4, with subcategories within each stage (e.g. Stage 1a, b, or c, and so on). Staging at surgery can also be subdivided into those patients where surgical stage is defined on macroscopic appearances at surgery, although more frequently the results of the pathology are also used to inform the final stage, with the prefix ‘p’ being used in front of the tumour–nodes–metastasis (TNM) stage to indicate that the pathology has been used to define the final stage. Staging has two purposes. The first is prognostication. Most def- initions of each separate stage within a system are based on retro- spective series where the prognosis is correlated with the criteria that define a particular stage +/- substage. The second and more im- portant use for staging is to guide treatment, which is why accurate staging for each patient is paramount and an absolute requirement before therapy is instituted. Management Planning treatment Multidisciplinary teams The biggest change in the delivery of cancer management in the last 20 years has been the recognition that cancer management is a multiprofessional and multidisciplinary effort. Patients need to be put at the heart of multidisciplinary teams, each participant bringing to bear their own knowledge, expertise, and experience. There is no longer any place for the sole specialist to define how an individual patient is managed. Management and treatment pathways need to be predefined for different situations, and each patient’s case should be discussed in a multidisciplinary setting in order to set that patient’s needs in the context of known evidence-based intervention. The multidisciplinary team for solid tumours typically consists of oncologists, surgeons, pathologists, radiologists, and specialist nurses, and in many incidences other healthcare professionals such as dieticians and speech/language therapists. All are specialists in the particular tumour type(s) related to that team. The United Kingdom has probably got the most developed and systematic multidisciplinary healthcare framework in the world, such that it is extremely difficult for any cancer patient in the United Kingdom not to be discussed at such a meeting and their diagnosis and treatment plan recorded prospectively. It is therefore some- thing of a paradox that survival rates remain lower in the United Kingdom than many other parts of Europe, and this does call into question the relationship between the apparent benefits of multi- disciplinary working and the outcome for individual patients. Criticisms of multidisciplinary working, particularly in the United Kingdom, do not relate to the existence of such arrangements, but rather to the detail of how these arrangements are put into practice. Multidisciplinary meetings are lengthy, and there have been sugges- tions that those patients whose management is not controversial or in question need not be discussed in detail, so that colleagues can discuss more complex and difficult cases at greater length. There are also other benefits of multidisciplinary working and meetings, such as the development of a collegiate and cohesive team, particularly as it is recognized that functional relationships between colleagues leads to better outcomes for patients. The aims of treatment There is a hierarchy of aims for cancer treatment which stretch from cure to palliation. There has been a move to try and prospectively define for each patient the precise aim of treatment (i.e. cure, life prolongation, palliation), but these divisions for some solid tumours can be blurred and confusing. For instance, patients with metastatic disease can be cured, and depending on the disease in question the percentage of patients with curable cancer varies. Thus, in a group of patients it may be known that 20% will be cured, but it is rarely possible to prospectively identify which individual patients fall into this category. The patient, however, may

have their life prolonged or their symptoms palliated. The treatment for that patient is the same even though there are three separate possible outcomes for them, none of which can be defined before treatment is instituted. The def- inition of the aims of a treatment thus need to be put into the context of probability, based on the knowledge of the pathology, molecular

5.5 Clinical features and management 491 definition, extent of disease, clinical prognostic factors of the pa- tient, and of course most importantly, the patient's wishes. The term 'curative intent' is very often used, but this term might perhaps best be replaced by a more descriptive one such as 'cura- tive potential' as we develop more therapies that have the potential of prolonging life, but we do not yet know whether a truly cured state exists. The main issue, however, does not relate to semantic definitions but to careful explanations to patients of the different probabilities of various outcomes and, of course, the toxicity of treatment. The use of best supportive care strategies is paramount in those situ- ations where it is clear that life cannot be prolonged and cure is out of the question. There also needs to be more understanding that the knowledge and techniques used in best supportive care situations should also come into play at earlier stages of a patient's treatment pathway. The early involvement of experts in palliative medicine has been shown to make a considerable difference to patient out- come and experience, and there are no discernible downsides to involving colleagues in palliative medicine in the management of patients going through curative, let alone palliative, therapies. Modalities of treatment There is an understanding that all modalities of treatment can be brought into play when managing patients. The use of chemotherapy and radiation together has been well described for many years, and how surgery relates to both these modalities has also been the subject of many randomized trials in many tumour types. There has been a change of philosophy recently in relation to the development of ablative therapies for patients with so-called oligometastatic disease. The ablation therapies can be used in con- junction with systemic treatments, and although there are (as yet) no randomized data on this potential change in therapeutic strategy, it is undoubtedly going to be increasingly used for patients with relatively slowly progressing oligometastatic disease. This change of philosophy in relation to oncology strategies is related to both technology and biology, for instance, the increasing availability of stereotactic radiotherapy and its ease of use, our increasing know- ledge of the genomic heterogeneity of tumours, and in relation to immunotherapy the possibility that neo-antigens can be exposed and created by both radiotherapy and physical ablative therapies such as cryotherapy or radiofrequency ablation. This is an exciting area of therapeutic strategy, not only because there is the theor- etical possibility of keeping disease under control for longer, but it suggests that if techniques are nonmorbid and associated with little risk, then treating tumours when they are small before they can cause local or systemic symptoms becomes a logical approach to cancer management. Furthermore, if for instance two separate clones expand while others are kept under control by systemic therapy, then logic would dictate that these two clones should be ablated by a local therapy. Surgery Surgery undoubtedly cures more patients than any other modality of treatment and is the pivotal initial therapeutic intervention in solid tumour oncology. Other treatment modalities, such as radiotherapy and systemic therapy, are often built around surgery. For instance, treatment given after surgery—whether radiotherapy or systemic therapy—is described as adjuvant therapy, and sometimes both are used in this setting. Increasingly, systemic therapies—particularly chemotherapy—are being given before surgery in order to 'down stage' the tumour and allow surgery to take place more easily and with less morbidity. Treatment prior to surgery is known as neoadjuvant therapy and applies to either systemic therapies or to a combination of chemotherapy and radiotherapy (known as 'chemorads').

Surgical techniques have improved remarkably in the past three decades. Use of minimally invasive techniques, such as laparoscopic surgery and robotically assisted surgery, have minimized the mor- bidity of interventions. An important use of surgery is in relapsed disease, and particularly in relapsed disease that is solitary and/or slow-growing. Frequently a single metastasis is observed for 3–6 months and then removed if it remains solitary. This strategy is known as ‘a trial of time’. Molecular imaging techniques such as PET scanning are able to define single site disease with a good degree of accuracy and allow better patient selection for surgery at relapse. Common problems in cancer management Cerebral metastases The development of cerebral metastases is an extremely serious oc- currence in every tumour type; indeed, even in curable cancers such as leukaemia and lymphomas, particular attention is required to prevent CNS disease from occurring. Successful prophylaxis against CNS involvement was the reason why childhood leukaemia became such a curable condition. In solid tumour oncology the presence of solitary cerebral metas- tases is an indication for surgery. Surgery to such recurrences has a surprisingly low morbidity, depending on the site of the disease. Increasing use of stereotactic radiotherapy techniques have made a strategy of regular surveillance of the CNS an important area of ex- ploration because such techniques can destroy small tumours very effectively without morbidity. The argument remains as to whether or not such approaches are associated with increased survival. They are certainly associated with less morbidity than waiting for metas- tases to become large and/or multiple for treatment to be instituted. Many stereotactic techniques can be used to treat more than one me- tastasis at any one time, or serial metastases that occur sequentially. The development of carcinomatosis meningitis, however, remains an extremely difficult complication to treat and in many tumour types is a preterminal event. Effusions The development of effusions in the serosal cavities of the body, namely the pleural, peritoneal, and pericardial spaces, are common complications of cancer. Symptoms include shortness of breath, car- diac tamponade, and abdominal discomfort. Patients require fluid to be drained so that their symptoms are palliated. It is often sensible to examine fluid drained from an effusion for infection (bacterial and TB) as well as the presence of malignant cells. In newly presenting patients, cytology can be extremely useful in making at least a preliminary diagnosis, and if cell blocks are used, considerable information can be gathered because such specimens are suitable for immunohistological examination and may facilitate accurate diagnosis.

