

## Context is everything: aneuploidy in cancer

Uri Ben-David<sup>1</sup>\* and Angelika Amon<sup>2,3,4</sup> 

**Abstract** | Cancer is driven by multiple types of genetic alterations, which range in size from point mutations to whole-chromosome gains and losses, known as aneuploidy. Chromosome instability, the process that gives rise to aneuploidy, can promote tumorigenesis by increasing genetic heterogeneity and promoting tumour evolution. However, much less is known about how aneuploidy itself contributes to tumour formation and progression. Unlike some pan-cancer oncogenes and tumour suppressor genes that drive transformation in virtually all cell types and cellular contexts, aneuploidy is not a universal promoter of tumorigenesis. Instead, recent studies suggest that aneuploidy is a context-dependent, cancer-type-specific oncogenic event that may have clinical relevance as a prognostic marker and as a potential therapeutic target.

### Aneuploidy

A chromosome number that is not a multiple of the haploid complement. In cancer genomics, the term often includes copy number alterations of chromosome arms. Note that the mechanisms that lead to whole-chromosome mis-segregation are very different from those that cause arm-level copy number changes.

Aneuploidy, an imbalanced complement of chromosomes, was identified as a distinct feature of cancer cells more than a century ago<sup>1</sup>, decades before DNA sequence alterations were shown to drive tumorigenesis. The process that causes aneuploidy, chromosome instability (CIN), has been studied extensively, and targeted therapies have been developed based on its biological understanding. By contrast, there has been rather limited progress in understanding how aneuploidy contributes to cancer initiation and progression, and therapeutics that exploit this hallmark of cancer have yet to be developed (reviewed in REFS<sup>2,3</sup>).

The challenge to understanding the role of aneuploidy in cancer, and how this disease feature can be exploited clinically, stems from the ‘aneuploidy paradox’<sup>4</sup>: aneuploidy is detrimental for primary cells during organismal and tissue development and when introduced experimentally, and it is associated with a substantial fitness cost under most circumstances<sup>5–8</sup>; however, aneuploidy is well-tolerated in cancer cells. Indeed, ~90% of solid tumours are aneuploid, ranging from 26% in some tumour types to 99% in others<sup>9</sup>. In a typical solid tumour, ~25% of the genome is altered at the copy number level through whole-chromosome or chromosome arm changes — a median of 3 gains and 5 losses of chromosome arms (or longer chromosomal segments) per tumour<sup>10,11</sup>. No other genetic alterations affect cancer genomes to this extent. The existence of characteristic aneuploidy patterns within a given cancer type<sup>9,11–14</sup> further suggests that specific aneuploidies drive tumorigenesis.

Aneuploidy is notoriously difficult to study, for several reasons. Firstly, large chromosomal changes affect, by definition, hundreds of genes (and sometimes more) at once, making it difficult to identify the genes that drive

the recurrence of a specific aneuploidy in a particular cancer. Secondly, aneuploidy can play distinct, often opposite, roles in different contexts. Thirdly, introducing or eliminating specific chromosomes remains technically challenging and laborious, despite tools such as microcell-mediated chromosome transfer<sup>15,16</sup>, Cre–Lox recombination<sup>17</sup> and CRISPR–Cas9 gene editing<sup>9,18,19</sup>. We therefore lack the ability to systematically characterize the consequences of aneuploidy across a wide range of chromosomes and cell types. Lastly, it is often difficult to disentangle the effects of CIN, the process that generates aneuploidy, from its product, an abnormal karyotype. Although CIN is highly correlated with aneuploidy levels, some cancer cells may be highly aneuploid but chromosomally stable<sup>20</sup>. For example, CIN may be a transient phenomenon that is counterbalanced during tumour evolution (reviewed in REF.<sup>21</sup>), but the resultant aneuploid karyotypes of cancer cells may persist long after CIN has been attenuated. Nevertheless, recent progress in our understanding of cancer aneuploidy paves the way towards tackling these challenges, in both the laboratory and the clinic.

In this Review, we summarize recent findings that highlight the importance of cellular context for determining the consequences of aneuploidy, and we discuss the clinical relevance of aneuploidy in cancer — both as a predictor of clinical outcome and drug response and as a potential therapeutic target. This Review does not cover the mechanistic basis of aneuploidy formation, which has been reviewed extensively elsewhere<sup>2,22–27</sup>.

