CHAPTER 1

An Introduction to Cancer Cell Biology and Genetics

IN BRIEF

I find it impossible to describe how targeted cancer treatments work without mentioning what it is they target. And when I try to explain what it is they target, I find myself going back to the beginning and explaining where cancers come from, what faults they contain, and why they behave as they do. And, to explain that, I need to explain concepts such as DNA damage, oncogenes, tumor suppressor genes, and the hallmarks of cancer cells.

In recent years, we've also made great progress in using a patient's immune system to treat cancer using immunotherapy. When explaining how immunotherapies work, I find it useful to offer at least a brief description of our immune system and the ways in which cancer cells and white blood cells interact. Armed with this knowledge, various strategies to use the immune system to destroy cancer cells begin to make sense.

In this chapter, my goal is to bring together much of this background knowledge. I hope it will provide you with a useful foundation that enables you to understand individual targeted therapies and immunotherapies that I mention in later chapters.

First, I run through the causes and consequences of DNA mutations in cells. I describe how even just a handful of mutations can force a healthy cell to become a cancer cell.

I also describe the cancer microenvironment – the cells and structures that cancer cells live alongside, including white blood cells of our immune system. Cancer cells have the ability to exploit their local environment and, in many instances, manipulate it. I explain what impact this has when doctors come to treat people with the disease.

In addition, I tackle topics such as genome instability and intratumoral heterogeneity. Perhaps these are topics that right now don't mean anything to you, and you're unsure of why you need to know about them. But it's only through understanding these concepts that you can appreciate the limitations of targeted (and standard) cancer treatments and grasp the potential of immunotherapy. It is also important to understand why cancer spreads and how cancers evolve and change over time.

A Beginner's Guide to Targeted Cancer Treatments and Cancer Immunotherapy, Second Edition. Elaine Vickers.

I then turn my attention to the unique properties of hematological cancers. I describe some of the types of mutation that drive their behavior and talk about why these mutations occur. I also explain their greater vulnerability to immunotherapy compared to solid tumors.

Finally, I wrap up the chapter with a brief overview of why cancer is so difficult to treat successfully and why so many people currently cannot be cured.

1.1 INTRODUCTION

This book is about the science that lies behind targeted cancer treatments and cancer immunotherapies. Almost without exception, **these treatments work by attaching to, or blocking the actions of, proteins**. So, to understand these treatments, it's first of all essential to understand what proteins are, how they work, and how the proteins found inside and on the surface of cancer cells differ from their healthy counterparts.

For this to make sense to you, I need to explain the different types of DNA damage that cancer cells contain, **because a cell's DNA is its instruction manual telling it how to make proteins**. If we know what DNA damage a cell contains, this will tell us what faulty proteins it's making. And if we know what faulty proteins it's making, we will have a better idea of which treatments might work against it.

So, **this chapter contains lots of information about cancer cells, DNA, and proteins**. However, even in this chapter, I've made some assumptions about what you do and don't know. For example, I've assumed that you have a rough idea of what DNA is and how cells use their DNA to make proteins. If you're not familiar with these concepts, I would recommend first taking a look at the Appendix, which contains a list of reading materials about cells, DNA, chromosomes, genes, and proteins. When you've had a look at that, you'll be ready to read further.

This chapter doesn't exclusively focus on individual cancer cells and their faults. **Cancer cells don't live alone, nor are tumors a homogenous mass of identical cancer cells**. Instead, cancer cells live among other types of cells, such as fibroblasts, fat cells, and numerous types of white blood cells. This composition changes over time and also in response to treatment. In addition, cancer cells themselves evolve and change over time, and this has an enormous impact on the effectiveness, or not, of many treatments.

In this chapter, I'll also provide you with some background information about **how cancer cells relate to, and influence, our immune system**. Why it is, for example, that in some people their immune system reacts strongly against their cancer cells, while in another person their immune system seems to essentially shrug its shoulders and carry on as normal. I'll also pay special attention to T lymphocytes (T cells), which are at the heart of many different forms of immunotherapy.

Some of the information in this chapter is relevant to all cancers, wherever they occur in the body and whatever type of cell they developed from. However, there are some features of hematological cancers (such as leukemias and lymphomas) that set them apart from solid tumors like breast or bowel cancer. Some of this difference comes down to the mutations that drive hematological cancers, but some of it is due to their accessibility to drugs, and to healthy white blood cells.

Along with the chapter that follows (which is all about the two main groups of cancer treatments in this book: monoclonal antibodies and kinase inhibitors), this chapter hopefully

provides you with all the background information you need to make sense of the rest of this book.

1.2 DNA DAMAGE IS THE CAUSE OF EVERY CANCER

Our cells' DNA is essentially a huge instruction manual telling our cells what proteins to make, how to make them, when to make them, what to do with them, and when to destroy them. In turn, the proteins our cells make dictate their behavior. For this reason, **if you damage a cell's DNA, it is likely to make the wrong, or damaged, versions of proteins**, leading to abnormal behavior (see Figure 1.1).

Cancer starts to develop when a single cell accumulates DNA damage to several important genes. This damage causes the cell to make faulty proteins that force it to behave abnormally. To result in cancer, the cell also needs to overcome whatever hostile forces are exerted by its environment and by neighboring cells. Thankfully, this normally doesn't happen. Instead, **a cell that finds its DNA damaged usually tries to repair the damage, or it self-destructs** through a process called apoptosis.[1] **Or, if the cell doesn't kill itself, it's usually kept in check by its environment or destroyed by white blood cells**. But, if a damaged cell survives, and if it avoids or overcomes its hostile neighbors, it might ultimately multiply and cause us to develop cancer.

Over the past 40 years or so, scientists have been gradually uncovering which gene mutations cause cancer. Genes only take up about 1%–2% or so of our cells' total DNA, so it's this DNA they have focused on [1].

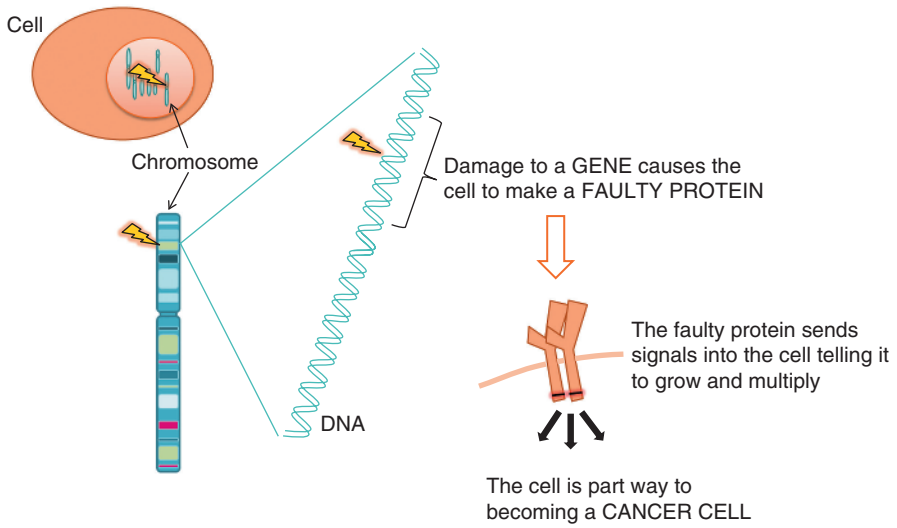

Figure 1.1 Gene mutations cause the production of faulty proteins. Chromosomes are long lengths of DNA found inside the nucleus of each cell. Within our chromosomes are regions of DNA called genes. These are stretches of DNA that contain the instructions to make proteins. If a gene is affected by a mutation (represented by a lightning bolt), the cell might then make a faulty protein. In this example, the faulty protein is a cell surface receptor that gives the cell a continuous signal to grow and multiply.

[1] Apoptosis is also referred to as "programmed cell death."

Box 1.1 The names of genes and their proteins

As you read this book, you might notice that protein names are written normally but that gene names are written in italics. For example, the *HER2* gene contains the instructions for making HER2 protein. You might also notice that sometimes the gene and the protein have different names. An example of this is the *TP53* gene, which contains the instructions for making a protein called p53. It's also possible for a gene to contain the instructions for making more than one protein. For instance, the *CDKN2A* gene (sometimes referred to as the *CDKN2A* locus) contains the instructions for making several proteins, two of which are called $p16^{INK4a}$ and $p14^{ARF}$.

To add to the confusion, some genes and proteins have more than one name. For example, the *HER2* gene is also called *ERBB2* and *NEU*. The reasons behind the various names often have a lot to do with what organism or group of cells the gene/protein was discovered in; if it's similar to another gene/protein that has already been discovered; what role the gene/protein is thought to play in the cells or organism it was found in; and whether or not abnormalities in the gene/protein cause disease. For example, HER2 stands for "human epidermal growth factor receptor-2," because it's similar in structure to HER1 (although we usually refer to HER1 as the EGF receptor or EGFR). *HER2* is also called *ERBB2* because a very similar gene, called *ERBB*, was discovered in a disease-causing virus called the avian erythroblastosis virus. *HER2* is also called *NEU* because a faulty version of it can cause a cancer called neuroblastoma in rodents.

A final point to note is that gene names are often written in capital letters, whereas protein names aren't. But this convention isn't always adhered to.

(What exactly the rest of our cells' DNA is for is a matter of continued debate among scientists.)

Through initiatives such as The Cancer Genome Atlas [2] and the International Cancer Genome Consortium [3], hundreds of scientists have amassed an incredible catalog of information about the thousands of different DNA mutations cancer cells contain [4, 5]. They've also discovered that **different types of cancer differ from one another in terms of the mutations they contain and the treatments they respond to**. In addition to these differences, we know that important similarities can exist between cancers that arise in different organs. For example, the cancer cells of some breast cancers overproduce[2] a protein called HER2, and the same is true of the cancer cells in some stomach cancers and other cancer types [6].

Because there's lots I want to say about the DNA mutations found in cancer cells, I'm going to split it up into different topics. First, I'll talk about what **causes the DNA mutations** found in cancer cells (Section 1.2.1). Then I'll describe what **types of mutation** occur (see Section 1.2.2), how the **number and pattern of mutations** in cancer cells varies (see Section 1.2.3), and **which mutations have the greatest effect on cell behavior** (see Section 1.2.4). Then I'll talk about some of the most **common gene mutations** in cancer cells and what impact they have (Section 1.2.5).

[2] Scientists generally talk about proteins being "overexpressed" rather than "overproduced," but they essentially mean the same thing.

All this information is gradually helping scientists create the new, more targeted cancer treatments described in this book.

1.2.1 Causes of DNA Mutations

There are many different reasons why our cells' DNA gets damaged. Much of this damage is **natural and unavoidable**, whereas some of it is down to our **lifestyle, behaviors, exposures, geographical location,** and even **local customs**.[3] We can also **inherit** damaged DNA from our parents. Depending on what sort of data scientists look at (e.g., whether they examine individual cells or whole organs or tissues, or look at populations of people in different countries), they end up drawing very different conclusions about what proportion of cancers could be avoided [7–10]. So, although I've listed some of the causes of DNA damage later, and in Figure 1.2, I haven't tried to pin down exactly how many cancers are caused by each one.[4]

Unavoidable Causes of DNA Damage

1. **The byproducts of chemical reactions.** Unfortunately for us (and for all living things), **our cells' DNA gets damaged every second of every day**. Scientists think that even without the influence of external factors, each of our cells sustains damage to its DNA roughly 20,000 times each day [15].

   Much of this damage is caused by the products of chemical reactions that are essential to keep us alive. For example, many of our cells' important chemical reactions produce oxygen free radicals[5] – high-energy oxygen atoms that essentially bash into and break DNA [16]. Our cells contain well over 100 different DNA repair proteins to fix this damage [17]. But sometimes they

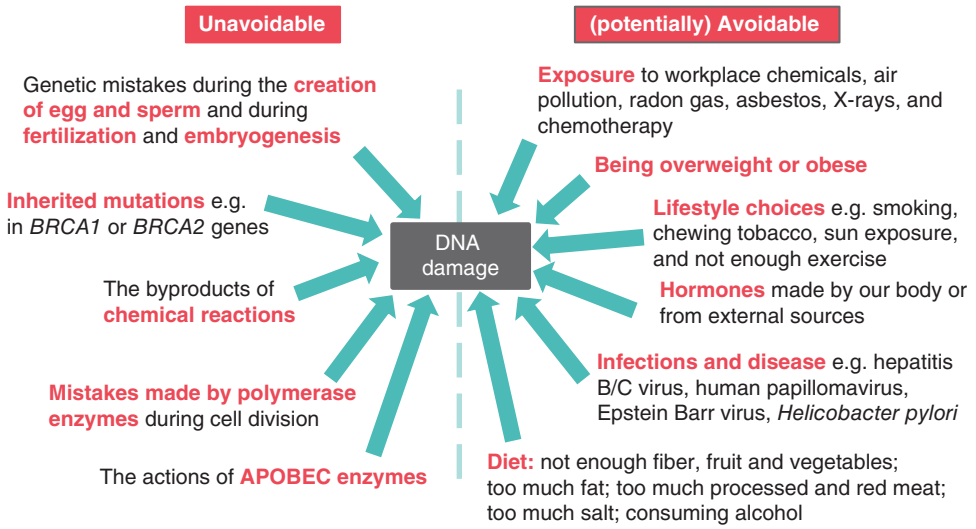

Figure 1.2 Some of the causes of DNA damage. *Source:* Adapted from Ref. [11–14].

[3] For example, in countries like Iran, people are used to drinking much hotter tea than people do in the United Kingdom, and this has been linked to a higher incidence of esophageal cancer.
[4] If you do want to learn more about what you can do to reduce your risk, I would recommend looking at the Cancer Research UK website: http://www.cancerresearchuk.org/about-cancer/causes-of-cancer/can-cancer-be-prevented.
[5] These are also called reactive oxygen species – ROS.

fail to spot all the damage, or they simply can't keep up.

2. **Cells make mistakes as they multiply**. Tissues that need to renew and replenish their cells often (such as the lining of our bowel, our skin, and those that comprise our immune system) are at the highest risk of cancer[6] [9, 18–20]. This is because for a cell to multiply, it has to make a complete copy of all of its DNA – all 3000 million base pairs of it. The enzyme that copies DNA, called DNA polymerase, although spectacularly fast and accurate, does occasionally make mistakes [18]. Therefore, **cells that need to multiply often are at a greater risk of becoming cancer cells** than cells that rarely, if ever, multiply.
3. **The actions of APOBEC enzymes**. APOBEC[7] enzymes are a family of proteins that our cells use to help protect them from viruses. **APOBEC enzymes attack viruses** by introducing mutations into their DNA. However, if an uninfected cell accidentally makes APOBEC enzymes, the enzymes will attack the cell's own DNA and introduce lots of mutations that could cause cancer [19]. Also, after a cell has become a cancer cell, APOBEC enzymes continue to add more and more damage to the cell's genes [20].
4. **Inherited mutations**. Some people are **born with DNA faults** that put them at a higher risk of cancer. Sometimes the fault has been passed down from generation to generation, with many family members affected. For example, actress and film director Angelina Jolie has inherited a fault in one copy of her *BRCA1* gene (we inherit two copies of each gene). Because this fault is shared by many of her relatives, she lost her mother, grandmother, and aunt to cancer [21]. Faults in high-risk genes such as *BRCA* genes are relatively rare, but they can have an enormous impact on a person's cancer risk. More commonly, subtle variations in many genes will combine to affect our risk.

Faults can also arise in an egg or sperm; if the faulty egg or sperm goes on to create an embryo, this fault will be present in every cell. Or, the fault might occur later, as the growing embryo is developing. For example, faults that occur in an embryo's white blood cells as its immune system forms can cause infant or childhood leukemia [11].

Potentially Avoidable Causes of DNA Damage

1. **Lifestyle and exposures**. Cells that are exposed to high levels of **carcinogens** (anything that causes cancer is called a carcinogen) are particularly vulnerable to becoming cancer cells. This includes cells that line our lungs, skin, bowel, and stomach. Carcinogens include various constituents of cigarette smoke, alcohol, UV light from the sun or from sunbeds, radiation from X-rays, some viruses, asbestos, and food toxins [13].

Our cancer risk is also linked to our **diet** (including our consumption of fruit and vegetables, red and processed meat, salt, and fiber), our level of **physical activity**, and our **weight**. This is a huge topic. If you would like to learn more, I suggest looking at the Cancer Research UK [22] and American Cancer Society [23] websites.

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[6] If this seems like a simple and straightforward association, don't be fooled. There is huge controversy around the exact relationship between cancer risk and tissue renewal, number of stem cells, and DNA damage by environmental versus natural mechanisms. I've supplied a handful of references if you want to explore further.

[7] In case you're curious, APOBEC stands for apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like.

2. **The influence of sex hormones**. When discussing the causes of cancer, we shouldn't ignore the influence of sex hormones such as **estrogen, progesterone, and testosterone**. These tiny, fat-soluble chemicals encourage cells that contain receptors for them to survive, grow, and multiply (estrogen can also cause DNA damage [24]). Cancers that develop from hormone-sensitive tissues in the breast and prostate often retain their sensitivity to hormones. These cancers often respond to treatments that block the production of hormones in the body or that block the impact of hormones on cancer cells.

   The risk of various cancers, including breast, ovarian, and endometrial cancer, is linked to a person's exposure to sex hormones such as estrogen. Reproductive factors (such as age of menarche[8] and menopause, along with the number of pregnancies and length of time they breastfed) and bodyweight affect a person's lifetime exposure to estrogen and thus also influence their cancer risk [25].
3. **The influence of inflammation**. For many people, their cancer diagnosis was preceded by **years of inflammation, infection, or irritation** [26]. For example, people with a chronic hepatitis B or hepatitis C virus infection are at high risk of liver cancer, whereas people with inflammatory bowel disease are at an increased risk of bowel cancer [27, 28]. It seems that the presence of white blood cells in a tissue can increase the DNA mutation rate in the tissue's cells and encourage the cells to multiply, raising the risk of cancer [28].
4. **Cancer treatments. Most chemotherapies and radiotherapy work by causing so much DNA damage** that cancer cells die. However, not every cell is killed. Cells that sustain damage to their DNA and yet survive may later become cancer cells. Because of this, people treated for cancer sometimes develop second cancers months or even many years later [29, 30].

Causes of DNA Mutations – Summary

Our risk of cancer in any particular place in our body is therefore a combination of the following [8–10]:

- The natural rate that the cells multiply in that tissue.
- The extent to which DNA polymerase, oxygen free radicals, and APOBEC enzymes have caused mutations in the tissue's cells (the amount of damage will gradually increase as we age).
- Our biological sex and our inherited genetic makeup.
- Our lifestyle and behaviors (which will be hugely impacted by our cultural background, physical location, personal choices, and opportunities).
- Our cells' exposure to carcinogens, hormones, and factors that cause inflammation.

Cancer Research UK estimates that **around 4 in every 10 cases of cancer diagnosed in the United Kingdom are potentially preventable** through changes to lifestyle, behaviors, exposures, and weight [31, 32]. However, we cannot influence factors such as the activity of APOBEC enzymes or the accuracy of DNA polymerase. As I said before, estimating what proportion of cancers can be prevented is an incredibly contentious topic, and estimates vary widely depending on how the research was done [7].

1.2.2 Types of DNA Mutations

DNA mutations come in many forms. For the sake of simplicity, I'm going to split them into two groups: (1) mutations affecting long

[8] The age at which a girl has her first period.