 For patients in whom active therapy is no longer having an ef- fect against the disease, the control of effusions in the three serosal cavities becomes paramount for successful palliation. Permanent pleural drains can be used in both the pleural and peritoneal cavities, and pericardial windows can be created surgically for uncontrollable pericardial effusions without excessive morbidity for the patient. An important aspect of the treatment of pleural effusions is that of pleurodesis. It is greatly preferable that this is attempted under direct vision through a video-assisted thoracic surgery (VATS) pro- cedure: using adhesive techniques through a simple chest drain may result in the patient developing loculated recurrent effusions, which are then very difficult to drain satisfactorily. Pleurodesis is only suc- cessful if the pleural cavity is drained to absolute dryness, and this can only be reliably accomplished under direct vision. Oncological emergencies The term oncological emergency requires precise definition because there are situations when intervention is required as an absolute im- mediate need, a true emergency, and others where treatment needs to be urgently instituted, within 24–48 hours. These latter clinical presentations are seen with uncontrolled, undiagnosed disease and include cachexia, anaemia, and those relating to the side effects of treatment such as nausea and vomiting. True

emergencies (i.e. those that require immediate and specific management) are in fact few and far between in oncology. The two main true oncological emergencies are neutropenic sepsis and cord compression. Superior venocaval obstruction is sometimes de- scribed as the third oncological emergency, but it rarely requires intervention within hours, although rapid intervention is desirable because patients can be extremely distressed and uncomfortable when this complication of cancer occurs. Neutropenic sepsis The survival of patients from neutropenic sepsis is directly related to the time between patient presentation and the institution of intra- venous antibiotics, and there has quite correctly been considerable emphasis placed on the importance of there being as short a time as possible between patients presenting with neutropenic sepsis and the institution of treatment. The best way of ensuring that patients do not die from neutropenic sepsis is to make sure that both pa- tients and healthcare workers, particularly in emergency rooms, are aware of this complication. The prophylactic use of granulocyte- colony stimulating factor has made an enormous difference to the incidence of neutropenic sepsis and in some healthcare economies it is used routinely, thus making this oncological emergency a rarity. Cord compression Management of patients with cord compression remains a challenge. Neurosurgical services may find it difficult to respond in a timely manner to requests for assessment because of the other demands that they face. Carefully designed networks and pathways for patients with suspected cord compression are paramount, and these should be en- gaged each time there is a suspicion of cord compression. There also needs to be a better understanding that the treatment of cord com- pression is best instituted when there is impending cord compres- sion, rather than waiting for overt physical signs of paralysis which inevitably results in a delay to recovery. Networked arrangements for the management of cord compression should include the efficient and timely availability of specific imaging, and assessment of patients by both radiation oncologists and neurological or spinal surgeons. Often the decision to engage radiotherapy or surgery is straightfor- ward, but in patients who have other sites of disease or perhaps more than one vertebral site of involvement, the decision between radio- therapy and surgery can be difficult and complex. Add to this the time-critical nature of the correct treatment being instituted, and the seamless networking of diagnosis and decision-making become one of the most important aspects of creating a functional cancer service. Other emergencies Rare emergencies such as hypophysitis, adrenal insufficiency, and insulin-dependent diabetes have emerged with the development of novel immunotherapies, and while the management of these is exactly the same as for their sporadic counterparts, their recognition may become an increasingly important issue as these agents become more frequently used. Cancer in particular patient groups The frail Cancer is often described as a disease of old age, with many solid tumours having their median age of onset during the sixth and later decades (see Chapter 5.1). This fact, together with the increasing in- cidence of cancer and age of the general population, means that more and more patients who are frail and have multiple comorbidities are going to be presenting to cancer services. Decisions about the appropriateness of both diagnosis and treat- ment are becoming ever more complicated, and while there is an im-portant drive to prevent discrimination in relation to the availability of cancer treatments based on age, there is also the tension between the ability to deliver effective cancer treatment in frail patients and the de- sirability to do so. We know that there has been discrimination against elderly patients in relation to the delivery of cancer treatments in that older patients are less likely to be offered a standard of care compar- able to that of younger patients, but to leap to the conclusion that all elderly patients should be offered all standard treatments is to deny the nuances of comorbidities as they relate to older people. Much needed attention is now being given to this area, and this is starting to help us make treatment decisions for older people and, in particular,

the challenge of those frail elderly patients who have reduced cognition. There are processes in place to protect such vulnerable adults from therapeutic discrimination. Nevertheless, there are still considerable social challenges relating to the care of the frail and elderly, as well as financial and physical ones. The area requires increased efforts in rela- tion to research and as healthcare systems are becoming increasingly stretched, it is encouraging that such research is becoming recognized by governments as something of an imperative. In pregnancy Cancer in pregnancy used to be thought of as an absolute indication to terminate the pregnancy if at all possible. However, in more recent times there has been increasing evidence that systemic therapies and surgery can be safely used in cancer patients who are also pregnant. Typically, surgery is safe in the early stages of pregnancy, while sys- temic therapies are safe after the first and early second trimester. There are some typical cancers that occur during pregnancy based on age, and these include the lymphomas, leukaemia, melanoma,

5.5 Clinical features and management 493 and germ cell tumours. The important strategic thinking for such patients is to design the therapeutic approach as if the patient were not pregnant, and then see if it is applicable to that particular stage of pregnancy. It is becoming less common to recommend termination to pregnant women with a concomitant cancer, although occasion- ally this is necessary. See Chapter 14.18 for further discussion. Hereditary cancer There is increasingly knowledge of the familial nature of some can- cers and an increasing knowledge of how patients are managed in this situation. In particular, knowledge of potential second or third malignancies associated with a genetic mutation in a cancer patient is becoming relevant as more primary tumours are becoming poten- tially curable. A classic example of this are BRCA mutations associ- ated with breast, ovarian, and prostate cancer. Where multiple cancers may occur with a hereditable mutation, screening may become complex, for instance, in patients with Lynch syndrome where very specific expertise is required to advise patients on how they should be managed following treatment of their pre- senting tumour. Cancer genetics goes wider than the individual patient and in- cludes the family. Family dynamics can be severely altered by the knowledge that a family member carries a potential hereditable mu- tation, and considerable care needs to be taken to treat each indi- vidual family member as such, an individual who requires separate and specific advice as it relates to them. It is not uncommon when a hereditable mutation is discovered in a family that the family attempt to take a ‘unified’ family approach, but this can be very damaging and the confidential provision of advice for individual family mem- bers is paramount. In conditions such as breast and ovarian cancer, where knowledge of a hereditable genetic mutation may impact the patient’s treatment (e.g. poly ADP ribose polymerase (PARP) inhibitors and BRCA- mutated ovarian cancer), there can be unforeseen consequences of wishing to detect such mutations in the patient who already has cancer. For instance, a daughter may then discover that she could potentially have inherited a high risk of breast or ovarian cancer and may be severely disturbed by such knowledge. Similarly, there may be an assumption from their mother that they would wish to know this so that prophylactic measures can be taken, but this can imme- diately bring mother and daughter into conflict. Experience, how- ever, suggests that these conflicts tend to be few and far between, and programmes that routinely screen patients for BRCA mutations have not reported detriment in terms of the psychodynamics within families. Information and support for patients and carers At diagnosis Many patients will have definite expectations of what a cancer diag- nosis will mean in terms of treatment, survival, and long-term ef- fects. The accuracy of these expectations will depend on many factors, such as past experiences with friends or family members in a similar situation, information from news or other media. It is extremely

important to communicate with cancer patients, their family, and friends, in an honest, open, and sensitive way. Important elements of successful communication include: • The patient will very often feel extremely vulnerable, and the sup- port of a family member or friend can be invaluable. • The meeting should take place in a quiet environment without interruption. • The doctor, clinical nurse specialist, and other staff involved should all be familiar with the patient's case and the manage- ment plan. • Providing 'chunks' of information rather than a single lengthy nar- rative. Similarly, it may be helpful to go over information on sev- eral occasions. • Written information sheets are an invaluable aide-mémoire and should complement rather than replace personal communication. High-quality information sheets are available on a very wide range of subjects from major cancer charities, with smaller specialist or local charities providing tailored support for the precise condition or locality. • The clinical team should provide a clear description of the patient's treatment options, including both standard of care and research options. Noting issues discussed earlier in this chapter, this de- scription should include the purpose of treatment—whether it is to palliate symptoms, prolong life, or to provide a cure. Unless starting treatment is an emergency, it is considered best practice to ask the patient to consider these options for a period of a few days. Further questions may arise and it is helpful to ask the patient to write them down so that the clinical team can answer them when the patient returns to clinic. • Patients should be fully involved in making decisions. Sensitivity and common sense are required in balancing an unbiased discus- sion of options with the patient's need to make an important deci- sion in an unfamiliar setting. It is entirely reasonable and indeed desirable for the clinical team to provide clear guidance. At different stages of the cancer journey Macmillan Cancer Support worked with the National Cancer Intelligence Network to provide a detailed survey of healthcare and outcomes for 85 000 patients in England and Wales between 2004 and 2011. This strongly suggested the need for four key measures to reduce the burden of cancer on the patient, their family, and on the health system: • Early diagnosis to maximize the chances of cure and minimize the morbidity of treatment; • Access to best treatment irrespective of chronological age or home address of the patient; • Increased physical activity; and • A 'recovery package' of care and support. Patients need different types and levels of support at different points during their cancer journey. Maher and McConnell estimated the number of people in six distinct periods of their cancer journey: (1) diagnosis and treatment; (2) rehabilitation; (3) early monitoring up to 5 years, and (4) up to 10 years; (5) progressive disease; and (6) end-of-life care (Fig. 5.5.1). They found that the greatest need for

 support was at times of transition. Most clinicians would recognize the need for support during the transition from healthy to diagnosis and treatment, and also from a period of monitoring to diagnosis of relapse and the need for further treatment. Fewer will be so aware of the need for support during transition from treatment to a period of rehabilitation. Understanding the individual needs of patients and their fam- ilies, particularly at these periods of transition, will enable health staff to provide necessary targeted support at the optimal time. The goal of empowering patients to deal with the medical, psycho- logical, social, and financial impact of cancer diagnosis and treat- ment is key. Diagnosis and treatment 41000 Diagnosis and treatment 40000 Rehabilitation 12000 Rehabilitation 28 000 Early monitoring: 2 ≥ 5 years 9000 Lung cancer (b) (c) Early monitoring: 2 ≥ 5 years 45 000 Colorectal cancer Early monitoring: 5 ≥ 10 years 6000 Early monitoring: 5 ≥ 10 years 51 000 Later monitoring 21000 Later monitoring 73000 Progressive illness ??? Progressive illness 24000 End-of-life care 35000 (28 000 year 1) End-of-life care 16000 (11 000 year 1) Diagnosis and treatment 48000

Rehabilitation 44000 (a) Early monitoring: 2 ≥ 5 years 100000 Breast cancer Early monitoring: 5 ≥ 10 years 122000 Later monitoring 226000 Progressive illness 24000 End-of-life care 12000 (2000 year 1) Fig. 5.5.1 Periods of the cancer journey. (a) Breast cancer care pathway: estimating the number of women in the United Kingdom, 2008*. (b) Colorectal cancer care pathway: estimating the number of people in the United Kingdom, 2008*. (c) Lung cancer care pathway: estimating the number of people in the United Kingdom, 2008*.