### Defining aneuploidy

To investigate the importance of aneuploidy in tumorigenesis and its potential prognostic value, we must first define the term in a clinically meaningful way (FIG. 1). Aneuploidy is classically defined as numerical

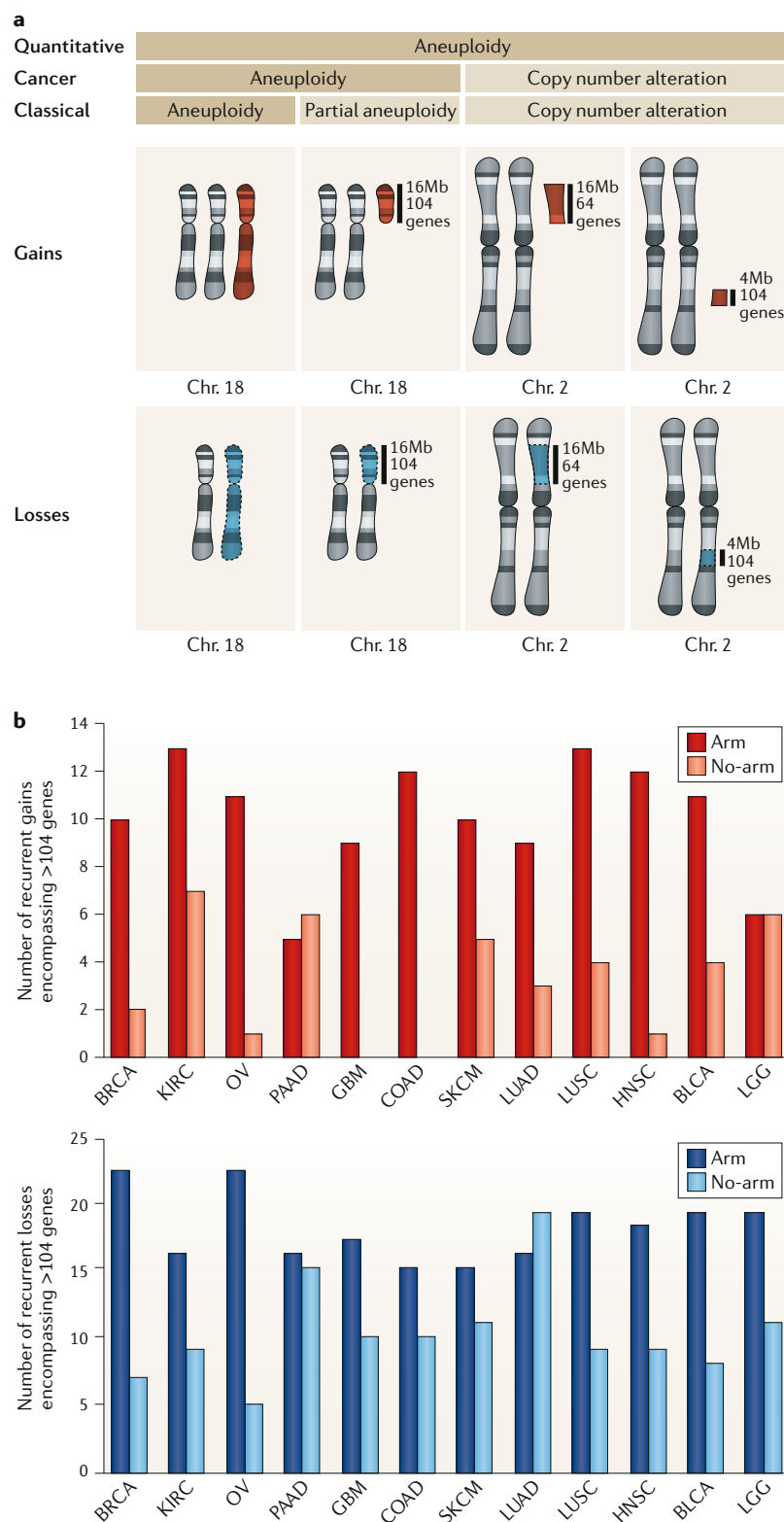
<sup>1</sup>Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

<sup>2</sup>Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA.