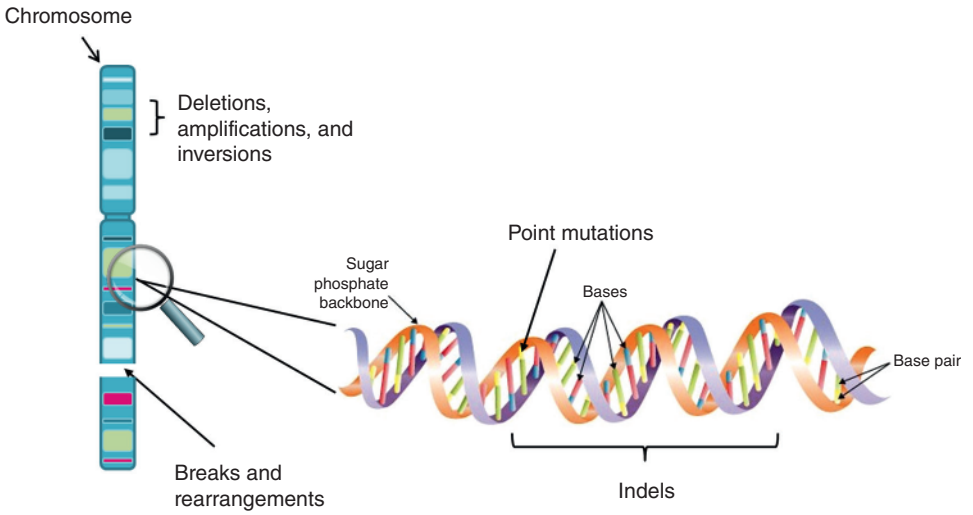

Figure 1.3 DNA damage can come in many forms. Deletions, amplifications, and inversions can affect tens of thousands of DNA bases at a time. Breaks and rearrangements affect whole chromosomes. Smaller-scale mutations include point mutations (insertions, deletions, or substitutions) affecting a single DNA base. Insertions and deletions that involve up to 1000 DNA bases are called indels. *Source:* Adapted from Ref. [33], the image of DNA double helix was created by the Genomics Education Programme and licensed under the Creative Commons Attribution 2.0 Generic license. https://www.flickr.com/photos/genomicseducation/13081113544/Image of magnifying glass from pixabay.com.

stretches of DNA and whole chromosomes and (2) mutations affecting under 1000 DNA base pairs (see Figure 1.3).

Mutations Affecting Long Stretches of DNA and Whole Chromosomes

For a start, **many cancers are aneuploid** – that is, the cells contain the wrong number (i.e., not the normal 23 pairs) of chromosomes [34]. Often, there is evidence that at an early point in the cancer's development, its entire genome – all 46 chromosomes – have been duplicated (called **whole-genome doubling** [35]). In addition, chromosomes often show signs of having shattered into small pieces and then been stitched back together in a haphazard fashion – called **chromothripsis** [36, 37]. These types of DNA damage are no doubt important. However, it's not always clear what impact they have on cancer cells, nor what we could do about them in terms of offering better treatments. Because detecting these types of DNA damage doesn't generally help doctors decide what treatment to give to their patients, I'm not going to talk about them further.

What can be more helpful is detecting chromosome faults such as translocations, inversions, insertions, deletions, and amplifications.

Chromosome Translocations and Rearrangements

A chromosome translocation is when two chromosomes break, and the cell accidentally sticks them back together incorrectly (see Figure 1.4). Chromosomal rearrangements are similar, but both breaks occur in a single chromosome. More often than not, the chromosomes break in regions that don't contain any genes (remember that the information to make proteins only takes up 1% or so of our chromosomes). However, **sometimes translocations and rearrangements do affect genes**, and this can have dire consequences.

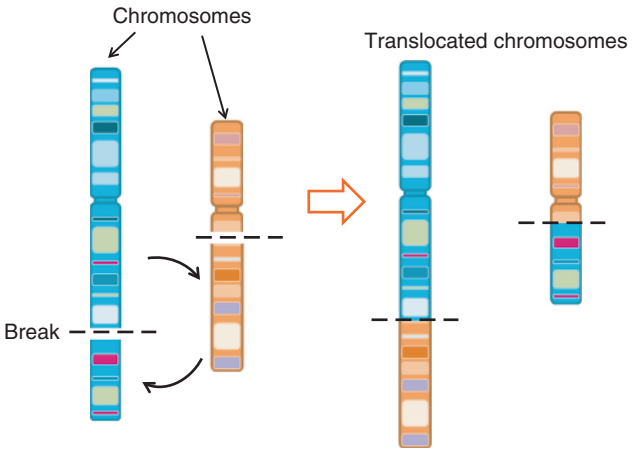

Figure 1.4 A chromosome translocation. Two chromosomes (colored turquoise and orange) break. The cell attaches them back together incorrectly. If the chromosomes have broken where genes are located, this may result in two genes from different chromosomes becoming fused together on the same chromosome (a gene fusion).

For example, the cancer cells of chronic myeloid leukemia (CML) almost always contain a translocation in which chromosome 9 and chromosome 22 have broken and been stitched back together incorrectly. This causes the *BCR* gene on chromosome 22 to become fused together with the *ABL* gene on chromosome 9. The fusion of these two genes causes the cell to make a Bcr-Abl **fusion protein** (a protein made using the information in the fusion gene), which forces the cells to grow and multiply [38, 39].

In some other cancers, you find translocations and gene rearrangements in which a control region from one gene (a promoter or enhancer[9]) has become fused to the protein-coding region[10] of a second gene. This has often happened during the development of prostate cancer and some forms of blood cancer such as non-Hodgkin lymphomas and myeloma (sometimes called multiple myeloma). In prostate cancer, the rearrangement often involves the *ERG* and *TMPRSS2* genes on chromosome 21. The rearrangement places the promoter from the *TMPRSS2* gene (a gene that is always active in prostate cells) next to the protein-coding region from a powerful, pro-growth protein called *ERG* [40] (see Figure 1.5). The consequence of this mutation is the massive overproduction of ERG protein, which forces the prostate cell to multiply.

Chromosome Insertions

An insertion is when **part of one chromosome is inserted into another chromosome** (Figure 1.6). It can also occur when part of a chromosome is reinserted back into the chromosome it came from, but in the wrong place. An example is the "internal tandem duplications" affecting the *FLT3* gene, which are found in the cancer cells of around a third of people with acute myeloid leukemia (AML) [41].

[9] The Khan Academy website has a nice description of gene regulation: https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/regulation-of-gene-expression-and-cell-specialization/a/overview-of-eukaryotic-gene-regulation.
[10] That is, the part of the gene that contains the instructions to make a protein.

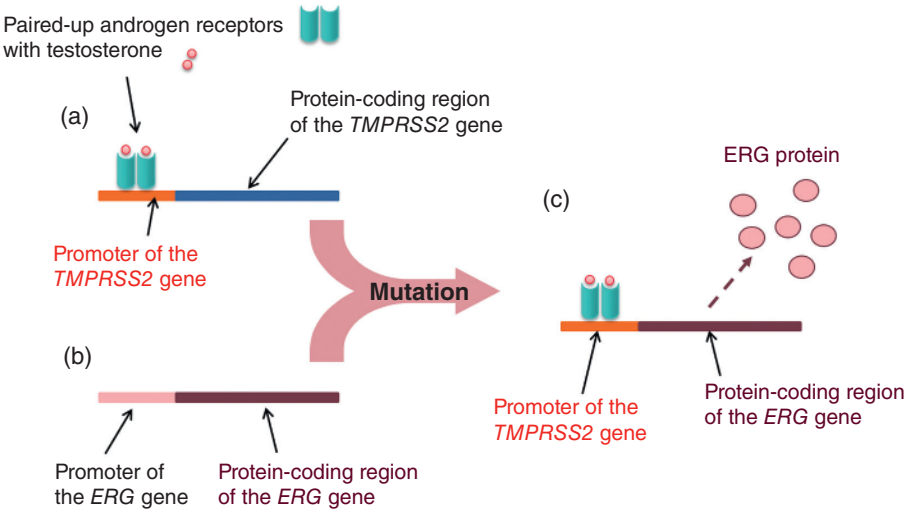

Figure 1.5 **The TMPRSS2-ERG gene fusion often found in prostate cancer cells. (a)** In healthy prostate cells, androgen receptors pair up due to the presence of testosterone. Paired-up receptors then attach to the *TMPRSS2* gene promoter and cause the cell to produce TMPRSS2 protein. **(b)** Prostate cells only rarely produce ERG because the *ERG* gene does not contain attachment sites for androgen receptors. **(c)** 50% of prostate cancers contain a mutation that puts the protein-coding region of the *ERG* gene under the control of the promoter from the *TMPRSS2* gene. This mutation causes the cell to overproduce ERG protein, which in turn forces the cell to multiply. *Source:* Adapted from Ref. [40].

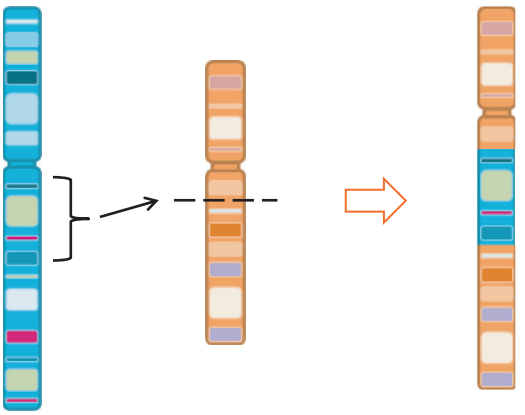

Figure 1.6 **A chromosome insertion** – part of one chromosome is inserted into another chromosome (as shown) or back into the chromosome it came from.

The insertion involves part of the *FLT3* gene, which is copied and reinserted back into the original gene. This causes the cell to make an extra-large, overactive version of FLT3 protein, which can be blocked with FLT3 inhibitors (see Section 3.6.8 for more on this).

Chromosome Deletions

Not surprisingly, a chromosome deletion is when **part of a chromosome gets deleted** (Figure 1.7a). An example is the deletion of the part of chromosome 17 that contains the *TP53* gene – this is often referred to as del(17p). *TP53* is a vital tumor suppressor gene that prevents faulty cells from becoming cancer cells (there is more about *TP53* in Sections 1.2.5 and 4.7). The loss of *TP53* means that part of the cell's protection against cancer has gone.

Chromosome Inversions

Inversions (Figure 1.7b), in which part **of a chromosome is cut out, flipped over, and then reinserted**, can also disrupt genes. For example, an inversion of part of chromosome 2 is found in about 4%–7% of non-small cell lung cancers (NSCLCs; this is the most common type of lung cancer). The inversion joins together the *ALK* gene with part of the *EML4* gene, creating an uncontrollable ALK fusion

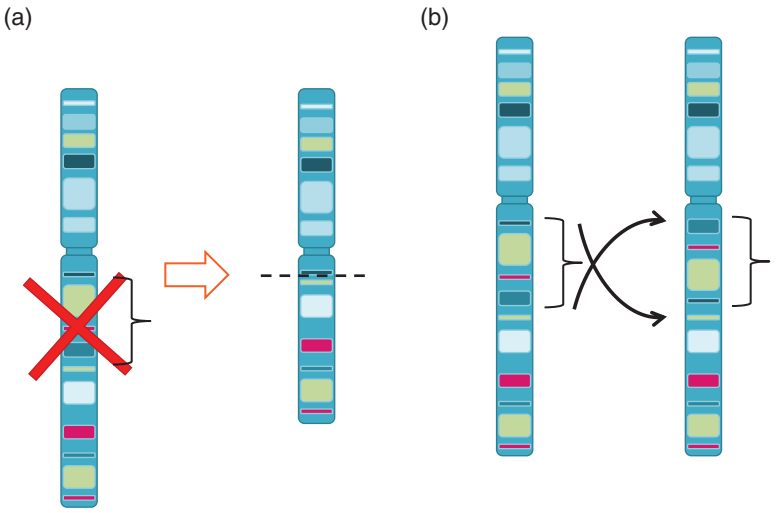

Figure 1.7 Chromosome deletions and inversions. (a) In a chromosome deletion, part of a chromosome is (not surprisingly) deleted. **(b)** Chromosome inversion – a segment of the chromosome is cut out, flipped over, and inserted back into the chromosome.

protein that forces the cells to multiply [42]. (For more about *ALK* mutations in lung cancer, and ALK inhibitors, see Section 3.6.5.)

Gene Amplification

Gene amplifications occur when a cell's DNA replication machinery **accidentally makes extra copies of a region of a chromosome** that contains one or more genes (see Figure 1.8). As a consequence, the cell overexpresses[11] the proteins made from the amplified genes. A common amplification is that of the *HER2* gene (also referred to as *NEU* or *ERBB2)*, which is amplified in about 15%–20% of breast cancers [43].

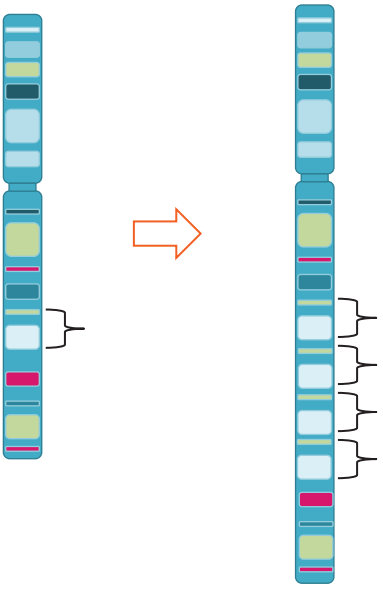

Figure 1.8 Gene amplification. The cell accidentally makes extra copies of a section of a chromosome. The duplicate segments are inserted into other chromosomes or back into the same chromosome.

Point Mutations and Indels

A point mutation is when **one DNA base is accidentally added, deleted, or swapped** for a different one. The term "indel" is used to refer to **small insertions and deletions affecting fewer than 1000 DNA base pairs**. Most point

[11] When a cell uses the information in a gene to make the corresponding protein, that gene is "expressed." So, if a gene is said to be overexpressed, that means that more of the corresponding protein is being made than is normal.

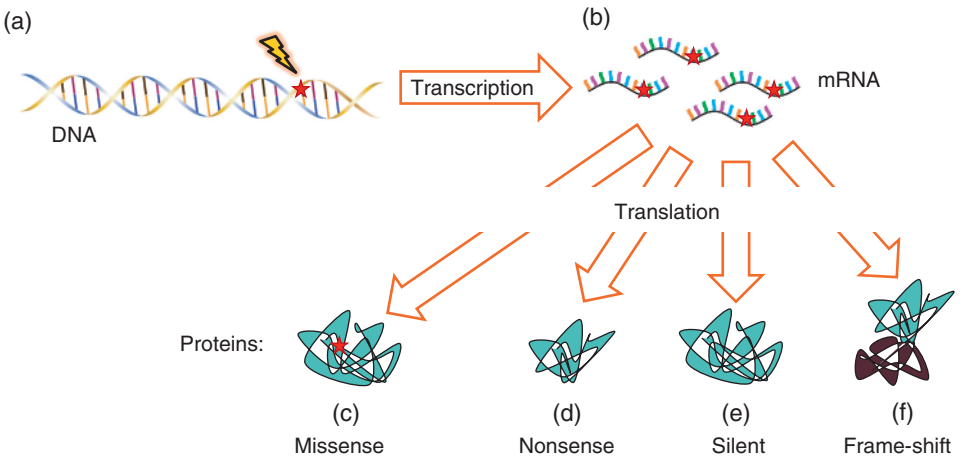

Figure 1.9 Point mutations. A point mutation (shown by a red star) is when one DNA base is added, deleted, or swapped for a different one in the cell's DNA. **(a)** If the mutation is in a gene, the mutation will be copied into the mRNA **(b)** and it may alter the resulting protein. The consequence might be that **(c)** due to a **missense mutation**, the protein made by the cell differs from the normal version of the protein by one amino acid, **(d)** **a nonsense mutation** in the DNA introduces a stop signal into the mRNA, and the cell makes an extra-short (truncated) protein, **(e)** a **silent mutation** has no impact on the protein produced, and **(f)** a **frameshift** mutation causes the cell to make a very different protein compared to the normal protein, which is only partly the same as the original. *Source:* DNA image from Pixabay. mRNA image by Christine I Miller, licensed under the Creative Commons Attribution-Share Alike 4.0 International license.

mutations and indels have no impact on the cell, as they occur outside of genes. However, if a mutation (such as a base substitution, addition, or deletion) occurs within a gene, it can have various consequences (see Figure 1.9).[12] Point mutations are classed as missense, nonsense, or silent, depending on what consequence the mutation has on protein production. They are also classified as "in-frame" or "frameshift" mutations [44]. All of these terms are explained in greater detail later.

Just one extra piece of information before I move on: The unmutated, normal version of any gene is often referred to as the "wild-type" version of that gene.

Missense and Nonsense Mutations

If one DNA base is substituted for a different one, this might change a single codon. As you might already know, proteins are constructed from long chains of 20 different amino acids. Each **set of three bases** (called a **codon**) in the mRNA strand tells the ribosome what amino acid to add next to the protein it's making.[13]

In a missense mutation, the change in a codon means that the **protein made from that gene differs by one amino acid** from the normal protein (Figure 1.9c) [44]. Two examples are the faulty version of the B-Raf protein (called V600E), which is often found in the

[12] If you need a refresher on gene transcription and translation at this point, I suggest taking a look at some of the resources suggested in the Appendix.
[13] If you're struggling to make sense of this, I would suggest looking at the Appendix and learning a bit about gene transcription and translocation.

cancer cells of people with malignant melanoma [45], and some of the faulty versions of EGFR, which are found in the cancer cells of some people with lung cancer [46]. In both cases, the faulty, cancer-causing versions of these proteins (both of which contain hundreds of amino acids) are just one amino acid different from the normal version of the protein. However, even changing that one amino acid is sufficient to create a massively overactive version of B-Raf or EGFR.

In contrast, nonsense mutations are those that cause the cell to make **a shortened (truncated) version of the protein** (Figure 1.9d). This happens because the original codon has now become a "stop codon." There are three codons (UAA, UAG, and UGA) that tell the ribosome to stop adding any more amino acids to a protein. If a DNA point mutation creates one of these stop codons part way through the mRNA, then the ribosome will stop part way through making the protein. For example, some of the inherited *BRCA* gene mutations that increase the risk of breast and ovarian cancer cause cells to produce a shortened version of a BRCA protein [47].

Silent Mutations

These point mutations **don't have any impact on the protein** the cell makes even if they occur within a gene (Figure 1.9e). For example, if a ribosome comes across the mRNA sequence CCC, this tells it to add a proline amino acid to the protein it's making. If a point mutation changes the mRNA from CCC to CCA, this has no impact because the sequence CCA also tells the ribosome to add a proline.

In-Frame and Frameshift Mutations

If one or two DNA bases are added to or deleted from a gene's sequence (or any number that isn't a multiple of three), this creates a **frameshift mutation** that is likely to have an enormous impact on what protein is produced (Figure 1.9f). An example is if one DNA base (a **C**) is added to a gene so that the mRNA goes from ….CGACGACGA…. to …CCGACGACGA…. Now, instead of adding three arginine amino acids to the protein (as directed by CGA-CGA-CGA), the ribosome adds a proline followed by two threonines (CCG-ACG-ACG). The ribosome carries on going from there, adding a completely different selection of amino acids from the normal sequence. As a result, **the protein the cell makes may bear very little resemblance to the normal protein**. Frameshift mutations also commonly introduce stop codons that create truncated proteins.

An "in-frame" mutation is opposite to a frameshift mutation in that it doesn't affect the rest of the protein. For example, if three bases are added to ….CGACGACGA…. so that it becomes ….CGA**CCC**CGACGA…, the ribosome will insert an extra proline in between the arginines, but it has no further impact.

1.2.3 Numbers and Patterns of DNA Mutations in Cancer Cells

In recent years, technologies have been developed that allow scientists to pinpoint the location and identity of thousands of DNA mutations inside cancer cells. They have discovered that **different cancers contain different numbers, types, and patterns of mutations that arise due to different mutational processes**. For example, lung cancers that develop in people with a history of smoking usually contain lots of point mutations in which a **C** DNA base has been changed to an **A**. A different pattern of mutations – where there are lots of insertions and deletions of more than three DNA bases at a time – is common in people with cancers associated with inherited *BRCA* gene mutations. Other patterns are linked to overactive APOBEC enzymes.

Scientists have discovered well over 100 different patterns of mutations in cancer cells, which they call "mutation signatures" [48–51]. It's possible for one cancer cell to contain multiple patterns of mutations because the cancer has arisen due to a combination of causes. This means that the various patterns of mutations found in a person's cancer cells are a bit like an archeological record showing what has happened inside the cell during the person's lifetime [48–52]. Some of the patterns will be due to natural aging processes, while others might reflect the person's choices through life, such as whether they took up smoking (Figure 1.10).

The amount of damage in cancer cells' DNA varies greatly from cancer type to cancer type, and from one person to another [5, 53]. Cancers that have come about because of the effects of powerful carcinogens often contain a vast amount of DNA damage. For example, lung cancers in people who are current smokers or have smoked in the past contain roughly ten times the amount of damage as lung cancers in people who have never smoked [54]. Melanoma skin cancers, which are almost always caused by UV light from the sun (or from sunbeds), also contain a vast number of mutations [5]. In general, cancers in older people contain more mutations than those in children and young adults simply because their cells have had more years in which to accumulate mutations.