- For each cancer type, the size of the boxes reflects the approximate proportion of people in each phase (with double counting for people who are diagnosed and die in the same year—these numbers are indicated in brackets; i.e., 'XX 000 year 1'). Median survival for incurable disease was taken from Frontier Economics (2010) 'One to one support for cancer patients: a report prepared for Department of Health' and is 3 years for breast cancer and 2.5 years for colorectal cancer. Estimates for progressive illness for lung cancer have not been made. Estimates for later monitoring for lung cancer exclude 8000 men more than 20 years from diagnosis. The total for men in this group was thought to be an overestimate and is likely to be nearer 6000 than the modelled 14 000. Various sources including Office for National Statistics, Cancer Research UK, Frontier Economics, Information Services Division (ISD) Scotland, Northern Ireland Cancer Registry, Welsh Cancer Intelligence and Surveillance Unit, London School of Hygiene and Tropical Medicine. Reprinted by permission from Macmillan Publishers Ltd on behalf of Cancer Research UK: British Journal of Cancer (Maher J and McConnell H, 2011. New pathways of care for cancer survivors: adding the numbers. Br J Cancer, 105, S5–S10), copyright © 2011.

5.5 Clinical features and management 495 While it is normal for patients to feel very upset by the news of a cancer diagnosis, it is important to recognize when psychological support is required. This may not be obvious and a holistic assess- ment of care needs early in the patient's cancer journey often reveals unexpected problems and fears. Helping a patient to deal with these can significantly boost quality of life under difficult circumstances. Many cancer units offer specialized psychological support services. For patients of working age, the ability to return to work is an extremely important element of rehabilitation. Evidence from the United Kingdom, the United States, and the Netherlands suggests that the impact of cancer on employment status is transient for most people, with around 80% able to return to work eventually, the corollary of which is that around 20% of people do not return to work. Four key factors determine the ability of somebody to return to work after a cancer diagnosis: • The organ of origin of the cancer: patients with lung cancer or myeloma were much more likely not to return to work than pa- tients with breast or urogenital tumours; • The intensity of treatment: patients presenting with late stage dis- ease requiring intensive treatment, or with tumours requiring aggressive therapies, are much more likely to have difficulties re- turning to work; • Occupational status: cancer survivors with physically demanding jobs are 20% less likely to be in employment 2–3 years after diag- nosis than age-matched controls. In contrast, those with sedentary occupations are only 7% less likely to be in employment; • The role of others in facilitating a return to work: employment protection and anti-discrimination legislation has greatly im- proved the position of cancer survivors wishing to return to work since the 1970s. After cancer Survivorship In 1970, the average life expectancy after a diagnosis of cancer was one year. By the 1990s this had doubled. Due to a combination of earlier diagnosis and more effective anti-cancer treatments, the average life expectancy for a patient

diagnosed with cancer was close to 10 years in England and Wales in 2010–11. Cancer survival in several other advanced health economies is even more impressive. Due to these improvements in survival, the number of people living with or after cancer has increased enormously. For example, in the United Kingdom 1.2 million were living with or after a diagnosis of cancer in the early 1990s, rising to 2.5 million by 2015. The number is projected to rise to 4 million in 2030. In themselves, the survival figures are most encouraging, but these crude statistics conceal a much more complex and difficult picture for patients with cancer. The Macmillan Cancer Support/ National Cancer Intelligence Network survey confirmed several well-recognized differences in clinical outcome between four cancer types: breast, prostate, lung, and brain/central nervous system malignancies. The main impact of the survey, however, was to reveal Table 5.5.1 Long-term sequelae of cancer treatment Consequence of treatment Notes Growth abnormalities Radiation involving a growing bone epiphysis may halt growth locally and result in unilateral shortening. Similarly, steroids and chemotherapy drugs can impair growth. Reduced fertility In men, rapid loss of fertility commonly follows treatment with alkylating agents or irradiation of the gonads. Sperm storage is essential before therapy begins. Some men will recover their fertility up to 2 years after treatment is completed. In women, chemotherapy and radiotherapy of the pelvis can lead to premature ovarian failure, especially high-dose treatments combining irradiation with alkylating agents. Strategies to maintain fertility should be implemented before therapy starts. The strategies may include ovarian suppression during therapy and, for women not requiring urgent cancer treatment, storage of frozen oocyte or embryo. In women who retain fertility, it is likely that menopause will occur some years earlier than would otherwise have been the case. Cognitive impairment Treatment of central nervous system tumours, especially in the young, may result in cognitive impairment with profound consequences for the patient and their family. Renal impairment Treatment with regimens involving cisplatin or ifosfamide may result in renal tubular damage and reduced glomerular filtration. Cardiac impairment Irradiation of the mediastinum and left chest wall, as well as the use of anthracyclines or vincristine can lead to cardiac toxicity including cardiac failure, arrhythmias, and risk of myocardial infarction. In adults, limiting the total dose of anthracyclines reduces the risk of cardiac toxicity. Peripheral neurological impairment Many chemotherapy agents are neurotoxic with cisplatin and vinca alkaloids being the best recognized. Recognition of this toxicity during treatment may lead to modification of dose or regimen. Respiratory impairment Fibrosis may result from radiotherapy or multiple drugs, notably bleomycin. Similarly, pneumonitis is a well-recognized complication of many therapies and may respond to dose interruption with steroids. Gastrointestinal impairment There are multiple causes of dysfunction, including pelvic irradiation. These symptoms need expert assessment. Immune dysfunction With the development of T-cell checkpoint inhibitors, we are noticing autoimmune phenomena, which may continue, or rarely start, after cessation of treatment. These toxicities include rash, colitis, endocrine abnormalities, and pneumonitis. Osteoporosis Chemotherapy, radiotherapy, and hormonal therapy such as aromatase inhibitors may result in osteopenia. Bisphosphonate treatment and calcium supplementation may be required to maintain bony integrity. In extreme cases, high-dose steroids may lead to osteonecrosis of weight-bearing bones. Young patients may require serial joint replacements. Second cancers Both radiotherapy and several chemotherapies increase the risk of second malignancies. Tumours may arise either within or on the edge of the radiation field, often with a delay of 10–30 years.

 a far less well-recognized issue, namely the extent to which people living with or after a diagnosis of cancer experience a range of other health and

social conditions, limiting both the quality and the length of their lives, and having a major impact on their carers and children. To take breast cancer as an example, most (69%) women who receive a diagnosis of breast cancer will survive at least seven years. Over a quarter of these women will have or will develop one or more serious comorbidities other than a relapse of their breast cancer. These conditions may both reduce the quality of life for the patient and make it more difficult to provide treatment. When compared with patients receiving hospital treatment for nonmalignant con ditions, cancer patients have a much higher risk of comorbidities. For those patients with breast cancer who do not survive seven years after diagnosis, the risks are substantially higher, with 67% of all people with breast cancer having or developing one or more serious health conditions. Late sequelae of treatment The main long-term sequelae of cancer treatment are noted in Table 5.5.1. With greater success in treating cancers, there is now greater realization of the problems that can be induced by current cancer therapies. These may occur in children and young adults through the effect of cancer and its treatment on the well-being and development of maturing people. Equally, with the successful treatment of older patients with multiple medical problems, there may be exacerbation of underlying nonmalignant conditions. As new therapies are introduced, clinicians need to remain vigilant as to their long-term side effects. Since cancer survivors are at increased risk of many malignant and nonmalignant conditions, it is important that they are encour- aged to have a healthy lifestyle by maintaining a normal weight, taking exercise, and avoiding smoking and excessive alcohol. FURTHER READING Maddams J, et al. (2009). Cancer prevalence in the United Kingdom: estimates for 2008. Br J Cancer, 101, 541–7. Maher J, McConnell H (2011). New pathways of care for cancer sur- vivors: adding the numbers. Br J Cancer, 105, S5–S10.

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ESSENTIALS Cancer therapy is underpinned by a detailed understanding of cancer biology and the complex interaction of tumour and host. Systemic therapies and radiation therapy are being used in many early stage cancers, with a ‘risk adaptive’ approach to maximize the likelihood of uncomplicated tumour cure, both as primary treatment and in the postoperative setting. Targeted therapies have changed the course of many common cancers. Patients with early stage disease benefit through enhanced cure rates, while rational treatment approaches to metastatic disease improve the outlook for many patients. Chemotherapy Chemotherapy drugs are cytotoxic agents which induce preferen- tial cell kill in tumour cells because of their increased rates of pro- liferation. Their mechanisms of action are diverse and complex, but typically involve: (1) impaired synthesis of DNA or nucleotides; (2) in- hibition of mitosis; or (3) damage to the DNA backbone or base pairs. Targeted therapies Targeted therapies have evolved alongside the discovery of key car- cinogenesis pathways and identification of druggable targets. They are designed to inhibit or interfere with a key pathway in tumour for- mation. The main classes of targeted therapy are: (1) small molecule tyrosine kinase inhibitors; (2) monoclonal humanized antibodies blocking growth factor receptors; and (3) immune-modulating antibodies. They are typically cytostatic, inhibiting tumour growth for the duration of the agent if prescribed, rather than inducing cell kill. Resistance to therapy is common and typically occurs after
9–12 months of treatment. Hormone therapies In some tumour types such as breast cancer and prostate cancer, differentiated tumour cells retain endocrine-dependent growth signalling mechanisms. Blockade of the hormonal signal, either by elimination of physiological production, or through a hormone re- ceptor antagonist, can inhibit tumour growth. Radiation therapy Ineffective DNA repair is a cancer hallmark, hence ionizing radi- ation induced DNA damage leads to preferential tumour cell death. Dose to normal tissues is minimized by shaping the radiation beams to match the shape of the tumour target (target conformation), imaging the patient treatment to ensure radiation is delivered to the target (image guidance), and—in some sites such as gynaecological malignancy—bringing the radiation source into close proximity with the tumour tissue (brachytherapy). Improvements in local disease control achieved by radiotherapy in breast, prostate, and head and neck cancer have translated into improved survival for these patients. Introduction Over 110 years have passed since the first use of X-rays as anticancer therapy, and

over 70 years since nitrogen mustards were first ob- served to induce remissions in a patient with refractory non-Hodgkin lymphoma. Since this time, developments in our understanding of the molecular biology of cancer have driven a change in the develop- ment pathway for systemic therapy. Many of the current chemotherapy agents were discovered using a large-scale screening approach, in which libraries of compounds were screened against tumour cell lines and in vivo rodent tumour models. Modern drug discovery, in contrast, is driven by a target- directed approach. The first step has shifted from discovery of lead compounds to the characterization of a specific druggable target that plays a key role in tumour proliferation. Once a suitable druggable target and its three-dimensional shape has been established, com- pounds directed against the target can be synthesized using rapid development approaches (Box 5.6.1). Conventional chemotherapy still plays a key role in tumour cell kill (cytoreduction) and is often used in combination with targeted agents. 5.6 Systemic treatment and radiotherapy Rajesh Jena and Peter Harper Box 5.6.1 Different approaches to drug discovery High-throughput screening—an established technique for drug dis- covery, using robotics and process automation to assay many putative compounds for activity against tumour cells. Fragment-based drug discovery—screening small molecules which bind weakly to the biological target, but which can be combined to form subunits of a novel drug. Diversity-oriented synthesis—large libraries of drug-like compounds are synthesized which are structurally similar to an intermediate agent, which has been proven to kill the target cells.

 Common principles of chemotherapy agents Most chemotherapy agents target processes relating to cell prolif- eration on the premise that cancer cells proliferate more rapidly than normal tissue cells. For both conventional agents and tar- geted therapies, the action is not entirely specific to cancer cells, resulting in normal tissue toxicity. Chemotherapy agents can be classified according to their rela- tionship with the cell cycle of tumours. Some agents are cell-cycle specific, acting only during certain phases of the cell cycle. Cell- cycle independent agents will have the same effect on cells, whether they are in resting phase or active proliferation. Optimum drug scheduling is dependent on the relationship of the action of the drug with the cell cycle. Phase specific agents will reach concentra- tion that gives a maximum effect, killing all cells in a specific phase of the cell cycle. Increasing cell kill requires a change in the duration of exposure of the drug. In counterpoint, cell-cycle independent drugs will typically have a linear relationship between dose and cell kill (Table 5.6.1). Chemotherapy can induce cell death by two mechanisms, namely apoptosis and necrosis. Apoptosis as a programmed mode of cell death, which is regulated by specific cell signalling pathways and energy (ATP) dependent. Both radiotherapy and chemotherapy typically induce DNA damage in tumour cells that is detected by the cell, leading to activation of the apoptosis pathway. In contrast, necrosis of cells is caused by gross mitotic catastrophe or injury to the cellular structure, and is associated with an inflammatory response. Principles of radiation therapy Radiation therapy utilizes ionizing radiation to induce tumour cell death through the formation of both single-stranded and double-stranded DNA breaks. DNA damage can be generated ei- ther through direct interaction of radiation with DNA (the direct effect), or by the interaction of radiation and water to produce free radicals, which in turn produce DNA damage breaks (the in- direct effect). Ionizing radiation can be in the form of electromagnetic radiation (X-rays when generated from electricity, γ rays when derived from naturally radioactive materials) or particle beams, such as electrons, protons, and heavier ions. For most radio- therapy in clinical practice, the indirect effect is the predominant mechanism of cell kill, and oxygen is a prerequisite for DNA damage via the indirect effect (Fig. 5.6.1). As a result, hypoxic

tumour cells are more resistant to the effects of radiation therapy. Heavier ions tend to cause more DNA damage by the direct Table 5.6.1 Cell-cycle phase specificity of chemotherapy agents Cell-cycle specific agents Cell-cycle independent agents Folic acid pathway Methotrexate, pemetrexed Alkylating agents Lomustine, carmustine, cyclophosphamide, procarbazine Pyrimidine analogues 5-fluorouracil, capecitabine Platinum agents Cisplatin, carboplatin, oxaliplatin Direct effect Indirect effect Fig. 5.6.1 Indirect and direct mechanisms of DNA damage by ionizing radiation. X-rays can interact directly with DNA, or hydrolyse water to produce intensely ionizing free radicals, which subsequently induce DNA damage. (Blue circles = oxygen; small orange circles = hydrogen.)

5.6 Systemic treatment and radiotherapy 499 effect, and are less dependent on the availability of oxygen to fix radiation damage. This may be advantageous in the treatment of hypoxic tumours. Radiation can trigger apoptosis or necrosis in tumour cells in the same way as chemotherapy. Furthermore, radiation sensitivity is not uniform through the cell cycle. Cells are most sensitive to radiation therapy during mitosis and late G2 phases, and most re- sistant to radiation during S phase. The SI unit of absorbed radiation dose is the Gray—defined as 1 joule of energy absorbed in 1 kg of tissue. Most tumours will exhibit a radiation dose response that is sigmoid in nature (Fig. 5.6.2). The curve can be described by two parameters the TCD50 (dose required to achieve 50% probability of tumour con- trol) and γ50, which is the gradient of the dose response curve at this point (or the change in the probability of tumour control for a 1% increase in radiation dose). Across a wide range of cancers, a dose of 60–75 Gy is required to control macroscopic (bulk) disease, 50–55 Gy is required to control microscopic tumour, and doses of 20–30 Gy are required for palliation of symptoms. Radiation dose can be delivered to the tumour target using one of three strategies (Table 5.6.2). Context of radiotherapy and chemotherapy Both radiotherapy and chemotherapy agents are used in a range of contexts as anticancer therapy. Understanding the context of therapy is vital in managing patient side effects and establishing appropriate ceilings of care (Table 5.6.3). 100 90 80 70 60 50 40 30 20 10 0 0 10 20 30 40 Radiation dose (Gy) Relationship between dose and tumour control Probability of tumour control (%) 50 60 70 TCD50 Fig. 5.6.2 Diagram of radiation therapy dose response for a hypothetical tumour. TCD50 is defined as the radiation dose required to yield a 50% probability of tumour control. The steepness of the dose response curve at the TCD50 point is defined as γ50. For many solid tumours, γ50 is between 1 and 2%, hence a 1% increase in dose to the tumour will yield a 1–2% increase in the probability of tumour cure. Table 5.6.2 Strategies for delivering radiation to tumour targets Teletherapy The tumour is targeted by X-ray beams at a distance from the patient (also known as external beam therapy). Teletherapy is used to treat a wide range of tumour types. Brachytherapy The tumour is irradiated by bringing a radiation source into close proximity to the tumour. Brachytherapy is used with great success in gynaecological tumours. Radionuclide therapy A radioisotope with a short half-life is selected, which either has specificity for a tumour target in its own right, or is bound to a biological ligand, which binds preferentially to tumour cells. Radioiodine with I-131 is used in the treatment of thyroid cancer. Table 5.6.3 Contexts of cancer treatments Primary Treatment used as a single modality to cure patient. Typically primary chemotherapy is used in haematological malignancies, germ cell tumours, and paediatric tumours. Primary radiotherapy can be used to cure head and neck cancers, cervix cancer, and anal cancer while preserving function of the organ. Neoadjuvant Chemotherapy or radiotherapy used prior to surgery, to downstage the tumour. Examples include breast cancer and sarcomas. Adjuvant Usage after macroscopic removal of tumour by surgery. Treatment is given to eradicate microscopic disease. Palliative Chemotherapy is used in patients with locally advanced or metastatic disease with the aim of disease stabilization

and symptom control, rather than cure. Radiotherapy can be used to alleviate symptoms of bone pain or visceral compression.