Although cancer cells often contain hundreds or even thousands of mutations, most of these mutations have no discernible impact on the cell's behavior. They have occurred because the cancer cell is damaged and unstable and is

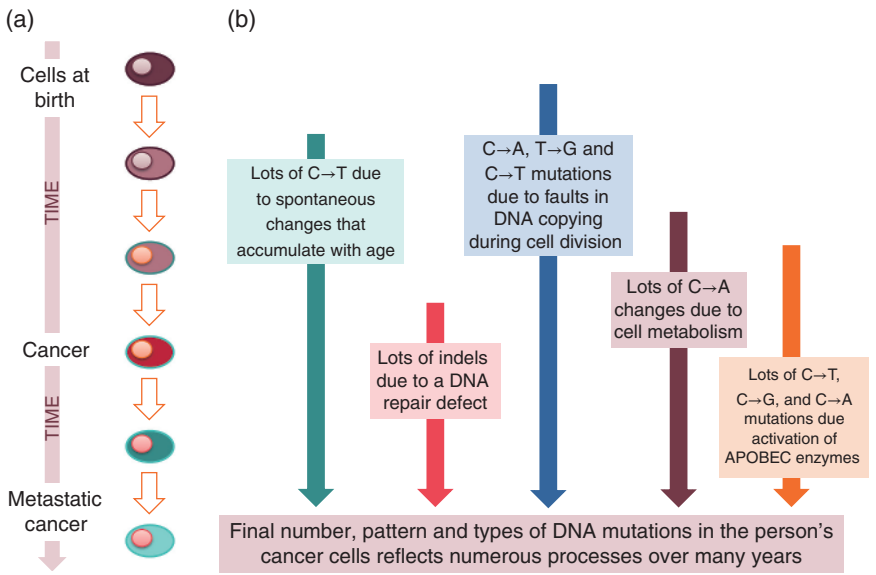

Figure 1.10 The mutations found in a person's cancer cells are a record of all the mutations that the cells have sustained throughout their lifetime. (a) At birth, the person's cells only contain the *germline* mutations they were born with. However, as they go through life, their cells accumulate additional *sporadic* mutations due to a variety of processes. Distinct patterns caused by different processes are called *mutational signatures*. The mutations in one cell may eventually cause it to become a cancer cell. The cancer cells later accumulate further mutations that cause metastasis. **(b)** Various mutational signatures are caused by different processes (e.g., aging and metabolism) and defects (e.g., faults in DNA repair processes and overactive APOBEC enzymes). *Source:* Adapted from Ref. [49].

picking up new mutations all the time. The mutations that are important in driving the cancer cells' abnormal behavior are referred to by scientists as **driver mutations**. Mutations that add little or nothing to the cells' behavior are called **passenger mutations**.

Perhaps not surprisingly, scientists are much more interested in finding a cancer's driver mutations than its passenger mutations. They want to know what's driving the cells' behavior so that they can do something about it.

1.2.4 Driver Mutations – Those that Affect Cancer Cell Behavior

For DNA damage to cause cancer, some of it must affect genes that control the cell's behavior. These "driver mutations" affect cell processes and behaviors such as:

- How fast the cell grows
- How frequently it multiplies
- The way it communicates with neighboring cells
- How often and how thoroughly it checks its own health and monitors and repairs DNA mutations
- Its ability to survive in adverse conditions such as low oxygen levels
- Its ability to extract itself from its normal environment and move elsewhere
- Whether it goes through all the normal checks and balances during the cell cycle[14]
- Whether it still has the ability to self-destruct by a process called apoptosis
- The way it produces energy
- Whether it can hide from or suppress the person's immune system.

The genes that control these behaviors are classed as oncogenes, tumor suppressor genes, and DNA repair genes.

Oncogenes

Many of the proteins made from oncogenes encourage our cells **to survive, grow, and multiply.**[15] Others can make cells more **mobile and invasive** or help them to **hide from the immune system**. All these genes need to be tightly controlled to avoid cancer. In cancer cells, the proteins that are made by oncogenes are often overproduced and/or overactive due to mutations. Examples of oncogenes include *EGFR*, *RAS*,[16] *BRAF*, *MYC*, *HER2*, and *SRC*.

Tumor Suppressor Genes

The proteins made from these genes **slow down or stop cell growth and proliferation and trigger apoptosis**. In cancer cells, they're damaged in a way that causes their protection to be lost. Examples include *TP53*, *PTEN*, *RB1*, and *APC*.

DNA Repair Genes

The proteins made from these genes **sense and repair DNA damage**. In cancer cells, they're damaged in such a way that they can no longer do their job properly. Because of this, cancer cells pick up more and more DNA damage as time goes on. Examples of DNA repair genes include *BRCA1*, *BRCA2*, *ATM*, *ATR*, *RAD51*, and *ERCC1*.

In healthy cells, the proteins made from DNA repair genes keep the cell's DNA free from faults. There is also a balancing act between the oncogenes and the tumor suppressor genes. For example, a protein called Bcl-2 protects cells from death, whereas a protein called p53 triggers death. The gene for making Bcl-2 (called *BCL2*) is an oncogene; the gene for making p53 (called *TP53*) is a tumor suppressor gene. Healthy cells contain

[14] The cell cycle is the normal, step-by-step process our cells go through when they multiply.
[15] For an explanation of oncogenes vs. proto-oncogenes, look in the Glossary.
[16] There are three main *RAS* genes: *KRAS*, *NRAS*, and *HRAS*.

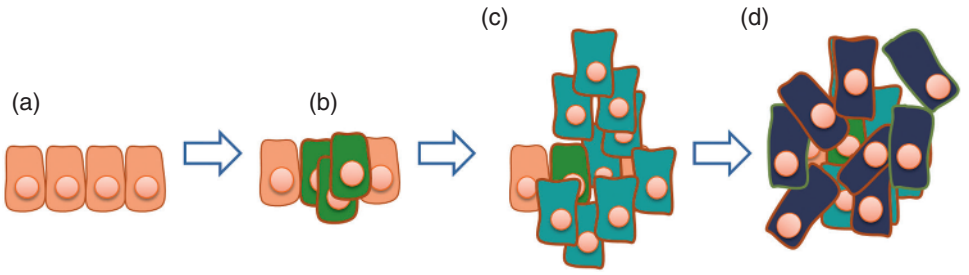

Figure 1.11 A series of mutations leads to bowel cancer [5]. **(a)** Orderly, well-connected cells in the lining of the bowel. **(b)** A random mutation in a bowel cell leads to loss of APC protein activity; this cell starts to multiply slightly faster than its neighbors, forming a little lump – an adenoma. The faulty cells are not cancer cells but, because they are multiplying more quickly than normal, they are prone to collecting more mutations. **(c)** Weeks, months, or years later, a mutation in the *KRAS* gene causes the K-Ras protein to become overactive; the cells now multiply rapidly and in a disorderly fashion. **(d)** Finally, genes like *TP53*, *PIK3CA*, and *SMAD4* are mutated. The faulty cells are now full-blown cancer cells, able to invade through local tissues, disrupt their function, and spread to other parts of the body.
Abbreviations: *APC* – adenomatous polyposis coli; *KRAS* – Kirsten rat sarcoma virus; *PIK3CA* – phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *SMAD4* – SMAD family member 4; *TP53* – tumor protein 53.

strict amounts of both proteins that balance each other out. But cancer cells often contain too much Bcl-2 and too little, or faulty, p53.

Multiple Driver Mutations Are Necessary for a Cell to Become a Cancer Cell

The gradual accumulation of mutations in several oncogenes, tumor suppressor genes, and DNA repair genes can ultimately cause someone to develop cancer. A sequence of events that frequently leads to bowel cancer is often given as an example (Figure 1.11).

Our bowel is lined by orderly layers of cells known as epithelial cells. Because bowel cells are constantly getting scraped off by food passing through, our bowel cells have to multiply pretty often in order to keep the number of cells constant. Cells that multiply are prone to picking up mutations. So, our bowel cells tend to contain more and more mutations as we get older. If a mutation affects a gene called *APC*, this is bad news as *APC* is an important tumor suppressor gene. But the situation isn't desperate as it's only one mutation, which isn't enough to cause bowel cancer. However, if it's followed by a mutation in *KRAS*, then the situation becomes worse; *KRAS* is a powerful growth-promoting oncogene that forces the cell to multiply more rapidly. As the cells multiply, they pick up yet more mutations. The cell still isn't a cancer cell because other protective proteins are still doing their job. But if genes such as *PIK3CA* (an oncogene), *SMAD4* (a tumor suppressor gene), and *TP53* (a tumor suppressor gene) become faulty, then the cell will become a full-blown cancer cell [5].

In other cancers, a similar **combination of mutations in a handful of important genes** is thought to drive their behavior [55].

1.2.5 The "Usual Suspects" – Genes Commonly Mutated in Many Cancers

Some gene mutations are common only in one or two types of cancer. These include the *VHL* mutations that are very common in kidney cancer and some of the translocations that are very common in hematological cancers (such as leukemias, lymphomas, and myeloma). But

other gene mutations crop up time and time again in many different cancer types. I'll be mentioning some of these gene mutations at various points in this book, so I've listed some of them in Table 1.1.

One thing that might (or might not!) jump out at you from the table is that many of the most commonly mutated genes in cancer cells are involved in **cell communication pathways**. These pathways are used by all our body's cells to sense and respond to changes in their environment, signals sent out by neighboring cells, the presence or absence of hormones, and signals sent out by white blood cells.

A wide variety of communication pathways exist in our cells, and they involve many different proteins. These pathways are often overactive in cancer cells, and they are the

Table 1.1 A selection of some of the most commonly mutated oncogenes, tumor suppressor genes, and DNA repair genes in human cancers.

Gene name (protein name)	What protein is made from this gene?	What is the consequence for the cell if the gene is mutated?
Oncogenes		
***RAS* (Ras)**	There are three main versions of the gene (*KRAS*, *NRAS*, and *HRAS*), which contain the instructions for making three Ras proteins (K-Ras, N-Ras, and H-Ras). They are enzymes that play a central role in cell communication [56].	All the proteins made from these genes are involved in cell communication pathways – the sequences of events triggered inside a cell when it receives a signal to grow and multiply from its neighbors. Therefore, all these proteins cause cells to grow and multiply. Overactive communication pathways also force cells to survive (even when damaged) and to become more mobile and invasive. For more on cell communication pathways and how they work, see Chapter 3, Section 3.2.
***PIK3CA* (p110alpha)**	The PI3K protein is an enzyme involved in cell communication. It comes in many different forms and is made up of two component parts: an enzyme part and a regulatory part. The *PIK3CA* gene encodes an enzyme part called p110alpha (p110α) [57].	
***HER2/NEU/ErbB2* (HER2)**	A receptor found on the cell surface, which activates cell communication pathways inside the cell [58].	
***MYC* (MYC)**	A transcription factor – it attaches to the promoters of various genes and triggers gene transcription. Many of the genes it controls are involved in cell growth and proliferation [59].	
***BRAF* (B-Raf)**	An enzyme involved in cell communication, activated by Ras proteins [60].	
***EGFR* (EGFR)**	A receptor found on the cell surface, which activates cell communication pathways inside the cell [58].	
Tumor suppressor genes		
***TP53* (p53)**	A transcription factor activated by DNA damage and other triggers – it attaches to various gene promoters and triggers gene transcription. The proteins produced as a result of p53 activity block cell proliferation and cause cell death [61].	If p53 is not working properly or is missing from a cell, the cell loses the ability to stop multiplying or die in response to DNA damage.
***PTEN* (PTEN)**	An enzyme involved in cell communication that blocks the activity of PI3K. PTEN also helps cells avoid DNA damage [62].	If PTEN is not working properly or is missing from a cell, the PI3K-controlled communication pathway becomes overactive.

(*Continued*)

Table 1.1 (Continued)

Gene name (protein name)	What protein is made from this gene?	What is the consequence for the cell if the gene is mutated?
***RB* (*RB*)**	RB has a pocket in its surface that fits E2F proteins, which control entry into the cell cycle[a]. RB holds onto and blocks E2F proteins, and this prevents cells from multiplying [63].	If RB is not working properly or is missing from a cell, E2F can force the cell into the cell cycle (for more about RB and E2F, see Chapter 4, Section 4.5).
CDKN2A **(p16 INK4a)**	$p16^{INK4a}$ is a protein that blocks a set of enzymes called the cyclin-dependent kinases (CDKs). CDKs force RB to let go of E2F proteins (see the description of RB mentioned earlier). By blocking CDKs, $p16^{INK4a}$ prevents cells from entering the cell cycle (see Chapter 4, Figures 4.18 and 4.19) [64].	If $p16^{INK4a}$ is not working properly or is missing from a cell, E2F proteins force the cell into the cell cycle.
NF1 **(neurofibromin)**	A large protein that inactivates Ras proteins (see the description of Ras earlier in this table) [65].	If neurofibromin is not working properly or is missing from a cell, Ras proteins become overactive.
***APC* (APC)**	The surface of the APC protein has various different regions through which it interacts with many different proteins involved in cell communication, mobility, adhesion to neighboring cells, and other processes [66].	If APC is not working properly or is missing from a cell, then levels of another protein, beta-catenin (β-catenin), rise. Beta-catenin causes cells to multiply.
DNA repair genes		
BRCA1 **(BRCA1) and** ***BRCA2*** **(BRCA2)**	BRCA1 and BRCA2 proteins are both necessary for a DNA repair process called homologous recombination (HR). Our cells use HR to accurately repair double-strand breaks in their DNA [67] (see Chapter 4, Section 4.3 for more information on BRCA proteins).	If either BRCA1 or BRCA2 is not working properly or is missing from a cell, the cell can no longer perform HR. The cell then has to rely on less accurate repair mechanisms and is liable to pick up further DNA mutations.
ATM* and *ATR	Cells trigger the activity of ATM and ATR proteins when they detect damage to their DNA. Together, these enzymes coordinate the cell's response to the damage [68].	If either ATM or ATR is damaged or missing from a cell, its ability to respond to DNA damage is compromised.

Abbreviations: APC – adenomatous polyposis coli; ATM – ataxia-telangiectasia mutated; ATR – ATM and Rad3 related; BRCA – breast cancer susceptibility gene; EGFR – epidermal growth factor receptor; HER2 – human epidermal growth factor receptor-2; NF1 – neurofibromatosis type 1; *PIK3CA* – phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN – phosphatase and tensin homolog; Ras – rat sarcoma virus; RB – retinoblastoma protein; TP53 – tumor protein p53.

[a] The cell cycle is the very orderly and precise sequence of events that a cell goes through in order to multiply.

target of various cancer drugs. The whole of Chapter 3 is dedicated to cell communication pathways and the drugs that block them.

1.3 THE DEFINING FEATURES (HALLMARKS) OF CANCER CELLS

All cancers are presumed to begin with a single cell that has sustained damage to its DNA and has multiplied out of control. As an adult, it's true that every cell in our body contains some sort of damage to its DNA [69, 70]. However, what sets a cancer cell apart from a non-cancer cell is the following:

- The **amount** and **type** of DNA damage the cells contain
- Damage in oncogenes, tumor suppressor genes, and DNA repair genes
- The **changes in behavior** that the damage causes
- The ability of the damaged cell to **overcome suppression** by neighboring cells and **avoid destruction** by the person's immune system.

The behavioral changes that set a cancer cell apart from a healthy cell are collectively known as "the hallmarks of cancer." These hallmarks are the brainchild of two scientists called Professor Douglas Hanahan and Professor Robert Weinberg, who came up with a list of six back in 2000 [71]. They added two more in 2011 and another two in 2022 [72, 73]. They also described four "enabling characteristics" – features of cancers and their environment that allow them to develop and that sustain their growth.

1.3.1 Ten Hallmarks of Cancer (Plus Four Enabling Characteristics)

The hallmarks can be summarised as: [71, 72, 74]

1. **They can tell themselves to multiply**. A normal cell only multiplies when it receives an instruction[17] to do so. A cancer cell can generate those instructions itself
2. **They are insensitive to negative feedback**, because proteins that would normally tell them to stop multiplying and die (like p53) have been lost or don't work properly.
3. **They resist death**. Every day, millions of cells in our body self-destruct because they have worn out or become damaged. Cancer cells have defects that make it almost impossible for them to do this.
4. **Cancer cells can multiply forever** because they contain a protein called telomerase. Healthy cells lack this protein and eventually stop multiplying.
5. **They develop a blood supply to gain access to oxygen and nutrients**. Cancer cells release a tiny protein called VEGF that tells nearby blood vessels to sprout and grow (a process called **angiogenesis** – see Section 1.6.3 for more on this process). They also take advantage of existing blood vessels.
6. **They can invade and spread**. Most of our body's cells are connected to each other in orderly arrangements. Cancer cells have lost connective proteins from their surface, and they are independent and mobile.
7. **They have changed the way they produce energy**. Healthy cells use sugars from our food to make energy using a highly efficient, oxygen-dependent process. Cancer cells use an inefficient process that requires less oxygen but helps them multiply more quickly.
8. **They can avoid destruction by the immune system**. White blood cells constantly patrol our body, looking for defective cells. Cancer cells hide from white blood cells, suppress cancer-fighting white blood cells and co-opt white blood cells for their own purposes (there is lots more about this in Section 1.5).
9. **They are more changeable than healthy cells**. Normal cells mature and specialize until they are perfectly adapted for a specific function. Cancer cells are more plastic – they adapt and change depending on their circumstances.
10. **Tumors contain senescent cells**. Senescent cells (those that have stopped multiplying and that have shut down many internal systems) appear to send signals that encourage cancer cells to multiply and protect them from the effects of treatments.

Enabling Characteristics

1. **Cancer cells are genetically unstable**. Cancer cells gain new DNA mutations all the time, and they evolve and diversify as time goes by. (I'll come back to this topic and its importance in Sections 1.4 and 1.9.4.)
2. **Cancer cells are also epigenetically unstable**. When a scientist talks about epigenetics, they're referring to chemical changes that influence how tightly packed DNA is. This influences whether the genes in that region

[17] This instruction is usually in the form of small proteins known as "growth factors" released by the cells' near neighbors – see Chapter 3, Section 3.2.1 for more on this.

of DNA are active and used to make proteins or not. Inside tumors, low levels of oxygen and nutrients and other cues cause cancer cells to alter their epigenetics. This is another cause of variation among cancer cells and their ability to adapt to changing circumstances.

3. **Cancer cells live in an inflammatory environment**. Normal inflammation occurs in response to tissue damage – it encourages repair and acts as a magnet for white blood cells that clear up any infection. This same mechanism is hijacked by cancer cells. Inflammatory white blood cells found inside tumors help cancer cells avoid destruction and support their growth and spread (for more on this, see Section 1.5.3).
4. **Cancer cells and cancer treatments are influenced by bacteria and other microbes**. The range and types of bacteria, fungi, and other microbes found inside a tumor differ from person to person and affect cancer cell behavior. The effectiveness of some cancer treatments is also influenced by the microbes living in the person's digestive tract (the gut microbiome). This is a new and complicated area of cancer research and biology, and we have a lot still to learn. I'll return to it in Chapter 5 when I discuss biomarkers of response to checkpoint inhibitor immunotherapy.

1.4 VARIATION AMONG CANCER CELLS IN A SINGLE TUMOR

A major reason why many tumors fail to respond to treatment or become resistant later is **intratumoral heterogeneity** – the fact that inside a tumor there are various populations of cancer cells that are different from each other. This variation is often genetic, with different populations of cells containing different combinations of DNA mutations (Figure 1.12).

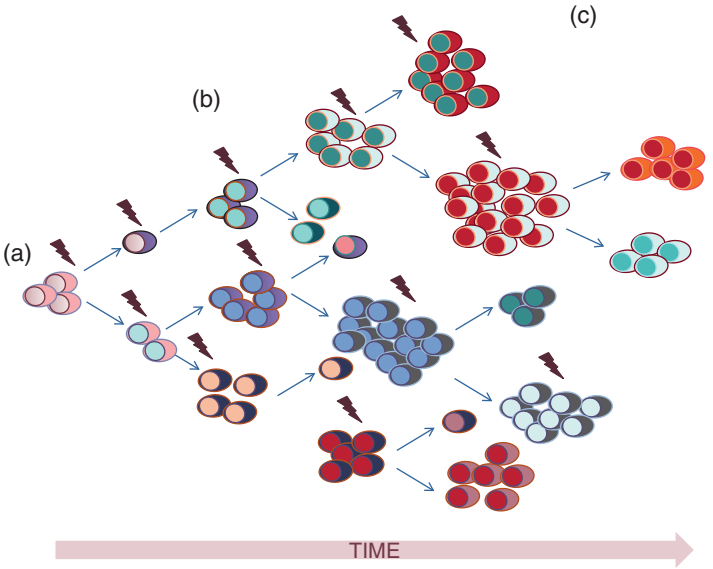

Figure 1.12 Genome instability causes intratumoral heterogeneity. **(a)** In a newly emerged cluster of cancer cells, all the cells are likely to contain the same genetic faults. However, each cell is genomically unstable and likely to pick up more mutations over time. **(b)** The cells start to evolve and become different from one another. **(c)** As time goes on, the cells diverge from each other more and more, creating distinct populations of cells driven by different sets of mutations.

For example, scientists analyzing multiple biopsies from a single tumor have found huge variations in the number, type, and chromosome location of genetic mutations in the person's cancer cells. One of the first and most comprehensive analyses of this phenomenon was conducted by a group of British scientists who studied tumor biopsy samples from people with kidney cancer [73]. When investigating 12 samples taken from one patient, they found that only a third of the 128 DNA mutations they discovered were present in all 12 samples. Similar studies investigating tumor samples from people with other cancer types have revealed similar stories [75–77].

1.4.1 Causes of Genetic Heterogeneity

It seems that as a cancer grows, the cells within it evolve and change. This is because cancer cells are **genomically unstable** – they accumulate DNA damage at a faster rate than healthy cells. There are various reasons for this instability, some of the most important of which are the following [78, 79]:

- Cancer cells contain faults in DNA repair genes and this compromises their ability to detect and repair DNA damage.
- Cancer cells' apoptosis machinery is faulty, which means they stay alive despite containing lots of DNA damage.
- The normal mechanisms that ensure each cell has the correct number of chromosomes and that help to avoid chromosome breakages and fusions are lost.
- The cells' ability to replicate their DNA accurately is compromised.
- Some cancer cells are continually exposed to mutagens such as tobacco smoke or UV light.
- Cancer cells contain mutations in powerful oncogenes that destabilize the cell and lead to further mutations.

Because of genomic instability, over the weeks, months, and years that go by before a cancer is diagnosed (and in the weeks, months, and years afterward), cancer cells emerge that have different combinations of mutations compared to their predecessors. And, as time goes on, the cancer cells within a tumor become more diverse.

1.4.2 Other Types of Heterogeneity

In addition to the cancer cells in a tumor (and any metastases[18]) being genetically diverse, they are also **epigenetically diverse**. In some parts of a tumor, certain genes might be suppressed because their DNA is too tightly coiled to allow gene transcription. In other parts of a tumor, or perhaps in a metastasis, those same genes might be relaxed and used to make protein.

Epigenetic gene regulation is influenced by a cell's environment (such as oxygen levels, nutrient availability, acidity, and the presence of chemical signals released by other cells). Many mutations found in cancer cells also affect epigenetic enzymes. As a result, epigenetic diversity can arise from both environmental and genetic influences on the cells' DNA [80].

Other causes of diversity include metabolic differences, with groups of cells performing all the chemical reactions they need to sustain life using distinct combinations of enzymes and chemicals. Cells can also differ in other ways, such as in terms of their appearance and what proteins they secrete into their surroundings [81].

[18] Metastases are tumors found in new locations of the body, in contrast to the primary tumor, which is where the cancer started. Metastases are also referred to as "secondaries."

The amount of variation among cancer cells in a person's body has a profound impact on the effectiveness of treatment, so I shall return to this topic in Section 1.9.

1.5 CANCER'S RELATIONSHIP WITH OUR IMMUNE SYSTEM

The fact that someone has cancer indicates that at least two important things have happened:

1. **One of their cells has sustained damage in the form of DNA mutations** that have caused it to become a cancer cell. This cell has then multiplied many times to become a detectable tumor or blood cancer.
2. For some reason, **their immune system has failed to recognize and destroy their cancer cells**.

So far in this book I have focused on the DNA mutations inside cancer cells. But now I want to shift my attention away from DNA mutations and look at the interaction between cancer cells and our immune system.

If you'd like to learn a bit about the immune system before we start, then you might want to get hold of an immunology textbook such as *How the Immune System Works* by Lauren Sompayrac [82] or, for more detail, the classic undergraduate textbook is Janeway's *Immunobiology* [83]. Or you could look on YouTube for immunology lectures. For example, a doctor in Australia called Armando Hasudungan has created a series of illustrated videos that introduce you to many immunology concepts (http://armandoh.org/subjects/immunology). As he says himself, his videos don't cover everything, but they're a great starting point if you've not learned any immunology before.

For the rest of this section, I'll focus on how cancer cells avoid getting destroyed by white blood cells, but also how they eventually use white blood cells for their own purposes.

I'll begin by describing how our immune system monitors our cells for signs of damage, and I'll introduce you to T cells. Then I'll look at the "cancer-immunity cycle" – the name given to the process through which our body generates a cancer-fighting immune response. From there, I'll describe the sorts of white blood cells you commonly find inside tumors, and their role in helping cancer cells thrive and survive. Lastly, I'll explain how cancer cells are ultimately able to hide from, suppress, and escape the immune system's control.

1.5.1 How Our Immune System Monitors for Signs of Damage and Destroys Faulty Cells

Probably the first thing to say is that **one of the roles of our immune system is to protect us from cancer** by detecting and destroying cancer cells.

We'll probably never know how often cancer cells pop up in our body. But immunologists reckon that our immune system is *"recognizing and destroying little cancers as they develop all the time. If we didn't have an immune system, then we would be developing cancer a lot more often"* [84].

Various types of white blood cells participate in destroying any cancer cells that emerge, but the most important are our T cells (aided by dendritic cells) and natural killer (NK) cells (see Box 1.2 for a description of various types of white blood cells).

At the heart of our immune system's ability to detect cancer cells is the fact that **all our cells are constantly showing our immune system what's going on inside them**. This means that passing white blood cells can instantly see which cells are healthy and identify any that have become faulty.

Box 1.2 Descriptions of some of the white blood cells mentioned in this book listed in alphabetical order

Antigen-presenting cells (APCs). These include dendritic cells and macrophages. These cells display tiny protein fragments (called **peptide antigens**[19]) to T cells. The peptides can have many different sources, such as being a fragment of a mutated protein from a cancer cell (sometimes called a neoantigen) or being part of a virus that is causing an infection. APCs "present" antigens to T cells using tiny cup-like structures on their surface called MHC proteins.

B cells (B lymphocytes). These lymphocytes have B cell receptors (BCRs) on their surface. Once they have matured in the bone marrow, they move to lymph nodes and other lymphoid organs (like the spleen) in search of infections. If their BCRs connect with an antigen (such as a protein fragment from a bacterium or virus), the B cell may become fully active. When fully active, some of these B cells become long-lived, antibody-producing B cells called **plasma cells.** These plasma cells move back to the bone marrow, from where they release millions of copies of their antibody into the blood.

Dendritic cells. Starfish-shaped APCs that shuttle between tissues and lymphoid organs (such as lymph nodes, spleen, and tonsils). A different set of dendritic cells – the follicular dendritic cells – spend their whole life in lymph nodes and other lymph tissues. They capture antigens delivered to them by the flow of lymph fluid and display them to B cells.

Leukocytes. A collective term for all white blood cells.

Lymphocytes. A collective term for B cells and T cells.

Macrophages. The most versatile, "jack-of-all-trades" cells of the immune system. They have the capacity to ingest and destroy invaders, act as APCs, activate T cells, and rid the body of cell debris. However, whereas dendritic cells can shuttle to lymph nodes and other lymphoid organs, macrophages generally stay put in our organs and tissues. Macrophages found in particular locations often look distinctive and have different names, such as Kupffer cells in the liver, Langerhans cells in the skin, and microglia in the brain.

Mast cells. Myeloid cells with histamine-containing granules inside them. The release of their histamine granules helps fight infections but also causes allergic reactions and inflammation.

Myeloid cells. A collective term for white blood cells that develop from myeloblasts. They include macrophages, basophils, neutrophils, eosinophils, and dendritic cells.

Myeloid-derived suppressor cells. Scientists don't really understand these cells, but large numbers of them are found inside tumors and in the blood of people with cancer. They can suppress cytotoxic T cells and also seem to be involved in angiogenesis.

[19] Any peptide (or other molecule) that triggers an immune response is called an antigen. If the source of the antigen is a mutated protein found inside a person's cancer cells, then it's often referred to as a **neoantigen**.

Neutrophils. Short-lived myeloid cells that enter tissues and destroy infections. They are the most numerous white blood cells in our body.

T cells (T lymphocytes):

Helper T cells. T cells that respond via their T cell receptor (TCR) to antigens presented to them by APCs via MHC class 2 proteins. They require further activation signals (called "costimulatory signals") from APCs to become fully active. Once active, they then activate B cells and enhance the activity of other white blood cells.

Cytotoxic T cells (CTLs). T cells that respond via their TCR to antigens presented to them by APCs via MHC class 1 proteins. Once they have received the necessary co-stimulatory signals from the APC, they directly destroy virus-infected cells and cancer cells in the affected tissue by releasing cell-killing enzymes.

Regulatory T cells (Tregs). T cells whose job it is to suppress any T cells that might otherwise attack the body's tissues and cause autoimmune diseases; they also prevent the overactivity of T cells that would otherwise cause tissue damage.

Natural killer (NK) cells. Short-lived T cells that enter tissues, release cytokines, and directly destroy virus-infected cells and cancer cells.

Source: Adapted from Ref. [85].

None of this would be possible without special proteins found on our cells' surface called **major histocompatibility complex** (MHC) class 1 proteins (see Box 1.3 for more about them). These proteins are like little cups sticking out from the cells' surface. Inside each of these cups is a tiny fragment from one of the proteins the cell is making. The fragments (called **peptides** or **peptide antigens**)[20] are usually just 8–11 amino acids long [86]. All our cells should be decorated with thousands of cups, which they use to show passing white blood cells tens of thousands of different peptides (Figure 1.13).

Specialized T cells (called cytotoxic T cells) that are passing by take a look at the peptide antigens presented to them by each cell. If the peptides displayed by a cell indicate that it is infected by a virus or has become faulty, the passing T cells immediately release toxic, cell-killing enzymes that destroy the faulty cell. **However, T cells are only able to do this if they have already been activated by a dendritic cell**.

Another type of white blood cell that can detect and destroy cancer cells is the NK cells. These cells destroy cells that have lost MHC class 1 proteins from their surface altogether and that have avoided destruction by cytotoxic T cells.

I apologize if this is already starting to feel very complicated. Immunology is one of those subjects that triggers long sighs and tired brains, and yet, once you get into it, it's

[20] Proteins are made from tiny chemical building blocks called amino acids, which are linked together in long chains to form proteins. There are about 20 or so different amino acids, and some proteins contain many thousands of them. A peptide is a short piece of protein – the term is usually used to describe a piece of protein containing anywhere from 2 up to 50 amino acids. "Peptide antigen" is the name given to any peptide that can trigger a response from the immune system.

Box 1.3 MHC proteins

MHC (major histocompatibility complex) proteins come in two main types: MHC class 1 and MHC class 2. Whereas all our cells have MHC class 1 proteins on their surface, only specialized cells called antigen-presenting cells (APCs) have MHC class 2 proteins on their surface. MHC proteins allow our cells to present small protein fragments (peptides) to one another.

MHC class 1 proteins are used by our cells to display peptides from proteins that they have manufactured themselves.

MHC class 2 proteins are found on APCs such as dendritic cells, which use them to display peptides that they have picked up from their environment.

Source: Adapted from Ref. [86,87].

hard not to be captivated by its beauty and complexity [88]. If you feel like you've already had enough for now, then I suggest moving on to the next section and coming back to this later, maybe once you start reading the chapters on immunotherapy. Or, if you're happy to prepare yourself with a cup of tea (I am from the United Kingdom after all), then do take a deep breath and prepare to continue!

T cell activation is a rather complicated process. But I do want to describe it to you as I think it will help you in a couple of ways: (1) It will help you to make sense of the sentence in which I said that only T cells that have been activated by dendritic cells can kill faulty cells, and (2) it will help you to understand how diverse forms of immunotherapy can all activate T cells (such as peptide and DNA vaccines, dendritic cell vaccines, oncolytic viruses, and checkpoint inhibitors).

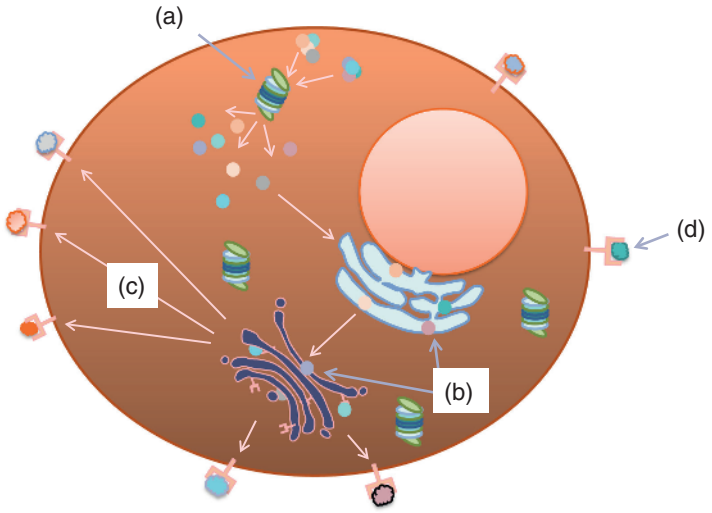

Figure 1.13 Cells use MHC class 1 proteins to display their inner workings to the immune system. **(a)** The cell breaks up a representative sample of its proteins into short peptides using its proteasomes. **(b)** These peptides are processed further in the endoplasmic reticulum and Golgi apparatus and assembled with MHC class 1 proteins. **(c)** Peptide-MHC class 1 complexes are transported to the cell's outer membrane. **(d)** Peptides are "presented" to passing T cells and then recycled and replaced with new ones. *Source:* Adapted from Ref. [86].

Let's start with the fact that dendritic cells activate T cells. Dendritic cells are starfish-shaped cells that act as our body's patrolmen. They are found throughout our body, taking position wherever we're most likely to be exposed to infections, e.g., in our digestive tract, skin, and airways. A key feature of dendritic cells is that they're constantly hoovering up stuff (like bacteria, viruses, and cell debris) from their surroundings. If they detect signs of an infection or another problem, they journey to nearby lymph nodes.[21] As they move, they become fully mature. They also transfer the debris to their surface in the form of short peptide antigens, which they assemble into their MHC proteins and display on their surface. During their journey, which typically takes a day or so, they also increase their production of B7 proteins [86].

When they reach a lymph node, fully mature dendritic cells search for T cells whose T cell receptors (TCRs) match the shape of the peptide antigens displayed by the dendritic cells' MHC proteins. (Apparently, each dendritic cell can scan the surface of about 1000 T cells per hour.) If such a match occurs, the T cell might become active. But to do so, it also needs to receive further activation signals, such as connecting with a B7 protein and receiving suitable signals in the form of cytokines from other white blood cells (Figure 1.14). Activated

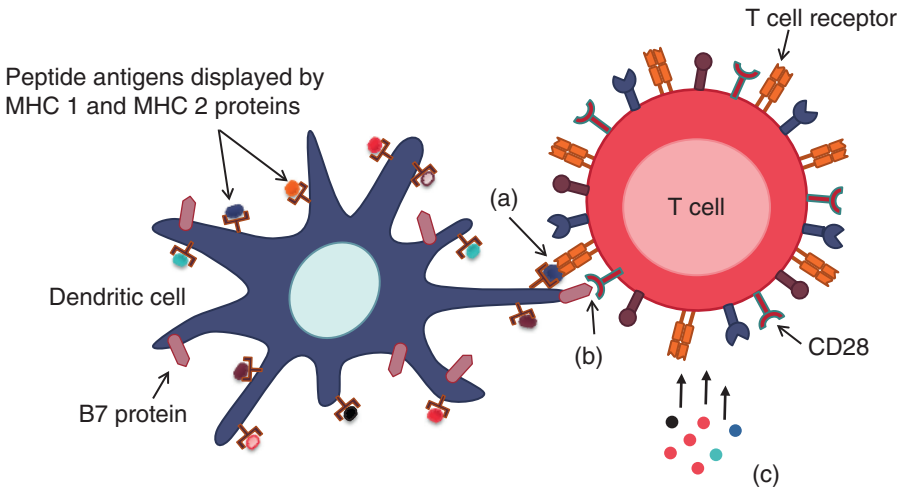

Figure 1.14 T cells are activated by dendritic cells. There are three signals that a T cell needs to receive to become fully active. **(a)** The first activation signal comes from a dendritic cell that displays a peptide antigen on its surface that matches the shape of the T cell's T cell receptor. **(b)** The second signal also comes from the dendritic cell in the form of a B7 protein on the dendritic cell's surface, which connects with CD28, found on the T cell. **(c)** The third signal comes from cytokines – a family of small proteins that act as signals sent out by white blood cells. They include interleukins (e.g., IL-6, IL-2, and IL-4). Interleukins connect with more receptors on the surface of the T cell, telling the T cell what sort of T cell to become (there are many subtypes of T cells) and encouraging it to multiply and survive. *Source:* Adapted from Ref. [89].

[21] Lymph nodes are small, bean-shaped patches of tissue, and we have hundreds of them scattered throughout our body. They are places where white blood cells congregate, and where bacteria and viruses get carried by the flow of lymph fluid. They are also where dendritic cells take everything they've picked up while out on patrol.

T cells now multiply rapidly. If they're cytotoxic T cells, they'll leave the lymph node and start hunting down and destroying cells that display on their surface the same peptide antigen shown to them by the dendritic cell [89].

You might have already noticed from Box 1.2 that there are various T cell types (and many subtypes) in our body. Out of all of them, it's the cytotoxic T cells[22] that can directly interact with and destroy other cells. They do this by getting close to the target cell and then releasing cell-killing enzymes called perforin and granzymes. These enzymes do things like drilling holes in the target cell's surface, causing it to become leaky and die – a process that takes about half an hour [90].

1.5.2 The Cancer-Immunity Cycle

Hopefully, by now, you're beginning to understand how our immune system can detect and destroy cancer cells. Perhaps the most famous journal article describing this process is the review written by two American scientists, Daniel Chen and Ira Mellman. They published their article in 2013 in the prestigious scientific journal "Cell" and coined the phrase, **The Cancer-Immunity Cycle** [91] (Figure 1.15).[23]

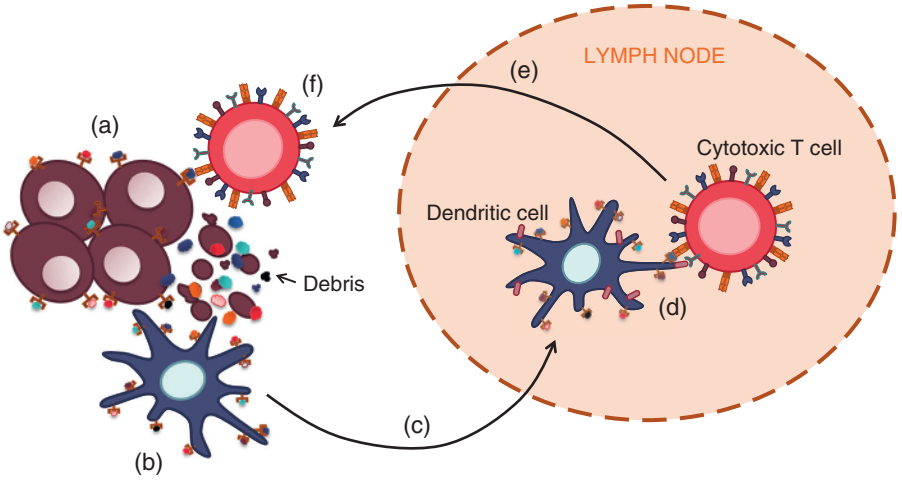

Figure 1.15 The Cancer-Immunity Cycle. **(a)** Tumors contain living cancer cells and a lot of debris from dead and dying cells. **(b)** Dendritic cells that find their way into a tumor may be activated by this debris, so long as it is accompanied by "danger" signals released by the dying cells and other cells in the tumor, such as various white blood cells. If the right signals are present, the dendritic cell hoovers up some debris. **(c)** The dendritic cell breaks up the debris to create short peptides that it displays on its surface using both class 1 and class 2 MHC proteins. It travels to a nearby lymph node. **(d)** In the lymph node, the dendritic cell presents the peptides on its surface to T cells, trying to find a match. T cells with T cell receptors that match the shape of peptides on the dendritic cell are activated and multiply. **(e)** Some of these activated T cells find their way into the tumor. **(f)** In the tumor, activated T cells destroy cancer cells with the same peptides displayed on their surface as those presented to the T cells by dendritic cells.