500 SECTION 5 Principles of clinical oncology Classes of chemotherapy agent Alkylating agents These agents were first isolated from the nitrogen mustards, fol- lowing observations of depleted white cell counts in Italian sol- diers exposed accidentally to mustard gas. They were used to treat advanced Hodgkin lymphoma in 1942 with short-lived but dra- matic disease remission. They are now used in a wide variety of solid tumours and haematological malignancies. Their mechanism of action is to form a covalent bond between an alkyl group and nucleic acids (Fig. 5.6.3). Many of the drugs in this class will have an alkyl moiety at each end of the molecule, allowing them to form cross-links in DNA. Antimetabolites These agents have a similar structure to key building blocks in DNA and protein synthesis, and compete with natural substrates for incorporation into DNA or RNA, or bind with key enzymes in biosynthetic pathways. Purine analogues of adenine (such as 6-mercaptopurine) and guanine (such as thioguanine) lead to defective DNA synthesis. Antifolates such as methotrexate in- hibit dihydrofolate reductase, a key enzyme in the production of purines (Fig. 5.6.4). Reduced availability of tetrahydrofolate re- duces the efficacy of thymidylate synthase, which in turns reduces production of dihydrofolate and thymidine monophosphate. Thymidine phosphate is a key enzyme in both DNA and RNA synthesis. The inhibitory effect of methotrexate can be partially reversed by administration of folinic acid to restore cellular pools of tetrahydrofolate. Pemetrexed is a novel antifolate therapy which targets multiple steps in the folate biosynthesis pathway. Cytotoxic antibiotics Several of the most successful chemotherapy agents have been isolated from cultures of fungi and bacteria (Table 5.6.4). The agents typically act by inhibiting DNA synthesis by a range of dif- ferent mechanisms. Anthracyclines (named after the red-coloured actinobacteria species from which they were originally extracted) have a range of cytotoxic effects, ranging from DNA intercal- ation and inhibition of replication, to inhibition of DNA uncoiling through the inhibition of the topoisomerase II enzyme, and free radical damage through the production of reactive oxygen spe- cies. Mitomycin C was also isolated from a Streptomyces species and leads to DNA cross-linking similar to the effect of alkylating agents. Bleomycin is a nonribosomal glycopeptide isolated from Streptomyces verticullus, which causes DNA strand breakage. Platinum compounds Organic heavy metal compounds containing platinum, such as cisplatin, carboplatin, and oxaliplatin, form DNA cross-links, interfering with mitosis. The platinum agents revolutionized the treatment of germ cell tumours and are utilized in a wide variety of solid tumours, as well as haematological malignancy. Mitotic spindle agents Both classes of agent in this group have an effect on the microtubules that form the mitotic spindle in preparation for cell division. Vinca alkaloids, such as vincristine, vinblastine, and vinorelbine, were iso- lated or derived from the periwinkle plant. They bind to tubulins and prevent further assembly of the mitotic spindle. The taxanes, such as paclitaxel and docetaxel, were originally derived from the bark of the pacific yew tree and have antimitotic effects through stabilization of the assembled microtubules, and preventing depolymerization at the end of mitosis. The taxanes have been used in a range of solid tu- mours, including ovarian, breast, lung, and pancreatic cancers. Topoisomerase inhibitors Topoisomerase enzymes control the winding of DNA, and facili- tate DNA transcription through DNA cleavage, unwinding, and rejoining. Cytotoxic agents have been developed to inhibit the action of two key topoisomerase enzymes. The Camptothecins (topotecan and irinotecan) were derived from the bark and stem of the Chinese tree Camptotheca acuminata. Irinotecan is used in the treatment of bowel cancer. Etoposide is a potent inhibitor of topoisomerase II, and is used in the treatment of a range of solid tumours, including germ cell tumours and small-cell lung cancer. It is a semi-

synthetic derivative of an epidophyllotoxin, derived from the mandrake plant. Hormone therapies Growth stimulus from endogenous hormone secretion can act as an important promoter of carcinogenesis in many tumours. In 1878, Thomas Beatson was the first to identify that modulation of en- dogenous hormone production could be used to treat advanced ma- lignancy by performing oophorectomies in women with advanced breast cancer. In 1941 Charles Huggins first utilized castration and Interstrand cross-linking Intrastrand cross-linking Intercalation Monoalkylation
Fig. 5.6.3 Mechanism of DNA damage induced by alkylating agents.

5.6 Systemic treatment and radiotherapy 501 exogenous oestrogen administration to induce disease regression in metastatic prostate cancer. Hormone therapies modulate the growth stimulus by reduction of endogenous hormone synthesis, or antagonism of a hormone receptor pathway. Hormone therapies are used extensively in the management of breast cancer, pros- tate cancer, ovarian and endometrial cancer, and a range of other malignancies. Oestrogen receptor modulators Tamoxifen is one of the most common hormone therapies used in the treatment of oestrogen receptor-positive breast cancer, both in early and advanced stages. It is one of the first approved agents of a class of selective oestrogen receptor modulators (SERMs), having an antagonistic effect against receptors in the breast, and a partial agonist effect against receptors in the endometrium and bone. The agonist action in bone is protective against postmenopausal osteo- porosis but increases the risk of endometrial cancer. Novel second- generation SERMs such as raloxifene and bazedoxifene have been developed as treatments for postmenopausal osteoporosis, and both have a preventive effect on breast cancer. Androgen receptor antagonists Androgen receptor antagonists are used in the treatment of pros- tate cancer. Cyproterone acetate was one of the first of this class of agent, having a combined androgen receptor antagonist and progesterone receptor agonist effect. The drug is associated with significant risk of hepatotoxicity, and has now been superseded by second-generation agents such as flutamide, bicalutamide, and enzalutamide. These agents are pure androgen receptor antagon- ists which bind the receptor with high selectivity. Aromatase inhibitors Aromatase inhibitors are a class of agents which act by inhibiting the aromatization of androgens into oestrogens. The agents are used for the treatment of breast and ovarian cancer in postmenopausal women by inhibiting extragonadal oestrogen synthesis. Nonster- oidal aromatase inhibitors such as anastrozole and letrozole in- hibit oestrogen synthesis through competitive interaction with the aromatase enzyme. Steroidal aromatase inhibitors, such as exemestane, bind and permanently deactivate the enzyme. 5-FdUMP (5FU) Methotrexate Pemetrexed dUMP N5, N10 methylene- tetrahydrofolate Glycine Serine Tetrahydrofolate 10-formyl-tetrahydrofolate PRPP GAR AICAR IMP NADP+ NADPH + H+ Dihydrofolate dTMP TS SHMT DHFR AICAR-FT GAR-FT Fig. 5.6.4 Chemotherapy agents targeting the folate biosynthesis pathway. 5-Fluorouracil is metabolized to 5-FdUMP, which is a competitive inhibitor of thymidylate synthase. Methotrexate is an inhibitor of the dihydrofolate reductase enzyme. Pemetrexed inhibits both of these enzymes and inhibits multifunctional synthetic enzymes in the purine biosynthesis pathway. Table 5.6.4 Cytotoxic agents: species of origin and their usage Species Cytotoxic agent Usage S. peucetius Daunorubicin, Doxorubicin, epirubicin Breast, ovarian cancer, sarcoma, leukaemia S. caespositosus Mitomycin C Anal, bladder cancer S. verticullus Bleomycin Hodgkin’s lymphoma, non-Hodgkin lymphoma, testicular cancer S. parvullus Actinomycin D Gestation trophoblastic tumours, Wilm’s tumour, sarcoma

502 SECTION 5 Principles of clinical oncology Inhibition of gonadotrophin production Inhibition of gonadotrophin production is a potent form of androgen and oestrogen inhibition. Gonadotrophin

release hormone (GnRH) agonists such as goserelin, leuprorelin, and degarelix are synthetic analogues of the decapeptide luteinizing hormone-releasing hor- mone (LHRH). The agents bind to LHRH receptors, leading to an initial increase in production of LHRH and downstream sex hor- mones. This increase can lead to a flare in tumour growth, particu- larly in men with advanced prostate cancer. The flare effect can be minimized by coadministration of an androgen receptor antagonist such as bicalutamide. After 14–21 days, downregulation of LHRH receptors leads to a profound suppression of sex hormone produc- tion. The peptide has been formulated for subcutaneous implant- ation, which can last for 12 months. Exogenous hormones Exogenous hormones may also be used an anticancer therapies. Steroidal progestogens such as medroxyprogesterone acetate are used in the treatment of breast, endometrial, and renal cancer. In breast and endometrial cancer, progestogens reduce production of oestrogen through reduced hypothalamic GnRH production. Megestrol acetate has similar progestogen and antigonadotrophic effects, and is used in the treatment of breast, endometrial, and prostate cancers. It also has a weak glucocorticoid effect, useful for constitutional effects and stimulation of appetite in patients with ad- vanced malignancy. Targeted therapies While conventional cytotoxic chemotherapy and hormone ther- apies are efficacious anticancer therapies, their action is not dis- ease specific, and drug–tumour combinations have been developed through large-scale screening of agents, both in the preclinical and clinical setting. Our enhanced understanding of the underlying gen- etic mechanisms in cancer provides a novel method for drug devel- opment. Targeted therapies are agents which have been designed to modulate specific targets that are known to be overexpressed or of vital importance in the proliferation and survival of a given tumour type. Development of predictive assays allows confirmation of the presence or absence of a target in tumour tissue from each patient. The targeted therapies typically inhibit key growth factor signalling pathways, either through competitive inhibition of a growth factor receptor or a key step in the signal transduction pathway, such as a tyrosine kinase enzyme. The targeted therapy agents follow a stand- ardized nomenclature (Table 5.6.5). Monoclonal antibody therapies are large proteins that can only act at the surface membrane of cells. They are given via subcutaneous infusion, typically have long biological half-life of 2–3 weeks, and are typically well tolerated. Many of the antibodies will also elicit a beneficial activation of the immune system, which enhances their antitumour effect. Small molecule targeted therapies are typically given as oral ther- apies and have a short half-life between 36 and 48 hours. They can pass into the cytoplasm and thus operate on intracellular targets. Side effects typically consist of cutaneous effects such as rash, acne and pruritus, and gastrointestinal toxicity such as diarrhoea, nausea, and vomiting. The targeted therapies can be grouped by class of action. Signal transduction inhibitors These agents work through inhibition of a transmembrane signal transduction pathway that provides growth stimulus to tumour cells. Examples include trastuzumab, which inhibits the HER2 receptor which is overexpressed in breast and gastric cancers. Antibodies may also prevent dimerization of surface growth factors and subsequent activation of signalling cascades. Pertuzumab inhibits dimerization of HER2 and HER3. Cetuximab is a monoclonal antibody targeting the epidermal growth factor receptor (EGFR), while gefitinib and erlotinib are tyrosine kinase inhibitors of the same receptor pathway demonstrating efficacy in head and neck cancers, lung cancer, and bowel cancer. The BCR-ABL fusion protein forms a constitutively active growth receptor tyrosine kinase, implicated in Philadelphia chromosome-positive chronic myeloid leukaemia. Imatinib inhibits this receptor tyrosine kinase pathway, also the c-kit pathway found commonly in gastrointestinal stromal tumours. ALK is a receptor tyrosine kinase which exerts oncogenic effects in large cell lymphoma, non-small-cell lung cancer, and a range of other tumours. Agents such as crizotinib and ceritinib are used to treat ALK

mutation-positive advanced lung cancers, typically found among nonsmokers with adenocarcinoma histology. ALK muta- tions and EGFR mutations are often mutually exclusive in lung cancer, thus patients with ALK mutation positive tumours typically do not respond to EGFR tyrosine kinase inhibitors. The BRAF protein kinase is an important regulator of cell growth, proliferation, and differentiation, and BRAF mutations are observed in approximately 50% of melanoma tumours. BRAK kinase inhibi- tors such as dabrafenib and vemurafenib have revolutionized the treatment of advanced melanoma. Angiogenesis inhibitors These inhibit tumour growth, typically through the vascular endo- thelial growth factor (VEGF) pathway. Bevacizumab is an inhibitor of circulating VEGF, thereby inhibiting tumour angiogenesis. It has been used successfully in a wide range of malignancies including colon cancer, breast cancer, ovarian cancer, and glioblastoma. Many tyrosine kinase inhibitors have been developed, targeting different isoforms of the VEGF receptor as well as other related receptors (Table 5.6.6). Proteasome inhibitors These block the action of proteasomes, leading to activation of proapoptotic pathways which have been suppressed in tumour cells. Table 5.6.5 Nomenclature of targeted systemic therapies Name element Meaning Example -mab Monoclonal antibody Trastuzumab -ib Small molecule inhibitor Erlotinib -ximab Chimeric human-mouse antibody Cetuximab -zumab Humanized mouse antibody Pertuzumab -ci- Circulating system target Bevacizumab -tu- Tumour target Cetuximab -tin- Tyrosine kinase inhibitor Afatinib -zom- Proteasome inhibitor Bortezomib

5.6 Systemic treatment and radiotherapy 503 Bortezomib is an example of such an agent, used in the treatment of multiple myeloma and mantle cell lymphoma. Targeted immunotherapy agents Immune modulation therapy through the use of naturally occurr ing cytokines such as interleukins and interferon has yielded poor response rates as anticancer therapy. Novel therapies target spe- cific checkpoints in immune surveillance which are used by tu- mour cells to bypass detection by the immune system. CTLA-4 is a receptor which mediates an inhibitory effect on cytotoxic T lymphocytes. Ipilimumab is a monoclonal antibody which binds to the receptor and blocks the inhibitory signal, permitting cyto- toxic T lymphocytes to destroy tumour cells. Ipilimumab has been used with great efficacy in advanced melanoma and renal cell carcinoma, though nonspecific T-cell activation can cause severe gastrointestinal toxicity. Programmed cell death protein-1 (PD-1) is a cell surface receptor expressed on T cells which plays a key role in immune regulation. It interacts with two ligands, PD-L1 (expressed on tumour cells) and PD-L2 (expressed on macrophages and dendritic cells). PD-1 inhibitors facilitate activation of the immune system to attack tu- mour cells. PD-1 inhibitors such as nivolumab have demonstrated durable disease remissions in melanoma, renal cancer, and lung cancer, but carry a high risk of immune-mediated hepatitis, colitis, and pneumonitis. Clinical applications of radiation therapy Radiation therapy can be utilized in both curative and palliative treatment for a wide range of tumour types. Approximately 50% of all patients will benefit from radiation therapy at some point in their cancer journey. In a curative setting, radiation therapy is often used alone or in conjunction with chemotherapy as an organ-preserving treatment where surgical excision would lead to sig- nificant loss of function. Examples include head and neck cancers, anal cancer, cervix cancer, and prostate cancer, as well as a range of brain tumours. Curative radiation therapy is typically given in daily treatment fractions in order to facilitate normal tissue repair and minimize late effects of therapy. Radiation therapy is often used in an adjuvant setting after sur- gery in the treatment of localized breast cancer, where local ex- cision and postoperative radiotherapy yield equivalent control rates to mastectomy. Adjuvant radiation therapy also be used in a range of other conditions to reduce the risk of local disease re-currence. Neoadjuvant radiotherapy can be used with great effect to downstage rectal cancers

prior to surgery. Radiation therapy can also be used effectively in the treatment of seminoma, early stage Hodgkin lymphoma and non-Hodgkin lymphoma, and in a range of paediatric tumours, although in modern practice con- cerns regarding the late effects of radiation therapy typically mean that radiation therapy in such conditions is reserved for patients with incomplete response to chemotherapy, using a risk adaptive approach. Rapid palliation of local symptoms can also be achieved by ra- diation therapy for symptoms such as bone pain, spinal cord com- pression, cerebral metastases, low-volume bleeding from tumour surfaces and ulcerating tumours. Palliative radiation therapy is often delivered in single large doses of radiation. Stereotactic radiosurgery uses high-precision delivery systems to achieve steep dose gradients of radiation dose between tumour targets and ad- jacent tissues. Uses include eradication of cerebral metastases as well as some brain tumours such as vestibular schwannoma and meningiomas of the skull base and pituitary gland region. Novel imaging techniques allow stereotactic body radiotherapy to be used to treat tumours in the lungs, liver, and spine. Particle therapy uses the characteristics of high-energy protons and carbon ions to improve conformation of radiation dose to tar- gets. The initial energy of a proton defines the depth to which it will penetrate in the body, allowing a sharp fall-off of radiation dose (Fig. 5.6.5). Particle therapy is of particular benefit in the treatment of tumours close to the spine or optic apparatus, where steep dose gradients are required between the tumour and nerve tissue. The lack of exit dose through healthy tissues is also benefi- cial in paediatric radiotherapy, reducing the risk of impaired organ growth and secondary malignancy. Brachytherapy utilizes naturally occurring radiation sources that can be placed inside or close to the surface of a tumour. As radiation dose falls with the square of distance, the dose to sur- rounding tissues can be minimized using brachytherapy treat- ment, allowing higher doses to be delivered to the tumour than would be feasible with external beam radiation therapy alone. Brachytherapy is used extensively in the treatment of gynaeco- logical malignancy, as well as early stage prostate cancer. Table 5.6.6 Multitargeted VEGF inhibitors Agent Target receptor Cediranib VEGFR-1, -2, -3, c-kit Sorafenib VEGFR-2, -3, Ras/Raf/Mek/ERK and PDGFR- β Sunitinib VEGFR-1, -2, -3, PDGFR -α and -β Pazopanib VEGFR-1, -2, -3, PDGFR -α and –β, c-kit 14 7 0.5 Relative energy 260 MeV proton ions 120 kV X-ray 18 mV X-ray Bragg peak Depth in water (cm) 1 Fig. 5.6.5 Dose deposition of low- and high-energy X-rays, and proton ions. Note that the Bragg peak defines a depth at which dose drops off rapidly in the path of a proton beam.

 Improving the specificity of treatments Chemotherapy Several strategies have been adopted to enhance the specifi- city of cytotoxic agents for tumour cells, reducing the incidence of dose-limiting toxicity and permitting safe escalation of drug dose. Tumours have an acidic and hypoxic environment due to poor vascular clearance and accumulation of extracellular lactate. Temozolomide is a prodrug with increased activation at low pH when compared to physiological pH. Similarly, hypoxia-activated prodrugs are activated at the low oxygen tensions found in the core of tumour tissues. Liposomal formulation of drugs can reduce bio- availability in healthy tissues by increasing the effective circulation time of the drug. Liposomal doxorubicin formulations reduce the risk of cardiac toxicity without loss of antitumour effect. Nanoparticles of metallic gold or platinum, measuring up to 100 nm in size, can be enveloped in peptides to facilitate entry into tu- mour cells and evade immune surveillance. Metal ions can be directly toxic to tumour cells through free radical production, and act as ra- diation sensitizers with X-rays through the production of secondary electrons. The coated particles can be conjugated to peptides, mono- clonal antibodies, or cytotoxic agents. Paramagnetic nanoparticles can also induce thermal injury when activated by nonionizing elec- tromagnetic radiation (laser light) or alternating