[22] Cytotoxic T cells are also referred to as "CD8-positive" T cells, or as "killer" T cells.
[23] You can find out how their famous article came about in this profile piece on the Genentech website: https://www.gene.com/stories/behind-the-cycle.

I hope you've noticed that this process has lots of "ifs, buts, and maybes" about it.

For example, IF a dendritic cell gets into a tumor, it MIGHT become active, but ONLY if it also receives other signals. Also, if an activated dendritic cell reaches a lymph node, and IF it finds suitable T cells to activate, those T cells MIGHT know where the cancer cells are located, and they MIGHT get into the tumor environment. In that new environment, they MIGHT manage to stay active long enough to kill some cancer cells. But there are no guarantees that any of this will happen. This brings us to the next Section: How cancer cells avoid detection and destruction by the immune system.

1.5.3 How Cancer Cells Avoid Destruction by the Immune System

There are many reasons why a person's cancer cells might not be destroyed by their immune system. Some of these reasons are important to understand as they can help us make sense of various forms of immunotherapy, such as the immune checkpoint inhibitors I describe in Chapter 5 like nivolumab, pembrolizumab, and ipilimumab.

These reasons can be categorized depending on whether they're directly to do with the person's cancer cells, their T cells, or a different mechanism. I've listed some of these reasons later, but this is by no means an exhaustive list.

Reasons Why the Person's Cancer Cells Aren't Detected by Their Immune System

- **The person's cancer cells might not contain the types of DNA mutations that lend themselves to recognition by the immune system**. For example, cancer cells with a handful of large-scale chromosome defects are much less visible to the immune system than those with thousands of small-scale mutations caused by powerful mutators such as components of tobacco smoke, UV light, defects in DNA repair processes, or overactive APOBEC enzymes [92].
- **If there's too much diversity in terms of the mutations the cancer cells contain, this can bamboozle the immune system**. The immune system has a better chance of detecting cancer if there are lots of mutations that are the same in every cancer cell in the tumor and in any metastases (called *clonal mutations*), rather than when there are diverse cancer cells with different mutations inside them [92, 93].
- **If the person's cancer cells have lost much of their ability to process peptides and display them via MHC class 1 proteins, they will be invisible to the immune system** [94].
- **Some of the gene mutations found in cancer cells seem to go hand-in-hand with a lack of immune response**. Examples include amplification of the *CCND1* gene, and mutations affecting *RAS* genes, *MYC, EGFR, HER2, PTEN,* and *TP53* [92, 95].

Sometimes Their Dendritic Cells Don't Present Peptide Antigens to T Cells

Reasons for this could be: [94]

- In some people, their cancer cells seem able to **prevent dendritic cells from becoming mature and active**, so they never pick up any tumor debris.
- Some cancer cells **limit the ability of activated dendritic cells to internalize and process cancer peptides**, reducing their ability to present peptide antigens to T cells.
- **The continued presence of cancer cells in a tissue can gradually lead to a phenomenon called immune tolerance** (see Box 1.4). This is a situation in which dendritic cells (and other white blood cells) start believing that the presence of cancer cells is normal and nothing to be concerned about, so they don't raise the alarm.

Box 1.4 Immune tolerance

Immune tolerance is essential to life. It describes the fact that our immune system ignores (and therefore doesn't attack) the normal proteins and other molecules that make up our body. If our immune system can't ignore our own healthy proteins, then we develop autoimmune diseases. However, cancers often hide from the immune system by inducing immune tolerance. If the immune system is tolerant to cancer cells and believes that they're normal, it will no longer try to attack them.

Box 1.5 Why do we have immune-suppressing white blood cells?

Our immune system fights off infections, destroys faulty cells, and keeps us healthy. However, it's important that immune responses don't outlive their usefulness. If immune responses last too long, the activated white blood cells will start doing damage to otherwise healthy tissues and organs. Hence, we have white blood cells such as **regulatory T cells** that exist to suppress and restrain other white blood cells. In addition, these restraining white blood cells prevent our immune system from destroying helpful bacteria in our gut, and they help prevent the growing embryo from being destroyed in the womb during pregnancy.

Sometimes the Person's Immune System Creates Cancer-fighting T Cells, But They Can't Get to Where They're Needed

Reasons for this could be: [93, 94]

- **Tumor blood vessels are strange and chaotic**. They can act as a barrier that prevents T cells from leaving the blood and getting in among the cancer cells (I come back to the strangeness of tumor blood vessels in Section 1.6.3) [93, 94].
- **Cytotoxic T cells that recognize peptide antigens displayed by cancer cells don't necessarily know where the tumor is** and may end up elsewhere in the body rather than inside the tumor.

Sometimes Cancer-Fighting T Cells Get into a Tumor But then Become Suppressed or Exhausted

Reasons for this could be: [96–99]

- In many tumors, cancer cells produce signaling molecules that attract and activate immune-suppressing white blood cells such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and certain types of B cells. **These white blood cells suppress any cancer-fighting cytotoxic T cells or NK cells in their vicinity** (see Box 1.5 for more about immune-suppressing white blood cells) [96–99].
- Activated T cells that enter tumors and start killing cancer cells eventually become **overworked, overstimulated, and exhausted**.
- Various types of cell in a tumor, including cancer cells and white blood cells, produce a range of small proteins called *growth factors* and other signaling molecules (e.g., prostaglandin E2, transforming growth factor-β, and interleukin-10) that **directly suppress cytotoxic T cells**.
- Cancer cells (and white blood cells) sometimes manufacture and release an enzyme called **indoleamine 2,3-dioxygenase (IDO)**, which suppresses the activity of T cells.
- Cancer cells sometimes display proteins on their surface that suppress the actions of T cells. These proteins, which are often found on the surface of other white blood cells in the tumor too, are known as **inhibitory checkpoint proteins**. They include PD-L1 and PD-L2 (I'll be coming back to this in Chapter 5).

- T cells become suppressed and inactive due to the toxic tumor environment, which is often acidic, low in oxygen, and lacking nutrients.

All or some of the mechanisms mentioned earlier are likely to be at play in someone with cancer. They explain why someone's cancer might not be controlled or destroyed by their immune system. But they don't explain how cancer cells use white blood cells to help them thrive, which is what we'll turn to next.

1.5.4 How Cancer Cells Ultimately Survive, and Thrive, Among White Blood Cells

Cancer cells in a tumor are often outnumbered by their non-cancer neighbors. Some of these neighbors are white blood cells that I've already mentioned, such as T cells and NK cells. But many of them are white blood cells we commonly think of as being involved in inflammation, such as macrophages and neutrophils. Instead of killing cancer cells, these inflammatory white blood cells often help cancer cells to survive and thrive [28, 96, 100]. They do this by producing:

- Small proteins known as growth factors that cause cancer cells to multiply[24]
- Small proteins and chemicals collectively called "survival factors" that help cancer cells stay alive despite being in a hostile and toxic environment[25]
- Small proteins and chemicals that promote cancer cell migration, invasion, and metastasis.

On top of this, as I mentioned earlier, cancer cells and other cells in the tumor environment actively recruit white blood cells that suppress T cells and prevent them from attacking and destroying cancer cells such as Tregs and MDSCs.

1.5.5 Elimination, Equilibrium, and Escape

So far, I have described how cancer cells interact with white blood cells and explained the role played by different white blood cell types. But what's true for a small cluster of cancer cells isn't necessarily going to be true for a large tumor that has developed over several decades. The relationship between cancer cells and the person's immune system changes over time as the cancer grows. Scientists often refer to this as a process of elimination, equilibrium, and escape (Figure 1.16).

These three phases may take days, weeks, months, years, or even decades. But by the time a person is diagnosed with cancer, generally because it is causing symptoms, their cancer will have reached the escape phase. In this phase, their cancer is no longer being controlled by their immune system.

Together, whether a person's cancer is hidden from T cells, whether T cells have been generated but then suppressed, and what other white blood cells are present in the person's tumor (and what they're doing there) will all influence a person's prognosis and whether they benefit from immunotherapy; something we turn to again in Chapter 5.

1.6 THE CANCER MICROENVIRONMENT

As I've already mentioned, tumors are not lumps of tissue made from millions of identical cancer cells. Instead, they **contain a variety**

[24] We return to the topic of growth factors in Chapter 3, as many cancer treatments work by blocking growth factor receptors.

[25] This might not seem obvious, but because cancer cells grow in a haphazard manner and there aren't enough decent blood vessels around to supply them with everything they want and to take toxins away, their environment is toxic.

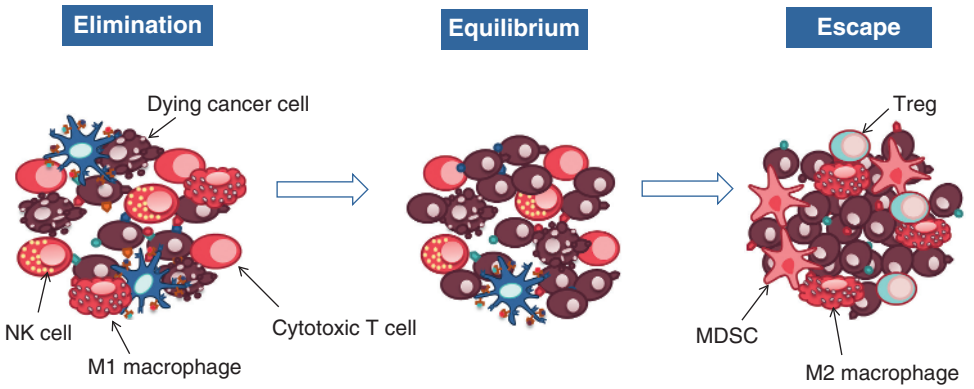

Figure 1.16 Elimination, equilibrium, and escape. In the initial *elimination phase*, cancer cells are successfully spotted and destroyed by both cytotoxic T cells and natural killer (NK) cells. However, any cancer cells that are hidden from the immune system will survive, such as those that present very few antigens on their surface via their MHC proteins. The tumor may then reach an *equilibrium phase*, where some cancer cells are destroyed but other, less visible, or more protected cancer cells are able to multiply. Finally, due to the accumulation of further mutations or changes in the tumor's microenvironment, the tumor reaches the *escape phase*. In this phase, cancer cells multiply at a faster pace than they are destroyed. In addition, immune-suppressing white blood cells such as MDSCs and Tregs accumulate, along with other cells that support, nurture, and protect cancer cells and aid tumor growth and metastasis. *Source:* Ref. [98, 99, 101, 102].

of non-cancer cells (collectively known as stromal cells) such as various types of white blood cells, fibroblasts (these are common, structural cells found in many locations around the body), cells that make up the blood vessels (endothelial cells and pericytes), fat cells (also called adipocytes), nerve cells, and other cell types (see Figure 1.17) [103].

The cells in a tumor are also embedded in a network of proteins and complicated sugar molecules known as the ECM – the extracellular matrix.[26] This intricate web surrounds the cells in all our tissues and organs, and its makeup and role differ from place to place around the body. When a cancer develops, cancer cells and non-cancer cells (which are now under the cancer cells' influence) cause the makeup and density of the ECM to change. For example, in breast cancer, the ECM becomes stiffer, and this seems to help cancer cells move and escape into the lymph vessels and bloodstream [104].

1.6.1 The Role of White Blood Cells

As I described in Section 1.5, each person's tumor will have a different collection of white blood cells inside it and at its outer fringes. The type of cells present, their number, and their behavior have a huge impact on how quickly or slowly the tumor grows and whether the person can be cured [28, 100, 105]. Some of these are "friends" to cancer cells; they protect cancer cells or encourage their growth. Others are "foes" that attack and destroy cancer cells (Figure 1.18).

[26] Examples of ECM proteins include collagen, fibronectin, laminin, and elastin. The ECM also contains long, complicated sugar molecules (called glycosaminoglycans) that are generally chemically linked to proteins to form protein-sugar hybrids called proteoglycans. These proteoglycans form a jelly-like substance in which the fibrous proteins like collagen are embedded.

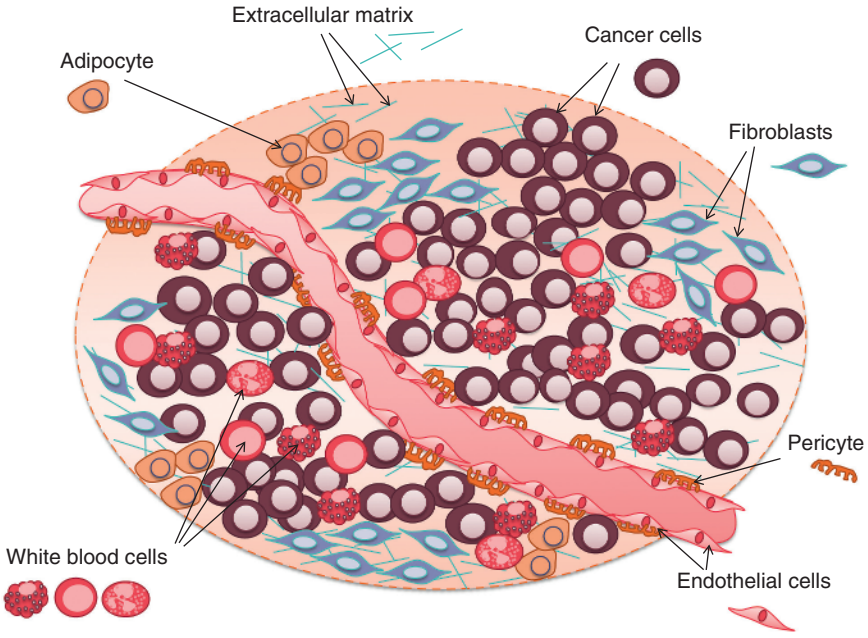

Figure 1.17 The cancer microenvironment contains many different types of cells. Tumors contain cancer cells, many different types of white blood cells, fibroblasts, fat cells (adipocytes), and other cell types (not shown). Winding their way through them are blood vessels, which are made up of endothelial cells and pericytes. Lymph vessels might also be present (not shown). All of these proteins are embedded in a complex network of structural proteins called the extracellular matrix.

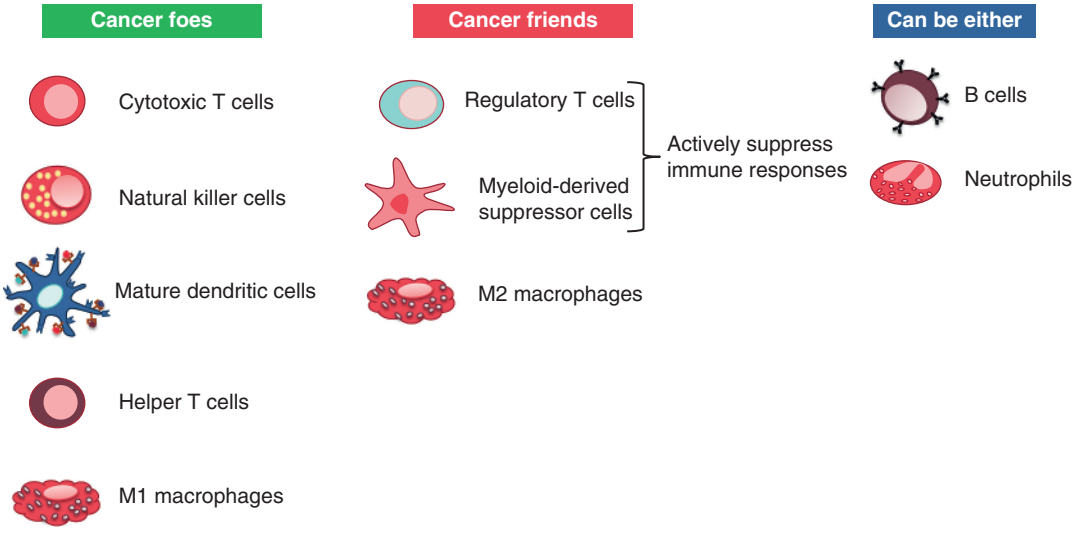

Figure 1.18 The influence of various infiltrating white blood cells on patient prognosis and response to treatment. The presence of some types of white blood cells (Cancer Foes) is generally a good sign for the patient as these create a cancer-fighting immune response and are linked to longer survival times. Whereas the presence of "Cancer Friends" is generally a bad sign and linked to shortened survival. Depending on cues from other cells, macrophages can become "M1" macrophages that produce molecules that kill cancer cells, or they can become "M2" macrophages that suppress immune responses and encourage angiogenesis. The presence of some other types of white blood cell can be a good or a bad sign depending on the cancer type and other influences. *Source:* Ref. [27, 101, 106, 107].

However, as with all things immunology-related there is a lot of subtlety and variation to this. For example, white blood cells that are located among lots of cancer cells will exert a different influence compared to white blood cells that are located around the tumor's fringes or trapped inside blood vessels [101].

1.6.2 The Role of Other Cell Types

Fibroblasts sit in our tissues, and they normally produce structural proteins that form the ECM [108]. In tumors, fibroblasts change in response to chemicals and other signals sent out by cancer cells. They become perpetually activated and behave as though they are in a damaged tissue. For example, they release vast quantities of ECM proteins – much more than normal – and they produce growth factors and chemicals that encourage cancer cells to multiply [103]. These same proteins can be an enormous obstacle to successful treatment [104].

Also found in some tumors are fat cells called adipocytes. Again, the adipocytes found within tumors aren't normal; they've been altered by signals sent out by cancer cells. And, like the fibroblasts in tumors, the adipocytes also encourage and help cancer cells to grow and multiply [103].

1.6.3 Angiogenesis

Angiogenesis (**the formation of new blood vessels**) is almost always necessary for a cancer to become life threatening. By the time a cancer has reached a few millimeters in size, the cells will be experiencing a drop in oxygen levels (hypoxia). Cancer cells then trigger angiogenesis to gain a blood supply and get access to oxygen and nutrients.

The most important trigger for angiogenesis is a tiny protein called vascular endothelial growth factor **(VEGF)**[27], which is released by cancer cells (and other cells) when oxygen levels drop. VEGF attaches to receptor proteins on the surface of endothelial cells – the cells that line our blood vessels. Once VEGF has attached to its receptors, the endothelial cells multiply and move into place to form a new blood vessel, which is supported by other cells called pericytes [107, 108]. VEGF isn't the only thing that triggers angiogenesis. Other triggers include angiopoietins, fibroblast growth factor, and ephrins. The fact that VEGF isn't in sole control will become important when we look at the class of cancer drugs called angiogenesis inhibitors (Section 4.1).

When properly controlled, angiogenesis is an important and entirely healthy process. It happens normally during the healing of cuts and wounds, during the menstrual cycle, during the formation of the placenta in pregnancy, and in a growing embryo [109]. The blood vessels that form during these healthy processes are evenly distributed and well supported by pericytes.

However, when angiogenesis happens in a tumor, it helps the cancer to grow and spread by supplying cancer cells with oxygen and nutrients and providing access to the bloodstream. In addition, tumor blood vessels tend to be lumpy, leaky, and disorderly [110]. Endothelial cells are no longer tightly connected to each other and are poorly supported by pericytes, which normally feed, protect, and physically support them [111]. On top of this, the supply of blood (and therefore oxygen) through tumor blood vessels is patchy and some areas in the tumor are constantly deprived of oxygen, changing the behavior of cancer cells nearby (Figure 1.19).

[27] There is in fact a whole family of VEGF proteins, called VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor.

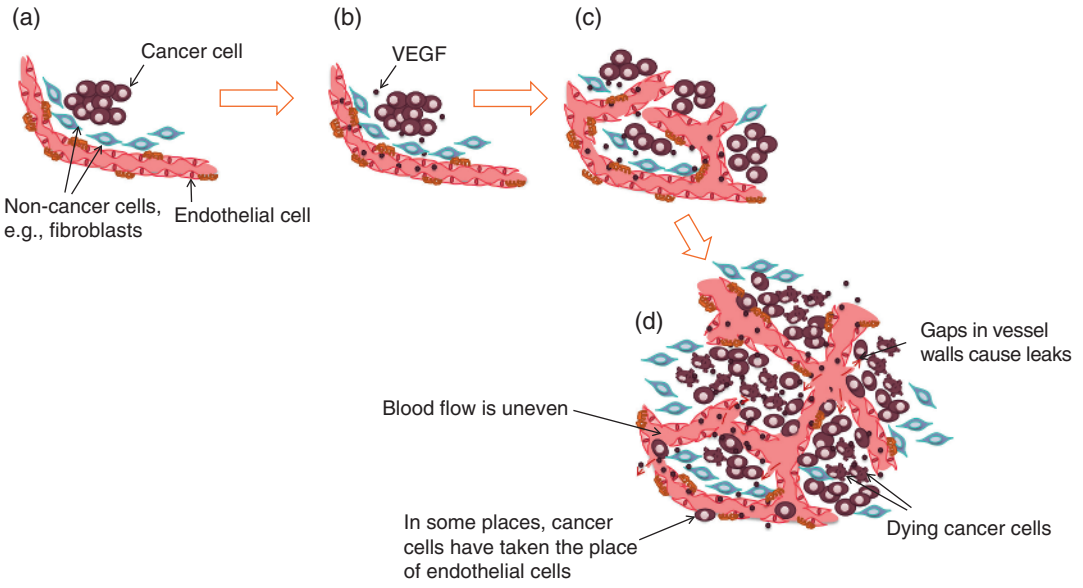

Figure 1.19 **Cancer angiogenesis**. **(a)** A cluster of cancer cells is too far away from the nearest blood vessel to receive an adequate blood supply. **(b)** Low oxygen levels trigger the cancer cells to release VEGF and other angiogenesis factors into their surroundings. **(c)** VEGF attaches to VEGF receptors on the surface endothelial cells, causing the blood vessel to sprout side branches and grow. **(d)** The tumor contains a convoluted, lumpy, leaky network of blood vessels; many cancer cells now have sufficient blood supply, but many others do not. **Abbreviations:** VEGF – vascular endothelial growth factor.

1.6.4 Two Examples of the Importance of the Tumor Microenvironment

Perhaps the best way to illustrate the importance of the makeup of the tumor microenvironment in determining how cancers behave and respond to treatment is to give a couple of examples. The two I've chosen are NSCLC and pancreatic cancer. The microenvironment of these two cancers is organized very differently. Each one has a distinctive set of non-cancer cells arranged in a particular way. As you'll hopefully see, this has a powerful impact on how these cancers respond to various treatments.

Non-Small Cell Lung Cancer

NSCLCs contain some of the highest numbers of infiltrating white blood cells, particularly T cells, of any cancer (Figure 1.20).

But perhaps equally important is the presence of tertiay lymphoid structures (TLSs). These are small patches of tissue within tumors that contain many white blood cells arranged in the same way as you normally find in lymph nodes (such as cytotoxic T cells, helper T cells, mature dendritic cells, and B cells). Our lymph nodes are where T cells are activated, and TLSs are the same. This means that cancer-fighting cytotoxic T cells are being generated within the tumor, and then they don't have far to go to find some cancer cells to destroy. The presence of TLSs in NSCLC and other tumors correlates with a better prognosis for the patient, and with a greater benefit from immunotherapy [27, 112–114].

Pancreatic Cancer

The cancer microenvironment of the most common sort of pancreatic cancer (pancreatic ductal adenocarcinoma) is very different from that of NSCLC (Figure 1.21).

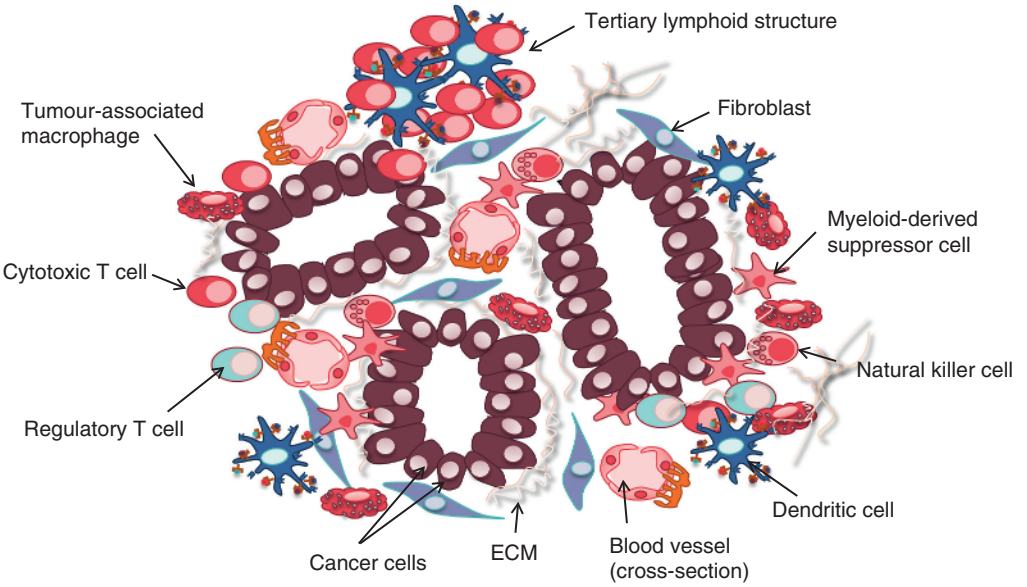

Figure 1.20 The microenvironment of non-small cell lung cancer (NSCLC). Inside the tumor are cancer cells and various non-cancer cell types, including various types of white blood cells. Dotted throughout the tumor are tertiary lymphoid structures (TLSs), and there is a profusion of cytotoxic T cells inside TLSs and elsewhere. There are relatively few macrophages compared to other tumor types.
Abbreviation: ECM – extracellular matrix; TLS – tertiary lymphoid structure.

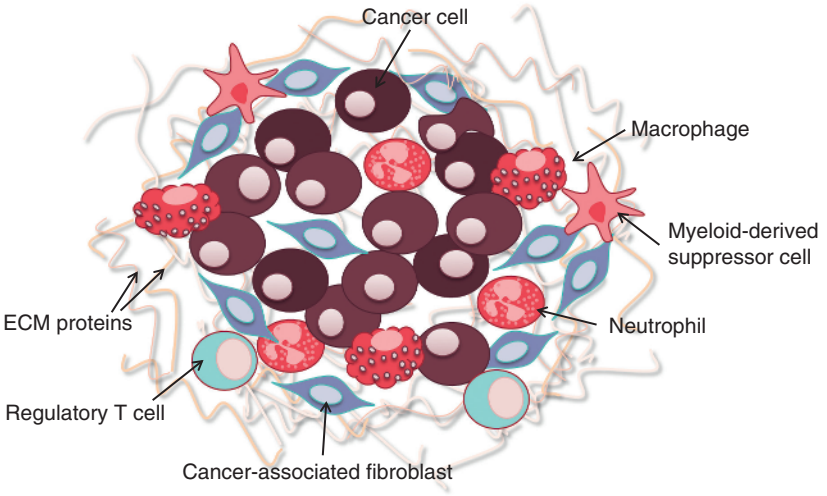

Figure 1.21 The pancreatic cancer microenvironment can protect cancer cells from the effects of treatment. Pancreatic cancers usually contain a fibrous network of ECM proteins that compress blood vessels and prevent cancer drugs from penetrating the tumor. Cancer-associated fibroblasts produce fibrous proteins and release pro-survival proteins such as growth factors. White blood cells such as macrophages secrete many small proteins and chemicals that protect cancer cells from treatments. Cytotoxic T cells are rare.
Abbreviation: ECM – extracellular matrix.

One important difference is the presence of **desmoplasia**. This refers to the dense accumulation of lots of tightly packed fibrous ECM proteins that have been created by **cancer-associated fibroblasts** (these are fibroblasts whose behavior is being controlled by cancer cells). Desmoplasia creates a physical barrier that prevents angiogenesis and that limits the number and types of white blood cells that can enter the tumor. Pancreatic tumors tend to contain lots of cancer-assisting macrophages, lots of immune-suppressing Tregs and MDSCs, and very few cytotoxic T cells [115]. As a result, pancreatic cancer is one of the most aggressive and difficult to treat types of cancer. In addition, immunotherapy has so far shown little sign of being an effective treatment approach.

1.7 CANCER SPREAD/ METASTASIS

As soon as a cancer spreads (metastasizes) to another part of the body, treatment becomes more complicated, and the person's likelihood of being cured of their disease drops dramatically [111, 112]. **Scientists estimate that metastasis is responsible for around 90% of cancer deaths** [116]. Sadly, once a cancer has metastasized, surgery is often no longer helpful and other treatments are likely to have limited impact. The various new cancer growths go on to disrupt and destroy vital tissues and organs.

Also, even when a cancer doesn't *appear* to have spread, there can be individual cancer cells, or microscopic clumps of cells that are circulating in the person's blood or lodged in distant organs or tissues [117]. These initially dormant cells can later cause metastasis and relapse.

There are numerous reasons why cancers metastasize. For example:

- Some cancer cells contain DNA mutations that force them into behaviors that cause metastasis.
- Cancer cells that are on the move might enter a blood or lymph vessel and get carried along by the blood/lymph to distant sites.
- The cells, proteins, and structures in the cancer cells' environment, and the cancer cells' limited access to oxygen, can encourage cancer cells to become more mobile or to move in specific directions.

One important thing to realize is that cancer cells that metastasize might contain lots of mutations and display behaviors that aren't present in cancer cells that stay put. As a result, a patient's metastases might behave differently and respond to different treatments than the primary tumor.

1.7.1 Routes Through Which Cancers Spread

There are five main routes through which a cancer can spread: [118]

- Local invasion
- Lymph vessels
- Blood vessels
- Nerves
- Fluid in the abdomen.

Routes of Cancer Spread – Via Local Invasion

"Local invasion" describes the process whereby cancer cells digest ECM proteins in their surroundings and gradually move into, infiltrate, and destroy nearby tissues. Local invasion is often the first step toward metastasis to distant organs.

Routes of Cancer Spread – Via Lymph Vessels (Lymphatic)

The fluid around our cells drains into lymphatic vessels and from there into lymph nodes (also called lymph glands), and finally

back into the bloodstream.[28] Cancer cells that have become detached from the cells around them are often caught up in this flow and carried to nearby lymph nodes.

Routes of Cancer Spread – Via Blood Vessels (Vascular)

Individual cancer cells (and small clusters) are sometimes able to squeeze their way into small blood vessels. The red and white blood cells in the vessel then sweep the cancer cells along until they get stuck somewhere else. Cancer cells that have found their way into the bloodstream are called **circulating cancer cells** or **circulating tumor cells** (CTCs).

Routes of Cancer Spread – Via Nerves (Perineural)

This is a relatively rare but dangerous route of cancer spread in which cancer cells spread along the course of nerve bundles. This type of spread is often very painful because cancer cells produce chemicals that trigger nerve activity.

Via Fluid in the Abdomen or (Transcoelomic)

Cancers that arise in the abdomen, particularly ovarian cancers, are liable to spread via the fluid that circulates within the abdomen. Cancer cells on the surface of the tumor break away and float in the abdominal fluid that bathes our internal organs. Cancer cells are carried along in the fluid and then adhere to tissues and organs in the abdomen such as the omentum[29] or bowel.

Once a cancer cell has reached a new location in the body, it won't necessarily cause a new cancer to grow. In fact, the vast majority of breakaway cancer cells die in the lymph or blood, are killed by white blood cells, or simply remain dormant (see Figure 1.22). In order for the cell to cause metastasis, it must survive and thrive in its new environment. And only a tiny proportion of breakaway cancer cells are ultimately able to go through this process.

1.7.2 Locations to Which Cancers Spread

Some cancers have particular routes of spread that are more likely than others (e.g., breast cancer commonly spreads via the lymph system). And each type of cancer is also more likely to spread to some locations than others [119]. For example:

- Breast cancers often spread to the bones, brain, liver, and lungs
- Prostate cancers often spread to bones
- Bowel cancers often spread to the liver, lungs, and the lining of the abdominal cavity (peritoneum)
- Lung cancers often spread to the adrenal glands, bone, brain, liver, and/or into the other lung
- Melanoma skin cancers often spread to the lungs, brain, other parts of the skin, and liver.

The preference that cancers have for spreading to some locations rather than others is often due to the anatomical layout of lymph and blood vessels. For example, the blood supply to the bowel goes from there to the liver, hence the liver is where bowel cancers often spread to first [120].

1.7.3 Reasons Why Cancers Spread

Many of the cells in a tumor seem to be relatively inert and dormant, perhaps because of low oxygen levels or due to signals sent out by their cancer and non-cancer neighbors.

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[28] For a colorful illustration of the lymph system, see the Cancer Research UK website: http://www.cancerresearchuk.org/what-is-cancer/body-systems-and-cancer/the-lymphatic-system-and-cancer [Accessed January 11, 2022].

[29] The omentum is a fold of fatty tissue that hangs down from the stomach and covers our intestines and other organs.

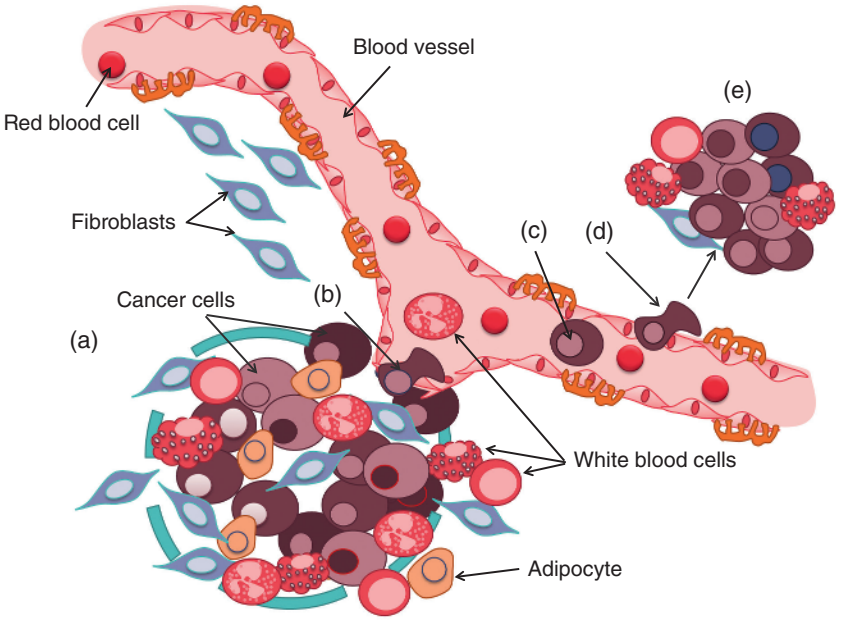

Figure 1.22 The path to metastasis. (a) A primary tumor containing many different cell types. **(b)** A cancer cell that is particularly mobile might invade locally and squeeze its way into blood vessels. **(c)** A cancer cell circulating in the blood. **(d)** The cancer cell squeezes out of the blood vessel into a new environment. **(e)** In its new location, the cancer cell may die or remain dormant for weeks or even years, kept in check by its new environment. However, eventually a change in its environment or the impact of new mutations might enable it to multiply and create a metastasis.

However, other cancer cells can be highly mobile and be much more likely to cause metastasis. Scientists believe that these mobile cells have gone through a change in appearance and behavior called **the epithelial-to-mesenchymal transition (EMT)** [117, 119] (Figure 1.23).

The EMT is a change that some healthy cells undergo in a developing embryo or in an adult when a tissue is damaged. It's when a stationary, well-connected epithelial cell[30] becomes more like a mobile, independent mesenchymal cell. During the EMT, the cell produces more ECM proteins, becomes more resilient, and changes shape [121].

The EMT is thus a natural process that is hijacked and reactivated by cancer cells. Understandably, if a cancer cell goes through this change, it's more likely to cause metastasis than other cancer cells.

Triggers that encourage cancer cells to go through the EMT include growth factors and other chemicals released by neighboring cells, low oxygen levels, and contact with various ECM proteins [122].

The EMT appears to be very important and it poses huge problems for doctors. For example, cancers that contain a high proportion of mesenchymal cells are more likely to resist treatment and spread quickly [123].

[30] Cancers that develop from epithelial cells are called carcinomas. These are the most common type of cancer diagnosed in the United Kingdom, accounting for about 85% of cancers [*Source:* Cancer Research UK].

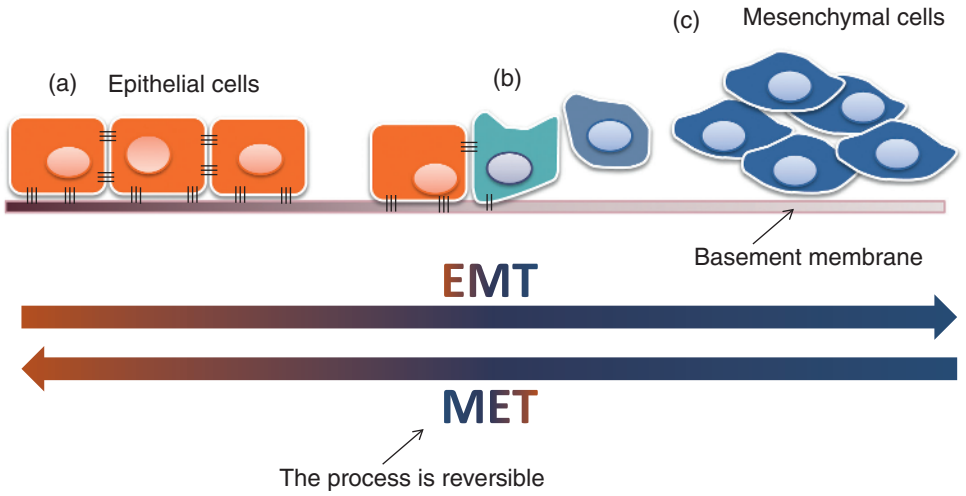

Figure 1.23 The epithelial-to-mesenchymal transition (EMT). (a) All our body's organs and tissues are lined with epithelial cells. Epithelial cells tend to be lined up and well connected to one another. They are also physically attached to the basement membrane (The basement membrane is a thin, dense sheet of ECM. It sits underneath layers of epithelial cells and anchors them to the tissue beneath. It also acts as a barrier separating different types of tissue. In addition, it wraps around blood vessels and provides structural support for endothelial cells.). **(b)** During the EMT, cells gradually stop making epithelial cell proteins and start making lots of proteins common in mesenchymal cells. **(c)** Mesenchymal cells are mobile and resilient and less well connected to one another and the basement membrane.
Abbreviations: EMT – epithelial-to-mesenchymal transition; MET – mesenchymal to epithelial transition.

Also, some treatments seem to cause cancer cells to go through the EMT, helping the cells survive the effects of treatment and causing metastasis [124, 125].

1.8 CANCER STEM CELLS

Over the past 20 years or so, scientists have increasingly become convinced that a proportion of cancer cells behave somewhat like our body's stem cells[31] and can be classed as **cancer stem cells** [126]. That is, they not only have the ability to multiply to generate further cancer stem cells, but they can also produce cancer cells with various other properties. Therefore, **if you kill all the other cells in a tumor but leave the stem cells behind, they will cause the cancer to return**. Evidence suggests that cancer stem cells are relatively rare, slow-growing, drug-resistant cancer cells that can survive many cancer treatments [126, 127]. The strength of evidence for their existence varies from cancer type to cancer type.

The precise properties of cancer stem cells and where they come from are hotly debated by scientists [128–130]. Some scientists suggest that they could start out life as healthy adult stem cells that, due to DNA mutations, start behaving like cancer cells. Other scientists

[31] Adult stem cells are slow-growing, versatile cells found in small numbers in our organs and tissues. When they multiply, they create mature, specialized cells that replenish, repair, and renew the tissue and keep it healthy. The number of stem cells differs from organ to organ and tissue to tissue around the body, depending on the turnover of cells in that tissue. For example, there are many stem cells in the lining of the bowel because cells are continually being scraped off as food passes through, and these cells need to be replaced.

point to the similarities between cancer stem cells and cancer cells that have gone through the EMT. They suggest that cancer stem cells are derived from cancer cells that have gone through the EMT and that have later undergone further changes [127, 130–132].

Two of the problems scientists face when trying to study cancer stem cells are that (1) these cells are highly changeable and adaptable and (2) what constitutes a cancer stem cell varies from cancer to cancer and even from patient to patient [127]. So, it's best not to get too worked up about the label "cancer stem cell." Instead, we will simply acknowledge that there are often cells in a cancer that are not easily destroyed by treatments and that can cause a cancer to return weeks, months, or years later.