magnetic fields. Radiation therapy Radiation therapy effects are not intrinsically specific to cancer cells, and normal tissue effects in structures adjacent to the tumour target limit the maximum radiation dose that can be delivered. Various strategies are employed to maximize the effect of radiation on tumour cells while minimizing the effect on normal tissues (Table 5.6.7). Late effects of cancer therapy Where cancer therapies are given with curative intent, care must be taken to consider long-term toxicities from treatment. Systemic therapies may be associated with organ-specific late effects such as dose-dependent cardiac toxicity from anthracycline chemotherapy, lung fibrosis from bleomycin, and sensorineural hearing loss from vincristine. Radiation therapy also carries a risk of normal tissue damage, dependent on the dose and volume of tissue that is irradiated. Common side effects in curative treatment include fibrosis fol- lowing breast irradiation, xerostomia due to irradiation in the salivary glands in head and neck cancer, and radiation proctitis fol- lowing pelvic radiotherapy. The mechanism of radiation injury is typically due to small vessel ischaemia and tissue fibrosis. Second malignancy is a rare yet important complication of both systemic therapies and radiation therapy. Alkylating agents, cis- platin, and etoposide have been associated with secondary leu- kaemia, typically between 2 and 10 years after treatment. Leukaemia may be preceded by a myelodysplastic syndrome. Ionizing radiation therapy also increases the risk of acute leukaemia, typically with ex- posures that irradiate large volumes of bone marrow. For targeted radiation therapy, second malignancy often manifests with earlier onset (especially breast cancer, lung cancer, and osteosarcoma). Unlike secondary leukaemia, epithelial malignancies typically occur decades after treatment. Patients undergoing cranial irradiation are also at increased risk of meningioma. FURTHER READING Begg AC, Stewart FA, Vens C (2011). Strategies to improve radio- therapy with targeted drugs. Nat Rev Cancer, 11, 239–53. Davita VT Jr, Chu E (2008). A history of cancer chemotherapy. Cancer Res, 68, 8643–53. Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next gen- eration. Cell, 144, 646–74. He J, Hu Y, Hu M, Li B (2015). Development of PD-1/PD-L1 pathway in tumor immune microenvironment and treatment for non-small cell lung cancer. Sci Rep, 5, 13110. Krause DS, Van Etten RA (2005). Tyrosine kinases as targets for cancer therapy. N Engl J Med, 353, 172–87. Table 5.6.7 Strategies to improve radiotherapy treatment Conformal radiotherapy This involves shaping of the radiation dose to match that of the target. At its simplest level, this can be achieved by shaping the radiation beam to match the shape of the target, known and 3D-conformal radiotherapy. The radiation field can be broken down into thousands of small beamlets directed at the tumour from different directions. The intensity of radiation of each beamlet can be controlled such that the summation of dose from all beams delivers a dose distribution that closely matches the shape of the tumour target. This technique is known as intensity modulated radiotherapy. Fractionated radiotherapy Radiation therapy is often delivered in a series of daily treatments, or fractions. This is because most tumour cells will have defective DNA repair pathways, and accumulate DNA damage from one treatment to the next. In contrast, the repair half- time for DNA damage in healthy mammalian cells is approximately 8 hours, hence in the 24 hours between treatments nearly 90% of the DNA damage caused by radiation will be repaired. Chemo-radiotherapy Low-dose chemotherapy can be used in conjunction with radiotherapy with specific synergistic effects. For example, cisplatin induces interstrand and intrastrand cross-links that interfere with double-strand break repair, leading to perpetuation of radiation-induced double-strand breaks.

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ESSENTIALS Early breast cancer Most patients with breast cancer are offered surgery, the main options being modified radical mastectomy, with or without immediate or delayed breast reconstruction, or breast-conserving surgery. All patients treated surgically for early breast cancer should be con- sidered for risk-reducing neoadjuvant (before surgery) or adjuvant (after surgery) treatments. Adjuvant radiotherapy should be considered for all patients who have undergone breast-conserving surgery. Adjuvant med- ical therapies include (1) endocrine therapy—should be given to all oestrogen-receptor positive patients (premenopausal— tamoxifen; postmenopausal—aromatase inhibitors); (2) anti-HER2 targeted therapy (e.g. trastuzumab) in cancers that overexpress the HER2 oncogene; (3) chemotherapy—selection is informed by clinic-pathological parameters and increasingly by molecular genetic platforms such as Oncotype DX; patients with oestrogen- receptor negative, node-positive disease should receive regimens containing sequential anthracyclines and taxanes. Regimens for neoadjuvant treatment are similar to those used in the adjuvant setting. Metastatic breast cancer Although local treatment of symptomatic lesions with radiotherapy or (in selected cases) with palliative surgery can be considered, the mainstay of treatment for metastatic disease is systemic therapy. Unless contraindicated, rebiopsy should be performed to confirm diagnosis and establish updated receptor immunophenotype. In oestrogen-receptor positive cases, first-line systemic therapy should be with endocrine agents, unless there is impending visceral or bone marrow crisis and/or rapid clinical response is required. Choice of chemotherapy regimen should take into account treat- ment given in the adjuvant setting and single agents are usually preferred to combination regimens. In HER2-positive disease, dual anti-HER2 blockade with trastuzumab and pertuzumab combined with a taxane is standard of care. Options for triple negative breast cancer may include platinum-based regimens and anti-PD-1 dir- ected immunotherapy. Patients should always be considered for participation in clinical trials. Pharmacogenomics to individualize systemic therapy is certain to become an integral part of the treatment decision-making process in the near future. Introduction More than one million women worldwide are diagnosed with breast cancer each year, with about 400 000 dying of the disease. Familial breast cancer, most commonly related to BRCA1 and BRCA2, accounts for only 5% of all cases. Many of the known risk factors are not modifiable because they are inherent or would require un- realistic lifestyle changes, but moderating alcohol consumption,

avoiding obesity, and increasing physical activity are all possibly useful interventions. See Chapter 5.1 for further discussion of the epidemiology of breast cancer. Screening for breast cancer is an effective means of achieving earlier diagnosis and provides the opportunity for reducing mortality: X-ray mammography screening alone can be expected to re- duce mortality by 30% in women aged 40–70 years who participate. Diagnostic assessment of symptomatic breast problems and screen-detected abnormalities is best carried out by multidiscip- linary teams following the principles of triple assessment, which in- volves (1) detailed history and clinical examination of both breasts, axillae, and supraclavicular regions; (2) imaging; and (where indi- cated) (3) cytology/core biopsy. The primary imaging techniques are X-ray mammography and ultrasonography, with MRI when there is diagnostic uncertainty. Ultrasound-guided core needle biopsy is the preferred method for sampling abnormalities. Once the diagnosis has been made, further imaging is used to assess the extent of cancer in the breast and detect the spread of disease to the axilla. The most significant clinico-pathological predictors of prognosis are lymph node stage, histological grade, and histological assess- ment of tumour size, but evaluation of tumour type and the absence or presence of lymphovascular invasion provide additional information. Tumour oestrogen receptor (ER) status and human epidermal growth factor receptor 2 (HER2) status have been used to guide se- lection of therapy for many years, and clinical practice is increasingly 5.7 Medical management of breast cancer Tim Crook, Su Li, and Peter Harper

 informed by analysis of expression of other genes (e.g. Ki67, which encodes a nuclear protein associated with cellular proliferation). In terms of management, most patients with breast cancer are offered surgery. The main options are modified radical mastec- tomy, with or without immediate or delayed breast reconstruction, or breast-conserving surgery. Metastatic involvement of axillary nodes is the best predictor of risk of recurrence and death, hence accurate assessment of axillary node status is important for sta- ging, prognosis, and guiding adjuvant treatment selection, and many regard sentinel lymph node biopsy as the best technique for establishing this. In this context, medical management of breast cancer has made remarkable progress since the first demonstration in 1975 that adju- vant chemotherapy in the form of cyclophosphamide, methotrexate, and fluorouracil (CMF) improved outcomes in node-positive breast cancer. In day-to-day clinical practice, systemic therapy of breast cancer continues to rely on a set of proven agents and regimens containing cytotoxic drugs, endocrine modulators, and targeted therapies. However, we are now entering a phase of rapid change in which these well-established approaches are increasingly being added to, and in some case replaced by, a more personalized ap- proach. Interesting and active drugs with novel mechanisms of action are emerging, which will change therapeutic algorithms. Moreover, as genomics allows molecular subclassification of breast cancer, it is certain that this will lead to more individualized treat- ment. Novel approaches (including immunotherapy) in specific patient subsets are starting to impact on potential management of metastatic breast cancer. Adjuvant therapy Although many patients with early breast cancer are undoubtedly cured by surgery alone, a significant proportion will ultimately re- lapse with metastatic disease, at which time they have incurable dis- ease. The term adjuvant therapy is used to describe treatment given after surgery. Adjuvant radiotherapy Adjuvant radiotherapy is indicated for all patients who have under- gone breast-conserving surgery. Chest wall radiotherapy is recom- mended for patients treated with mastectomy for larger cancers (>50 mm) or with four or more involved axillary lymph nodes. Over and above radiotherapy, the task of the oncologist in early breast cancer is to assess the risk of relapse/death for an individual patient and to initiate appropriate (adjuvant) systemic therapy to reduce this risk.

Endocrine therapy, chemotherapy, and targeted therapy all have important places in the adjuvant treatment of early breast cancer and are considered individually. Adjuvant endocrine therapy Strategies to block the effect of oestrogen are fundamental to the ad- juvant therapy of ER-positive early breast cancer. In the premeno- pausal patient, tamoxifen remains the most commonly used agent, and the large ATLAS and aTTom trials support the use of tamoxifen for 10 rather than the previous standard of 5 years. In postmenopausal ER positive patients, the aromatase inhibitors are the drugs of choice. These agents inhibit the enzyme aromatase, which catalyses the conversion of androgens into oestrogen in the peripheral fat. Many large clinical trials show superior efficacy of aromatase inhibitors over tamoxifen in the postmenopausal patient population, and recent studies suggest that extending adjuvant use of aromatase inhibitors up to 10 years is superior to 5 years, pre- dominantly as a result of prevention of contralateral breast cancer. Patients who have completed 5 years of adjuvant aromatase in- hibitors should therefore be considered for extended use, taking into account patient factors such as bone health and tolerability. Treatment with bisphosphonates or denosumab not only promotes maintenance of bone mineral density but also improves outcomes in postmenopausal patients taking aromatase inhibitors. Adjuvant therapy for HER2-positive breast cancer About 20% of primary invasive breast cancers overexpress the HER2 oncogene. If this is demonstrated, either by histochemical staining or fluorescent in situ hybridization (FISH) analysis, an adjuvant regimen containing trastuzumab should be offered to all women with node-positive disease, and to women with node-negative disease who have larger tumours. Trastuzumab improves survival outcomes for HER2 positive patients with breast cancer (number needed to treat is eight for patients with high risk), but with a chance of 2–20% of inducing congestive heart failure (depending on prior risk of that condition), hence routine cardiac monitoring is recommended. Adjuvant chemotherapy Adjuvant chemotherapy is given with the aim of reducing future disease recurrence by eliminating subclinical micro-foci of meta- static cancer cells which are present but not detectable at diagnosis. Traditionally, patients being considered for adjuvant chemotherapy were risk-stratified using clinically based systems such as the St Gallen criteria and the Nottingham Prognostic Index (NPI), which incorporates the size of the tumour, the number of involved lymph nodes, and the tumour grade to generate a value predictive of 5- year survival. Subsequently, multiparameter models have become available. The first of these was Adjuvant! on Line (https://www. adjuvantonline.com), and this has been joined recently by the NHS Predict model (http://www.predict.nhs.uk). Unlike previous sys- tems, NHS Predict incorporates HER2 and Ki67 status and is useful in the decision-making process for adjuvant therapy. Notwithstanding the utility of these systems in facilitating patient selection for adjuvant chemotherapy, a major evolving theme in recent years has been the drive to personalize treatment decision- making using molecular genetic rather than clinico-pathological parameters. Several platforms are available, of which Oncotype DX is the most well-known (and the sole product currently avail- able in the National Health Service wherein its used is restricted to ER positive, node-negative patients). Other testing systems include Endopredict, which incorporates the traditional prognostic factors of tumour size and lymph node status with analysis of an eight gene panel to derive a measure of risk of future relapse and how this can be offset by chemotherapy. Drugs used in the adjuvant setting are those previously shown to have efficacy in treating metastases, and adjuvant chemotherapy for breast cancer continues to rely on established agents such as the anthracyclines (doxorubicin [Adriamycin], Epirubicin), taxanes (docetaxel and paclitaxel), and the bifunctional alkylating agent cyclophosphamide.

5.7 Medical management of breast cancer 507 Several pivotal trials have provided guidance on which regimens are appropriate for specific subsets of patients. The prognostic sig- nificance of lymph node status is well-recognized, and it is now widely held that patients with node-positive disease should re- ceive both taxanes and anthracyclines, particularly if they have ER- negative cancers. In addition to lymph node status, specific subsets of patients have a higher probability of relapse and accordingly have a lower threshold for the use of adjuvant chemotherapy. For ex- ample, in patients with triple negative breast cancer there are (by definition) no endocrine or anti-HER2 treatment options, and adju- vant chemotherapy is indicated for most patients. Of note, in triple negative breast cancer there is now good evidence that adjuvant paclitaxel given in the dose-dense (accelerated) or (even better) on a weekly regimen is superior to docetaxel. Similarly, cases positive for HER2 should usually be offered adjuvant chemotherapy together with trastuzumab, even if small, ER positive and node negative. Neoadjuvant systemic therapy The term neoadjuvant therapy is used to describe treatment given before surgery with the intention of ‘down staging’ the tumour and allowing surgery to take place more easily and with less mor- bidity. Neoadjuvant treatment of breast cancer is now established as a (frequently) effective strategy for early breast cancer, particularly in larger cancers, in locally advanced cases, and in many patients with HER2-positive disease. There are several factors which make neoadjuvant therapy an attractive option for selected patients: • Cancers can often be downsized to facilitate more cosmetic- ally acceptable completion surgery and, in some cases, breast conservation. • Use of systemic therapy ‘upfront’ provides a rapid indication of the sensitivity of individual cancers to chemotherapy and may there- fore guide adjuvant treatment options. • Achievement of a pathological complete response is a useful prog- nostic parameter and may, at least in part, inform the decision to offer additional (postoperative) chemotherapy. Regimens for neoadjuvant treatment are often identical to those used in the adjuvant setting. However, in HER2-positive disease, dual blockade with the combination of trastuzumab and pertuzumab (initially given together with taxanes) achieves a significantly higher rate of pathological complete response than trastuzumab alone. Metastatic breast cancer Approximately 6% of patients with metastatic breast cancer present with de novo disease and metastatic relapse will ultimately occur in 10–40% of patients treated (apparently successfully) for early breast cancer. Although local treatment of symptomatic lesions with radio- therapy or (in selected cases) with palliative surgery can be con- sidered, the mainstay of treatment is systemic therapy. As in the adjuvant setting, there are three modes of systemic treat- ment for metastatic disease, namely endocrine therapy, chemo- therapy, and anti-HER2. There are, however, several important differences in the way these agents are deployed in the metastatic set- ting. Oncological orthodoxy has long considered metastatic breast cancer to be an incurable disorder, the goal of management being extension of life with control of symptoms, and selection of regimens must reflect these different therapeutic objectives and be adaptable to widely differing patient factors, including performance status. Rebiopsy of a metastatic lesion should be performed wherever possible, not only to confirm the diagnosis of breast cancer, but also to obtain an up-to-date picture of the expression of ER and HER2. It is now well-recognized that expression of ER not infrequently changes with tumour evolution, and inappropriate and ineffective endocrine therapy could be given if ER status is assumed from the primary cancer. Furthermore, HER2 status flips from negative to positive in a few cases, affording new anti-HER2 treatment options. Endocrine therapy In cancers shown to be ER positive, first-line therapy is with endo- crine agents unless there is visceral crisis (bone marrow failure, lymphangitis carcinomatosis, or impending liver failure), evidence of rapidly progressing disease, or progression through multiple lines of endocrine therapy. Selection of drugs for endocrine therapy will depend on menopausal status and

previous/ongoing therapy. Those who have received tamoxifen in the adjuvant setting can be retreated with this agent if there is a long interval since completion of adjuvant therapy. Otherwise, initial endocrine therapy of meta- static breast cancer in premenopausal patients is with ovarian sup- pression together with an aromatase inhibitor. In postmenopausal patients, aromatase inhibitors are superior to tamoxifen and pre- ferred as first-line therapy. Patients who received aromatase inhibitors in the adjuvant setting can be rechallenged if there is a disease-free interval of at least one year. An alternative approach is the use of fulvestrant (Faslodex), whose mechanism of action is to promote degradation of the ER. The 500 mg dose of fulvestrant is clearly more active than the 250 mg previously used, and recent data reveal its superiority to aromatase inhibitors in the first-line setting in metastatic breast cancer. Resistance to endocrine therapy is a major problem. Up to 50% of ER positive metastatic breast cancers exhibit de novo resistance to endocrine therapy, and acquired resistance is almost certain in patients whose disease is initially responsive. In patients progressing on first-line nonsteroidal aromatase inhibitors, the combination of exemestane and everolimus improves progression-free survival and is currently recommended in routine clinical practice. Chemotherapy When chemotherapy is required, most treatment guidelines propose the use of single agent rather than combination regimens, because although the response rate may be higher with combination chemo- therapy there is increased toxicity without a significant survival benefit. There remains an absence of biomarkers to inform first-line chemotherapy. This situation will undoubtedly change to a more genomically informed approach in the future, but at present, choice of agent continues to be tailored to the patient rather than to the mo- lecular genetic parameters of the cancer. In the ER-positive, HER2-negative metastatic breast cancer pa- tient population, many agents can be given in the metastatic setting, including the taxanes, capecitabine, vinorelbine, doxorubicin, and the microtubule growth inhibitor eribulin (the latter after at least two previous lines of chemotherapy). Selection and sequencing of agents is in part influenced by drugs given in the adjuvant setting.

 For example, patients given docetaxel in the adjuvant setting are suitable for consideration for weekly paclitaxel, and anthracyclines should be considered in naïve patients. As with adjuvant therapy, special considerations are applicable for HER2 positive and triple negative breast cancer. In HER-2 posi- tive metastatic breast cancer, first-line therapy with double HER2 blockade via trastuzumab and pertuzumab (combined initially with a taxane) has produced unprecedented improvements in out- comes as shown by data from the Cleopatra trial, and this is now standard of care. Trastuzumab emtansine (T-DM1, Kadcyla) is now established as optimal second-line treatment, relegating the com- bination of lapatinib and capecitabine to third-line use. In triple negative breast cancer there is evidence that a platinum doublet may have efficacy, particularly in patients with germ-line mutations in BRCA1 and BRAC2. Eribulin also shows increased efficacy in the triple negative breast cancer patient population compared to ER positive cases. Patients with bone metastases should receive bisphosphonates or denosumab. Future developments Management of metastatic breast cancer is rapidly progressing and several new agents show considerable promise. In ER-positive disease progressing despite adjuvant aromatase inhibition, palbociclib (the first in class CDK4/CDK6 inhibitor) given as first line with letrozole or fulvestrant shows a significant improvement in progression-free survival and is sure to find a place in the man- agement of this patient population. Novel treatment approaches are emerging in triple negative breast cancer. For example, several anti- PD-1 immunotherapeutic antibodies show promise. These include pembrolizumab, atezolizumab (in combination with Abraxane), and the bifunctional anti-PD-1 avelumab, all of which have activity in PD-L1 positive

triple negative breast cancer (see Chapter 5.4 for further discussion). There is also evidence that anti-androgens may benefit some patients with triple negative breast cancer, both bicalutamide and enzalutamide having efficacy. FURTHER READING Cardoso F, et al. (2017). 3rd ESO-ESMO international consensus guidelines for Advanced Breast Cancer (ABC3). Ann Oncol, 28, 16–33. Santa-Maria CA, Gradishar WJ (2015). Changing treatment para- digms in metastatic breast cancer: lessons learned. JAMA Oncol, 1(4), 528–34. Sparano JA, et al. (2015). Prospective validation of a 21-gene expres- sion assay in breast cancer. N Engl J Med, 373(21), 2005–14.

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