1.9 UNIQUE PROPERTIES OF HEMATOLOGICAL CANCERS

Much of the information I've provided so far has been more relevant to solid tumors than to hematological cancers – those that develop from faulty white blood cells. Hematological cancers have unique features that set them apart from solid tumors, and I'll describe them here.

1.9.1 Introducing Hematological Cancers

Hematological cancers all develop from **faulty white blood cells or hematopoietic stem cells** (see Figure 1.24). The type of cancer the person develops, and how it behaves and responds to treatment, depends on factors like:

- What type of white blood cell went wrong and caused the person's cancer.
- How **mature or immature** the cell was when it went wrong.
- What **combination of mutations** or other faults the cell contains.
- **Where it was** when it went wrong (e.g., the bone marrow, a lymph node, or lymphoid tissue in the gastrointestinal tract).
- Whether it was **in the process of responding** to an infection when it went wrong.
- Whether it had responded to an infection **in the past**.
- Whether the infection the cell had responded to **is still around** (e.g., most MALT (mucosa-associated lymphoid tissue) lymphomas are linked to an ongoing *H. pylori* infection [133]).

For example, a very immature white blood cell that has just begun to specialize to become some sort of myeloid cell might give rise to acute myeloid leukemia, whereas a fully mature B cell in a lymph node might cause a non-Hodgkin lymphoma.

In addition to being derived from white blood cells or hematopoietic stem cells, hematological cancers have other important characteristics. I've listed a few of these later, as they will hopefully help you make sense of the treatments I mention in later chapters.

1.9.2 Most of Them Develop from Faulty B Cells

Most hematological cancers develop from faulty B cells. Only a minority are T cell cancers or cancers that develop from faulty myeloid cells or stem cells (see Table 1.2).

B cells seem to be more prone to going wrong than other white blood cells for a couple of reasons: First, because of the processes they go through as they mature in the bone marrow. Second, because of the activation steps involved in responding to an infection.

Both processes (maturation and activation) involve the B cell deliberately cutting up, mutating, and rejoining sections of its DNA (see Figure 1.25). The crucial thing here is that unlike most of our cells, which take great pains to prevent their DNA from changing, **B cells alter their DNA deliberately**. They do this during V(D)J recombination in the bone marrow. They also do it during class switching and somatic hypermutation as they become active in response to an infection.

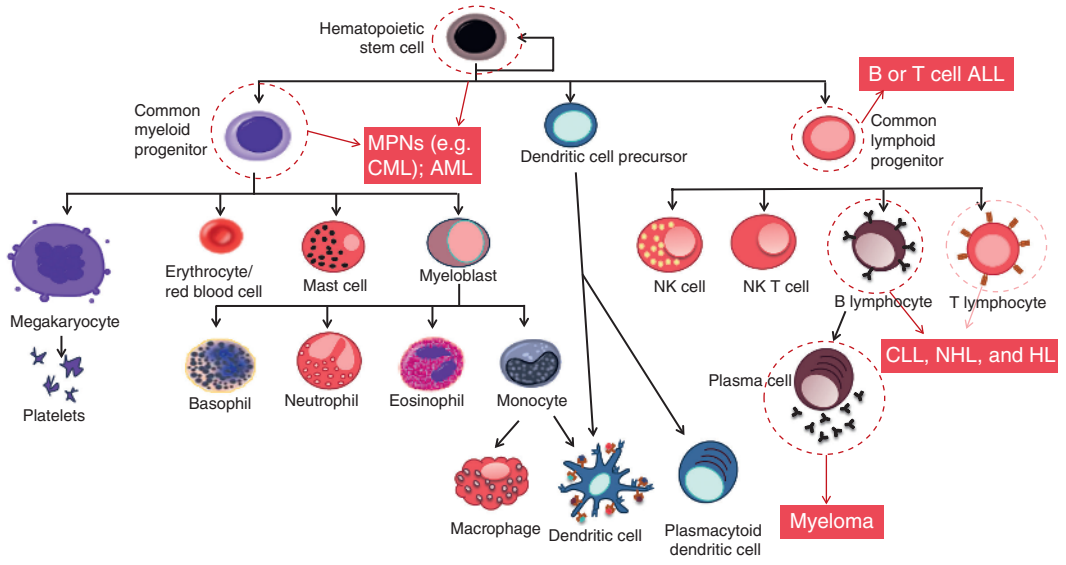

Figure 1.24 Diagram of hematopoiesis showing the cell of origin of common hematological cancers. Stem cells (top) create progenitor cells that gradually specialize and become mature myeloid or lymphoid white blood cells. If a stem cell or immature myeloid progenitor cell goes wrong, this may cause a myeloproliferative neoplasm (MPN) such as chronic myeloid leukemia (CML), or it could cause acute myeloid leukemia (AML). If an immature lymphocyte goes wrong, this may lead to a B cell or T cell acute lymphoblastic leukemia (B cell or T cell ALL). Mature T cells rarely lead to cancer; in the rare cases when this happens, they may cause one of various types of T cell non-Hodgkin lymphoma (T cell NHL). Mature B cells are the cell of origin of the vast majority of chronic lymphocytic leukemias (CLLs); they also cause B cell non-Hodgkin lymphomas (NHLs) and Hodgkin lymphoma (HL). B cells that are releasing antibodies to fight an infection are known as plasma cells. Myeloma is a cancer that develops from faulty plasma cells. There are many rare hematological cancers and disorders that are not shown in this diagram. **Abbreviation:** NK – natural killer. *Source:* Original figure taken from Wikipedia: https://en.wikipedia.org/wiki/Haematopoiesis#/media/ File:Hematopoiesis_simple.svg.

In addition, during B cell activation, B cells multiply rapidly and receive protection from death from their environment. This combination, rapid proliferation, protection, and deliberate DNA mutation, makes them vulnerable to cutting/mutating their DNA in the wrong places. It also makes them prone to aneuploidy (when a cell ends up with the wrong number of chromosomes) [134, 135]. Perhaps it's not so surprising that B cell cancers like chronic lymphocytic leukemia (CLL) and B cell non-Hodgkin lymphoma (NHL) are quite so common.

The last thing to mention about B cell cancers is that many of them rely on the BCRs on their surface for survival. I'll come back to this in Section 4.8 when I discuss treatments that target BCR-controlled signaling pathways.

1.9.3 Certain Translocations Are Common to Each Type and Subtype

Chromosome translocations (where two chromosomes break and end up stuck together incorrectly) are a common feature of hematological cancers.

Many types, and subtypes, of hematological cancers have characteristic translocations that exist alongside other mutations and abnormalities. Table 1.3 lists a few of the most common

Table 1.2 The expected incidence of various hematological cancers in the United Kingdom.

Type of cancer	Expected number of cases each year in the United Kingdom
Cancers that develop from stem cells or myeloid white blood cells:	**9010**
Acute myeloid leukemia	2890
Chronic myeloid leukemia[a]	720
Myeloproliferative neoplasms (MPNs): myelofibrosis, polycythemia vera, essential thrombocythemia, or MPN – unclassifiable	4530
Myelodysplastic syndromes	870
Cancers that develop from B cells:	**23,140**
B cell acute lymphoblastic leukemia	630
Chronic lymphocytic leukemia	4720
B cell non-Hodgkin lymphomas (marginal zone, follicular, mantle cell, large B cell, and Burkitt's)	11,260
Hodgkin lymphoma	1870
Myeloma	4660
Cancers that develop from T cells and NK cells:	**1140**
T cell acute lymphoblastic leukemia	150
T cell non-Hodgkin lymphomas	990
Proportion of cancers in this table that are of:	
Myeloid/stem cell origin	27%
B cell origin	70%
T cell origin	3%

[a] Chronic myeloid leukemia is often classified as a form of MPN.
Source: Data are from hmrn.org.

translocations [134, 135]. It seems that for some hematological cancers, a translocation between two chromosomes was the first mutation that occurred in an otherwise normal cell that put it on the path to becoming a cancer cell. However, all of us will have some white blood cells in our body that contain the same chromosome translocations found in cancer cells. So, the translocation itself is generally insufficient to cause cancer [135, 143].

Knowing what translocations have taken place in a patient's cancer can provide important information as to the likely future course of their cancer, how aggressive it's going to be, and whether it will respond to certain treatments. The discovery of translocations has also led to the discovery of important genes and proteins that have since led scientists to create new treatments. Sometimes, such as in CML, mantle cell lymphoma, and follicular lymphoma, there's just one specific translocation found in the cancer cells of virtually every person. In other cancers, you find a range of different translocations.

The Three Main Types of Translocations

As I outlined in Section 1.2.2, translocations can have a variety of consequences for a cell. In hematological cancer cells, you find three main types of translocations:

1. Translocations (such as the t(9;22) translocation found in CML) that create a **fusion protein that is a faulty version of a kinase**. Examples include Bcr-Abl and NPM-ALK, which force cells to grow and multiply.

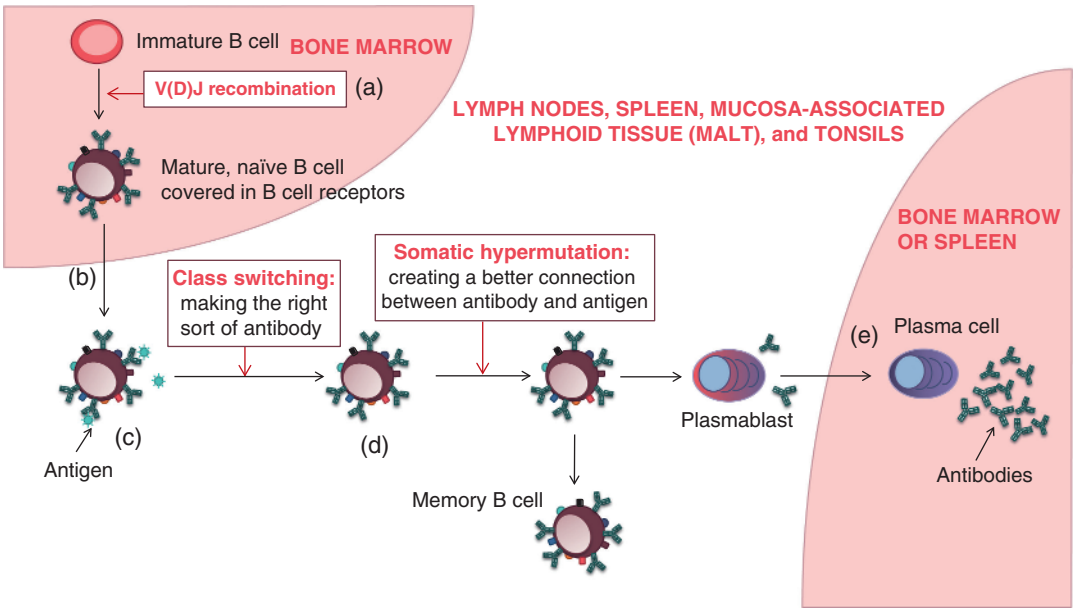

Figure 1.25 The maturation and activation of B cells is a multistep process. (a) As a B cell matures, it creates a unique B cell receptor (BCR) gene by a process called V(D)J recombination. A fully mature B cell has thousands of copies of the unique BCR protein made from this gene on its surface. **(b)** Mature B cells leave the bone marrow and travel to lymph nodes and other immune tissues. **(c)** Mature B cells are constantly meeting bacteria, viruses, and other pathogens, all of which have potential antigens on their surface that the B cell's BCRs might be able to recognize. **(d)** If a B cell does recognize an antigen, it might enter a multistep activation process involving somatic hypermutation and class switching. **(e)** Finally, the B cell might become a fully fledged plasma cell capable of releasing thousands of copies of a soluble version of its BCR protein (now called an antibody) into the blood. Plasma cells are mostly found in bone marrow and spleen. Activated B cells can also become long-lived memory B cells, ready to react if they encounter the same antigen again in the future. **Abbreviation:** BCR – B cell receptor.

2. Translocations that create a **fusion protein that is a faulty version of a transcription factor**. The faulty transcription factor suppresses genes and prevents the cell from making proteins that would help it mature properly. Examples include TEL-AML1, AML1-ETO, and PML-RARA.
3. Translocations that put together the control region of one gene with the protein-coding region of an oncogene, causing **overproduction of an oncogenic (cancer-causing) protein**. Examples include the t(14;18) translocation in follicular lymphoma (a form of B cell NHL), which causes overproduction of Bcl-2.

However, do remember that translocations aren't the only mutations found in hematological cancer cells. For example, in the cancer cells of CLL, the region of chromosome 17 that contains the *TP53* gene is commonly deleted or mutated. Lack of functional p53 protein in CLL cells causes the disease to be aggressive and resistant to chemotherapy [144].

1.9.4 They Have CD Antigens on Their Surface

All the proteins (and other large, complex molecules) found on the surface of our white blood cells have been allocated a number

Table 1.3 Some of the common translocations found in hematological cancer cells.

Translocation	Genes affected	Type of cell	Name of cancer
t(**14**;18)	*BCL2* (Bcl-2)	B cell	Follicular lymphoma
t(8;**14**)	*MYC*	B cell	Burkitt's lymphoma
t(3;**14**)	*BCL-6*	B cell	Diffuse large B-cell lymphoma
t(9;22)	*BCR-ABL* fusion	Myeloid cell	Chronic myeloid leukemia
t(11;**14**)	*CCND1* (Cyclin D1)	B cell	Mantle cell lymphoma
t(10;**14**)	*HOX11* (TLX1)	T cell	T cell ALL
t(2;5)	*NPM-ALK* fusion	B cell	Anaplastic large cell lymphoma
t(12;21)(p12;q22)	*TEL-AML1*[a] fusion	B cell	B cell ALL
t(1;19)(q23;p13)	*E2A-PBX1* fusion	B cell	B cell ALL
t(8;**14**)(q24;q32)	*MYC*	B cell	B cell ALL
t(15;17)(q21;q21)	*PML-RARA* fusion	Myeloid cell	Acute promyelocytic leukemia[b]
t(8;21)(q22;q22)	*AML1-ETO* fusion	Myeloid cell	Acute myeloid leukemia

Abbreviation: ALL – acute lymphoblastic leukemia.
Translocations that create an overactive kinase are in orange; those that create a faulty transcription factor are in purple; those that cause the overexpression of an oncogenic protein are in turquoise.
[a] This fusion protein created is also called ETV6-RUNX1.
[b] Acute promyelocytic leukemia is a rare subtype of acute myeloid leukemia.
Source: Ref. [135–142].

known as a "CD antigen" number.[32] (CD stands for "cluster of differentiation," but it doesn't mean anything very much.)

CD numbers correspond to the order in which the proteins were discovered (CD1 came first, then CD2 was discovered, then CD3 …). Scientists have now discovered and numbered over 370 CD antigens; regular workshops are held to discuss CD antigens found since the previous meeting.

So, the number assigned to a CD antigen doesn't tell you anything about that antigen itself (other than give you a rough idea of when it was discovered). But the range of CD antigens on the surface of a white blood cell can tell you things like:

- What type of white blood cell it is (e.g., only B cells have CD20 on their surface).
- Whether it's a mature, fully functioning white blood cell, or an immature one, or somewhere in between.
- What its job is.
- Whether, if it's a B or T cell, it has recognized and responded to an antigen.[33]

Each type of white blood cell has a wide variety of different CD antigens on its surface. Many of these proteins help our white blood cells communicate with one another. Other CD antigens transport things in and out of the cell, help the cell move through the bloodstream and into tissues, or help it destroy invaders.

[32] Numbering and naming of CD antigens are the responsibility of the participants of workshops run by the Human Cell Differentiation Molecules (HCDM) organization. Details of every CD antigen are available on the HCDM.org website.
[33] Although "antigen" and "CD antigen" sound like they're very similar to one another, it's probably easiest to think of them as two entirely different things: "antigens" being things that can trigger an immune response, and "CD antigens" being proteins and other large molecules found on the surface of white blood cells.

Box 1.6 The conventions of writing down translocations

When someone writes **t(9;22)(q34;q11),** they are giving you detailed information about the translocation that has taken place. First, **t** stands for translocation. Second, the translocation involves chromosomes 9 and 22. The term **q34** tells you that it was the long arm (rather than **p** – the short arm) of chromosome 9 that broke, specifically at position 34. And **q11** tells you that it was the long arm of chromosome 22 that broke, at position 11. All chromosomes have a long arm and a short arm, which are separated by a narrow region of the chromosome called the centromere.

It's worth noting a couple more things about CD antigens at this point:

- They're found on the surface of white blood cells, and they are therefore accessible to monoclonal antibody treatments.
- Many of them are not necessary for the survival of the white blood cells that they are found on; therefore, blocking them with an antibody won't necessarily kill the cell.

Each type of white blood cell has a very particular set of CD antigens on its surface. If a cell goes wrong and becomes a cancer cell, the cancer cell often has (more or less) the same CD antigens on its surface as its healthy counterpart. Knowing what CD antigens a person's cancer cells have on their surface can therefore tell you things like what sort of white blood cell their cancer developed from and which antibody-based treatments might be helpful for them.

1.9.5 They Live in Close Proximity to Other White Blood Cells

As with cancer cells in solid tumors, hematological cancer cells live in an environment that contains lots of other white blood cells. And, like solid tumors, hematological cancer cells influence and reshape their environment to suit their purposes and to avoid destruction. For example:

- In CLL, the person's cancer cells are generally found in protected environments within the bone marrow, lymph nodes, and spleen (as well as accumulating in the blood). The cancer cells are surrounded by T cells, NK cells, macrophages (called nurse-like cells), endothelial cells, and other cell types. These cells provide support, protection, and encouragement to CLL cells [145, 146].
- In ALL, AML, and other leukemia types, leukemic cells hijack and destroy the normal bone marrow environment. Normal bone marrow supports and guides the development of hematopoietic stem cells. In leukemia, the normal balance between white blood cell creation and death changes, and the microenvironment supports the rapid multiplication of cancer cells and protects them from the effects of treatment [147].
- Different types of B cell NHL, such as follicular lymphoma and diffuse large B cell lymphoma (DLBCL), contain different types of non-cancer white blood cells. For example, follicular lymphomas tend to contain lots of helper T cells, but these are virtually absent from the DLBCL environment. Follicular lymphoma cells are also very dependent on signals from their environment for their survival. In contrast, the cancer cells of DLBCL are more resilient and independent [148].
- In Hodgkin lymphoma, only 1%–10% of the cells in the person's tumor are cancer-causing (these are known as Reed-Sternberg cells). The rest are T cells, B cells, plasma cells, and other white blood cells that cluster around the cancer cells and provide support and protection [149].

The consequences of all of this are twofold:

1. To cure someone with a hematological cancer, you need to give them treatments that destroy their cancer cells and that overcome the protection provided by other white blood cells in their environment.
2. There are possibilities for immunotherapy for hematological cancers that don't exist with solid tumors. Although the microenvironment of hematological cancers does contain lots of altered white blood cells, fresh white blood cells are coming and going all the time. After all, the bone marrow, lymph nodes, and other lymph tissues (like the spleen, and Peyers patches in the intestines) are places where white blood cells are constantly congregating and being refreshed. As a result, there is a wider range of successful immunotherapy strategies for people with hematological cancers compared to those for people with solid tumors.

1.10 OBSTACLES THAT PREVENT US FROM CURING CANCER

In this chapter, I've explained some of what we now know about how cancers come about and why cancer cells behave as they do. I've also described some of the behaviors that cancer cells exhibit. In addition, I've tried to portray the diversity that often exists within tumors in terms of the types of cells found in them and the genetic diversity among cancer cells. Armed with all this knowledge about cancer, it's tempting to believe that we might know enough to cure everyone affected by the disease. However, as I'm sure you are fully aware, this sadly isn't the case.

So, what is it that still thwarts us? What features of cancer cells and cancer behavior are responsible for our inability to cure it, particularly when it has metastasized?

As a conclusion to this introductory chapter, I'm going to go through some of the chief obstacles to curing more cancer patients:

1. The similarities between cancer cells and healthy cells
2. The great dissimilarities between different types of cancer
3. The fact that cancer spreads
4. Intratumoral heterogeneity
5. The tumor microenvironment

There are, of course, other obstacles to successfully curing a patient of cancer. Not least are the issues of late diagnosis, the impact of racial, sex, and socioeconomic disparities, and the fact that many people who develop cancer are relatively elderly and frail and have other medical complaints that often preclude the use of aggressive treatments. However, these issues are beyond the scope of this book, so I'll stick to describing the five obstacles I listed above. If you are interested in age and other disparities, I would suggest taking a look at the following references [150–158].

1.10.1 The Similarity Between Healthy Cells and Cancer Cells

All our cells, cancer cells and non-cancer cells alike, **have the same repertoire of roughly 21,000 genes**. These genes contain the instructions for making all the proteins our cells will ever need. As you might have already gathered from the rest of this chapter, cancer cells never do anything completely new. Instead, they overproduce or produce faulty, overactive versions of proteins that help them grow, multiply, and stay alive. They also underproduce or produce dysfunctional versions of proteins that would normally limit their growth or encourage them to die.

The result of this is that although we might think that cancer is an unnatural aberration that needs destroying, a patient's body doesn't necessarily think the same. So, although it's true that our immune system is powerful

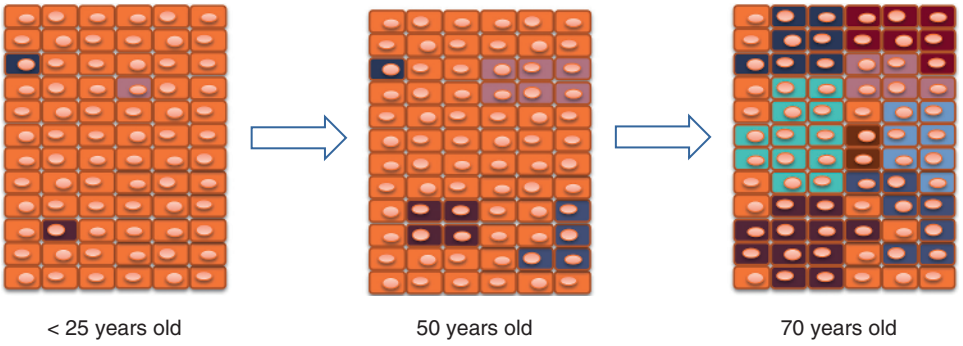

Figure 1.26 As we age, our tissues become a patchwork of colonies of mutated cells. In our youth, our cells contain relatively few mutations. However, as we enter middle age and beyond, mutated cells have outcompeted and outgrown their near neighbors, creating colonies of mutated cells. These cells might never become cancer cells, but they often contain many of the same faults. *Source:* Ref. [159–165].

enough to rid the body of cancer, it often doesn't do so (although it's impossible to say exactly how many of us have avoided cancer thanks to the vigilance of our immune system).

Because **cancer cells are very similar to healthy cells**, it's very difficult to create drugs that can kill one without the other. Newspapers and websites are often littered with stories about chemicals from many different sources that can kill cancer cells grown in a lab. But that isn't difficult. The difficulty is finding chemicals that can kill the cancer cells in a person **while leaving their healthy cells alone**. And this is virtually impossible. So, every treatment, no matter how targeted we might think it is, will kill some healthy cells alongside killing cancer cells. That means that every cancer treatment causes side effects. The severity of a treatment's side effects often limits how much of the treatment can be given to a patient safely, and that ultimately compromises the treatment's ability to cure them.

Another aspect to this is that our **so-called "healthy" cells often aren't all that healthy** [159–162]. Every cell in our body sustains DNA damage every day we're alive, and not all of it gets repaired. This damage might not be enough to cause a cell to become a fully fledged cancer cell, but it might be enough to make the cell a bit weird and cause it to multiply faster than normal, creating a group of mutated cells. The consequence of this is that as we get older our tissues and organs gradually become a patchwork of groups of mutated cells (Figure 1.26) [163]. These cells might never cause cancer, but they often contain some of the mutations that we typically find inside cancer cells and that might be the target of some cancer treatments [164–167]. This adds to the difficulty in selectively targeting and destroying cancer cells while leaving our "healthy" cells unharmed.

1.10.2 Differences Between Different Cancer Types

I'm often asked whether there will ever be "a cure for cancer." And if all cancers shared the same DNA mutations and behaviors, my answer might perhaps be "yes." But as it is, there are many, many different types of cancer, and **each cancer has its own unique vulnerability to different treatments**. Additionally, not only is it possible to develop liver cancer, stomach cancer, bowel cancer, skin cancer, and so on, but there are also **many different types of cancer that can occur in each location**. For example, there are adenocarcinoma and squamous cell carcinoma

versions of NSCLC, estrogen receptor-positive and estrogen receptor-negative breast cancer, and various types of skin cancer.

In recent years, scientists have uncovered more and more information about the various forms of cancer, what drives them, and what impacts their behavior. Thankfully, this knowledge is gradually improving our ability to treat people more effectively. However, the complexity is mind-blowing. Even when two cancers appear to be driven by the same mutations, it's not necessarily the case that they will respond to the same treatments. It depends on precisely how the cells' internal proteins interact with one another, and how the cancer cells interact with the cells around them. For example, in 50% of people with melanoma skin cancer, the cancer cells contain a mutation in a gene called *BRAF*. Treatment with a B-Raf inhibitor shrinks 50%–80% of these cancers (described in Section 3.7.4) [168]. The same *BRAF* mutation is also found in the cancer cells of 8%–10% of people with bowel cancer. But, in bowel cancer, a B-Raf inhibitor does not work, at least not unless it's combined with a treatment that targets the EGF receptor [169–171].

So, for every cancer, and for every subset of every cancer, we have to discover exactly how the cells are wired up – what's driving them and what's protecting them – before we can uncover how best to treat them. As a result, there will never be "one cure" for all cancers.

1.10.3 Cancer Spread

Scientists have made lots of progress in identifying the gene mutations that cause cancer and that drive its growth. They've also created many treatments that target the consequences of these mutations. However, a lot less progress has been made in identifying the mutations that drive metastasis. Very few treatments that specifically target metastatic cancer cells have been developed [117]. So, once a cancer has metastasized and become resistant to treatment, doctors currently have very little to offer their patients.

Also, there is often a lag between the cancer cells' arrival in a new location and their growth into a metastasis. During the lag period, the cancer cells are dormant and unlikely to be killed by chemotherapy or other cancer treatments [172, 173]. The length of time the cancer cells remain dormant, and the likelihood that they will cause metastasis, varies from cancer to cancer. For example, relapses several years after surgery are common in people with breast, prostate, kidney, or melanoma skin cancer.

In addition, cancer cells that have traveled to locations like the brain or bone marrow **receive protection and support from their new environment** [172–175]. The brain in particular is difficult for drugs to penetrate, has a large nutrient supply, and is relatively protected from the immune system [172, 174]. Likewise, the bone marrow is full of white blood cells and other cells that churn out substances like various cytokines that can help cancer cells survive and multiply [175].

Lastly, cancer cells in distant organs might contain different mutations compared to those in the original (primary) tumor. Consequently, they might not be destroyed by a cancer treatment chosen by a doctor for its ability to target the person's primary tumor [117].

1.10.4 Intratumoral Heterogeneity

Intratumoral heterogeneity[34] is a huge obstacle to curing people of cancer. As I described in Section 1.4, heterogeneity comes in many

[34] As you might remember from Section 1.4, intratumoral heterogeneity is the phrase scientists use to describe the fact that most cancers contain multiple populations of cancer cells driven by different combinations of gene mutations. Cancer cells in a single patient can also differ in terms of the proteins they make, their epigenetics, their metabolism, and their ability to change and adapt in response to changing circumstances.

different forms. It can be genetic, with different pockets of cancer cells containing different combinations of DNA mutations. Cancers can also vary internally in terms of their epigenetics or in their metabolism. And of course, cancer cells don't live alone. Various parts of a person's tumor, and any metastases, are going to have variations in their blood supply, in the number and behavior of fibroblasts and adipocytes, and in what white blood cells are present and what they're doing there. These variations make it impossible for cancer treatments to have a uniform effect on every part of a tumor and any metastases.

The Impact of Heterogeneity on the Effectiveness of Targeted Cancer Treatments

As we create treatments that precisely target cancer cells, it becomes more and more likely that our treatments will kill some cells in a tumor while leaving others unharmed [79, 81, 176–179].

If someone has millions upon millions of cancer cells in their body, it is inevitable that among the different populations of cells there will be some that contain mutations that make them resistant to treatment (see Figure 1.27a & b).

Thus, the precise targeting that is a feature of many of the treatments mentioned in this book is also the treatments' greatest weakness. **The more precisely targeted a treatment is, the less likely it is to kill every cancer cell in a person's body** [79, 178, 180].

In fact, there are often multiple treatment-resistant clones of cancer cells in the person's tumor and in any metastases. Each clone may have a different resistance-causing mutation [181, 182]. Following treatment, if the cancer reemerges, it's likely to be these resistant cancer cells that are the cause. In addition,

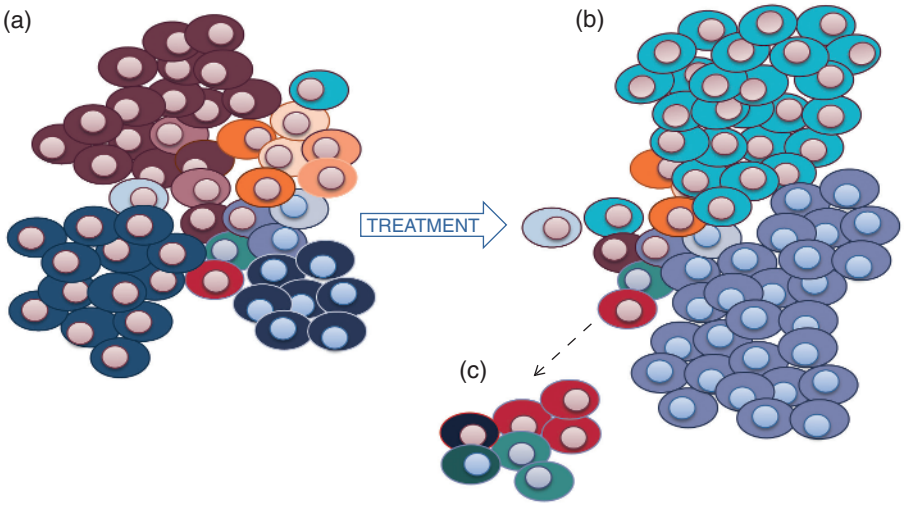

Figure 1.27 Intratumoral heterogeneity is an obstacle to effectiveness of targeted cancer treatments. **(a)** Due to the genomic instability of cancer cells, cancers generally contain multiple populations of cancer cells driven by unique combinations of mutations (represented by the different colors). Each population exhibits a different level of sensitivity to any particular cancer treatment. **(b)** Some populations of cancer cells have been killed by the treatment the person was given. However, some cells contained mutations that made them resistant and able to survive. Some of these resistant cells have multiplied and caused the person's disease to return. **(c)** Cancer cells that leave the original tumor and create a metastasis elsewhere in the body may have different properties from the original tumor.

because the reemergent clones contain different combinations of mutations, a single treatment approach is unlikely to help [181].

Another problem that intratumoral heterogeneity causes is that **a biopsy sample from a patient's cancer might not give an accurate picture as to the presence or absence of a particular mutation** [176]. It might be that the targetable mutation picked up in the biopsy analysis is only present in a proportion of the cancer cells and absent in others. This would mean that targeting the mutation in question is doomed to fail.

The opposite situation is when a biopsy sample contains **such a small proportion of cells with a particular mutation that the testing doesn't pick it up**. For example, a colorectal cancer sample might appear to be free of *KRAS* mutations, suggesting that an antibody treatment targeted against the EGF receptor will work [183]. But even a tiny number of *KRAS*-mutant cells that survive treatment might cause recurrence later.

Another problem caused by intratumoral heterogeneity is the way it enables cancers to change over time. Therefore, the cancer cells that drive recurrence and metastasis might contain different gene mutations and have different survival mechanisms than the cancer cells that were first present (Figure 1.27c) [81]. So, when a cancer starts growing again, it's likely to be impervious to the treatments used previously (any cancer cell that was vulnerable to that treatment is already dead); hence, **the cancer gets harder and harder to treat**

The Impact of Heterogeneity on the Effectiveness of Immunotherapy

The degree of heterogeneity in a tumor also influences whether immunotherapy is likely to work [182]. Because different pockets of cancer cells contain different combinations of mutations, they also differ in their visibility to the person's immune system. As time goes by, visible cancer cells will be destroyed, leaving less visible cells behind – a process called **immuno-editing**.

In addition, different parts of a tumor will vary in terms of their accessibility and hostility to different types of white blood cells. Thus, one part of a tumor might be full of cancer-fighting T cells, while another part is full of MDSCs and Tregs (both of which actively suppress T cells) [184].

The degree and pattern of genetic heterogeneity in a tumor appear to influence how likely it is that immunotherapy will work.

If a person's cancer cells all developed from a single cancer cell with many mutations (Figure 1.28a), every cancer cell in their body will also have all these mutations inside them (called **clonal mutations**). T cells that recognize any of these clonal mutations have the potential to seek out and destroy cancer cells wherever they might be in the body [93, 185]. Excitingly, they can even cure someone with metastatic disease.

In contrast, if the person's cancer cells diversified very early on, and most mutations are only present in a few subsets of cells (called **subclonal mutations**), then their immune system has a much harder job (Figure 1.28d). Some mutations might fail to elicit an immune response at all; others are so rare that, even if suitable T cells are activated, they have virtually no impact on the tumor as a whole. Pockets of cells with unique mutations might go undetected. Cancers with a high proportion of subclonal mutations are therefore less likely to respond to immunotherapy than those with lots of clonal mutations [186, 187].

Strategies to Overcome the Problem of Intratumoral Heterogeneity

Thus, intratumoral heterogeneity is a huge barrier to the successful treatment of patients with cancer. Efforts to overcome this problem center on the following: [82, 148], [155, 156]

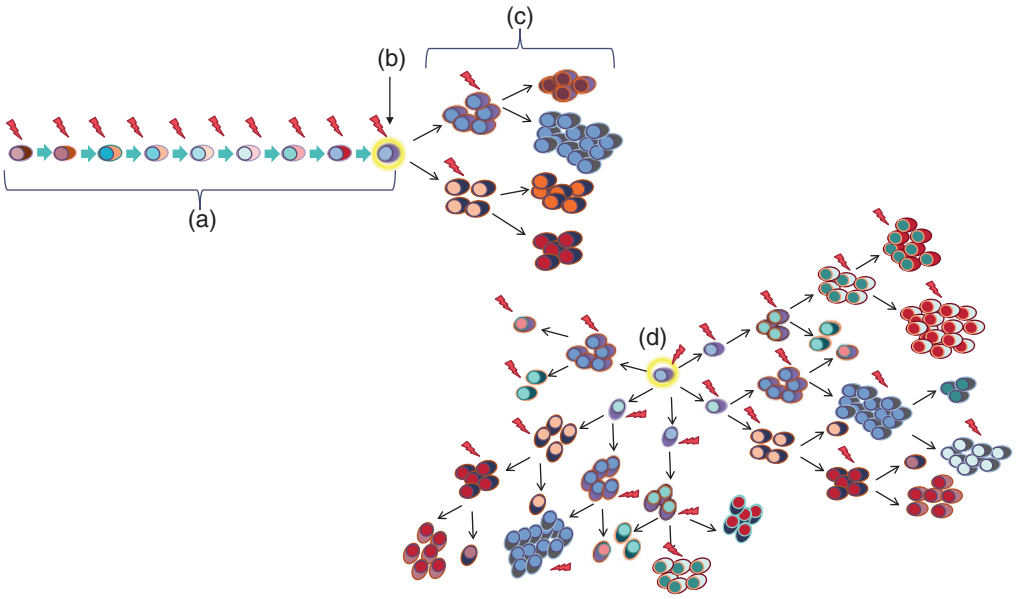

Figure 1.28 The proportion of clonal vs. subclonal mutations influences the effectiveness of immunotherapies that boost T cells. **(a)** A cell may accumulate many mutations before it finally becomes a cancer cell. **(b)** The first, fully fledged cancer cell. **(c)** Cancer cells are unstable and pick up new mutations, causing heterogeneity. However, all the mutations present in the first cancer cell **(b)** are still present in every cell descended from it – the cancer cells in the person's body contain many **clonal mutations**. T cells activated in response to clonal mutations can destroy any cancer cell in the person's body, and an immunotherapy that boosts T cell activity is likely to work. **(d)** If a person's cancer cells evolve and diversify right from the start, there will be very few clonal mutations. Most mutations will be subclonal. T cells activated in response to subclonal mutations will only be able to destroy pockets of cancer cells, making it less likely that the person's cancer will be controlled with a treatment that boosts T cell activity.

- Using logical combinations of drugs that target different faulty proteins and pathways and that synergize with one another to kill a more diverse range of cancer cells than any individual treatment used on its own.
- Innovations in the analysis of cancer cells or cancer cell DNA (often called ctDNA – circulating tumor DNA) in a patient's bloodstream, and using these cells/DNA to track the cancer cells' evolution and predict drug resistance-causing mechanisms.
- Taking multiple biopsies from a tumor and its metastases to gain a fuller picture of the mutations driving the cancer.
- Developing treatments such as immunotherapies that are less selective and may be able to kill a broad range of cancer cells driven by different mutations (see Chapters 5 and 6).
- Using mathematical methods to model the outcome of different treatment approaches. The timing, dose, and combination of drugs given to each patient are then chosen to kill the highest proportion of cancer cells over the longest possible period of time – called **adaptive therapy**.
- Implementing a broad range of tests looking for potentially hundreds of DNA mutations, proteins, and other possible

biomarkers, to match a patient's treatment to the features of their cancer as closely as possible.

1.10.5 The Cancer Microenvironment

The environment in which cancer cells live can have an enormous impact on whether a treatment given to a patient is effective. Even if a drug is theoretically highly effective against a patient's cancer, it still might have no impact if the cancer cells' microenvironment is protecting them. Two main issues that affect a drug's effectiveness are (1) **the physical environment** in which the cancer cells live and whether the treatment can reach them and (2) **the behavior of the non-cancer cells** that live alongside the cancer cells. For example [95]:

- Growth factors and other proteins released by non-cancer cells such as fibroblasts, white blood cells, endothelial cells, and adipocytes (fat cells) can protect cancer cells from the effects of various treatments.
- In some cancers, the cancer cells' microenvironment contains a dense network of structural proteins (called **desmoplasia**) that compresses blood vessels and prevents cancer drugs from reaching the cancer cells.

As I mentioned in Section 1.6.4, a classic example of the problems posed by the cancer microenvironment is pancreatic cancer. Many scientists have found combinations of chemotherapy and other treatments that can successfully kill pancreatic cancer cells cultured in a lab or grown in mice (called xenografts). However, these same treatments have failed to improve the survival times of most patients with pancreatic cancer [158]. One of the chief obstacles that stop treatments from working against pancreatic cancer is its microenvironment (Figure 1.21). It's not unusual for non-cancer cells to outnumber the cancer cells in these tumors, and the microenvironment is awash with a diverse array of cells and densely packed structural proteins that together prevent drugs from penetrating and protect cancer cells from death. Treatments that remodel the microenvironment might be our best chance at improving the situation, but many have already been tried and have failed [114].

1.11 FINAL THOUGHTS

In this chapter, I have tried to give you a good idea of why cancers come about, what drives them, how they behave, and why we can't yet cure everyone who develops this disease.

Do be aware, though, that this chapter covers just a small percentage of all the knowledge that scientists have accumulated about cancer. There are some big areas of science that I have missed out, such as most of the research on epigenetics; micro-RNAs; exosomes; the role of metabolic pathways and of viruses and infections; the similarities and differences between cancers in different organs; the difference between a benign tumor, a precancerous lesion, and an invasive cancer, etc.

Therefore, **this chapter is just a selection of information that I have chosen because I think it might come in handy when you read later chapters**.

Throughout the rest of this book, I'll be focusing much of my attention on cancer treatments. Most of these treatments target just one protein, or one cell process that is faulty in cancer cells or that controls their relationship with the immune system. However, the proteins and processes that are targeted by these treatments represent just a small proportion of all the faulty proteins and processes that drive cancer cells and are responsible for the way they behave. I hope that in this chapter I have given you a sense of this complexity.

Even so, the treatments described in the rest of this book target a range of different features

of cancer cells. These include treatments that target aspects of cell communication, the cell cycle, DNA repair, angiogenesis, and the interaction between cancer cells and the immune system. Despite mentioning them briefly in this introductory chapter, I will explain these processes in more detail when I come to describe the various treatments in later chapters.

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