<sup>3</sup>Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA.

<sup>4</sup>Howard Hughes Medical Institute, Chevy Chase, MD, USA.

\*e-mail: [ubendavid@tauex.tau.ac.il](mailto:ubendavid@tauex.tau.ac.il); [angelika@mit.edu](mailto:angelika@mit.edu)  
<https://doi.org/10.1038/s41576-019-0171-x>



**Fig. 1 | Definitions of aneuploidy. a** | The classic definition of aneuploidy refers to changes in the copy number of whole chromosomes. Of all the chromosomal aberrations shown, only the gain or loss of chromosome 18 would be considered an aneuploidy under this definition. Recent genomic analyses of aneuploidy in cancer have extended this definition to include chromosome arm gains and losses. According to this cancer definition, the loss or gain of arm 18p would also be considered an aneuploidy. A quantitative approach to aneuploidy would ideally take into account parameters such as the fraction of the genome that is altered, the number of genes affected and the number of discrete events. Under this definition, changes to chromosome 2 that affect regions that pass a threshold size (here, 16 megabases (Mb), the size of 18p) or number of genes (here, 104, the number of genes on 18p) would also be considered aneuploidy. However, given that most cancer surveys have defined aneuploidy as chromosome arm gains or losses, it would be most practical to continue to use this definition. **b** | The bar plots show the number of recurrent DNA copy number gains (top, in red) and losses (bottom, in blue) that encompass  $\geq 104$  genes, the number of genes residing on chromosome arm 18p, across 12 cancer types. Approximately one third of these recurrent alterations are not chromosome-arm-level events. These copy number alterations are expected to have effects on cellular fitness similar to those of chromosome arm alterations in the size range of chromosome 18p, demonstrating the limitation of an arm-focused definition of aneuploidy. The data were extracted from the GISTIC 2.0 analysis of The Cancer Genome Atlas data, provided by the [GDAC portal](#). BLCA, urothelial bladder carcinoma; BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; GBM, glioblastoma multiforme; HNSC, head-neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; LGG, low-grade glioma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OV, ovarian cancer; PAAD, pancreatic adenocarcinoma; SKCM, skin cutaneous melanoma.

Although this qualitative definition of aneuploidy is operationally convenient, it is ambiguous. Most, probably all, aneuploidy-driven phenotypes are caused by copy number changes of genes. It follows that the more genes are affected the greater the phenotypic consequences. In light of this argument, it needs to be considered whether there is a conceptual or functional difference between an  $\sim 16$ -megabase (Mbp) gain or loss encompassing the entire chromosome 18p arm — a chromosomal alteration defined as aneuploidy in cancer genome studies — and a similarly sized aberration that occurs within the  $\sim 250$ -Mbp chromosome 2q arm — defined as a CNA (FIG. 1a). In other words, should aneuploidy be considered a quantitative trait, in which the size of the alteration determines whether or not a cell is defined as aneuploid? Already, most analyses of aneuploidy in human cancers do not consider changes involving only the short (p) arm of acrocentric human chromosomes (13, 14, 15, 21 and 22) as aneuploid<sup>9,11</sup>, because they are small and lack functional genetic elements. If such a quantitative approach to defining aneuploidy is adopted, further questions arise. Should the number of CNAs, the fraction of the genome that is altered or the number of coding genes that are affected be included in the definition of aneuploidy?

## Complement

The set of all chromosomes. The haploid complement consists of one chromosome each, the diploid of two, and so forth.

aberrations of whole chromosomes. More recently in the cancer genome literature, this definition has been extended to include gains or losses of chromosome arms<sup>9,11</sup>. The term ‘focal copy number alterations’ (focal CNAs) is usually used to describe smaller copy number changes that encompass fewer genes, and unlike ‘aneuploidy’, the term frequently refers to gene amplifications beyond an additional copy.

**Chromosome instability (CIN).** A high rate of chromosome mis-segregation that gives rise to aneuploidy.

**Microcell-mediated chromosome transfer**  
A technique to transfer a chromosome from a donor cell line to a recipient cell line.

**Cre–Lox recombination**  
A technique to introduce deletions, insertions, translocations or inversions at specific chromosomal locations.

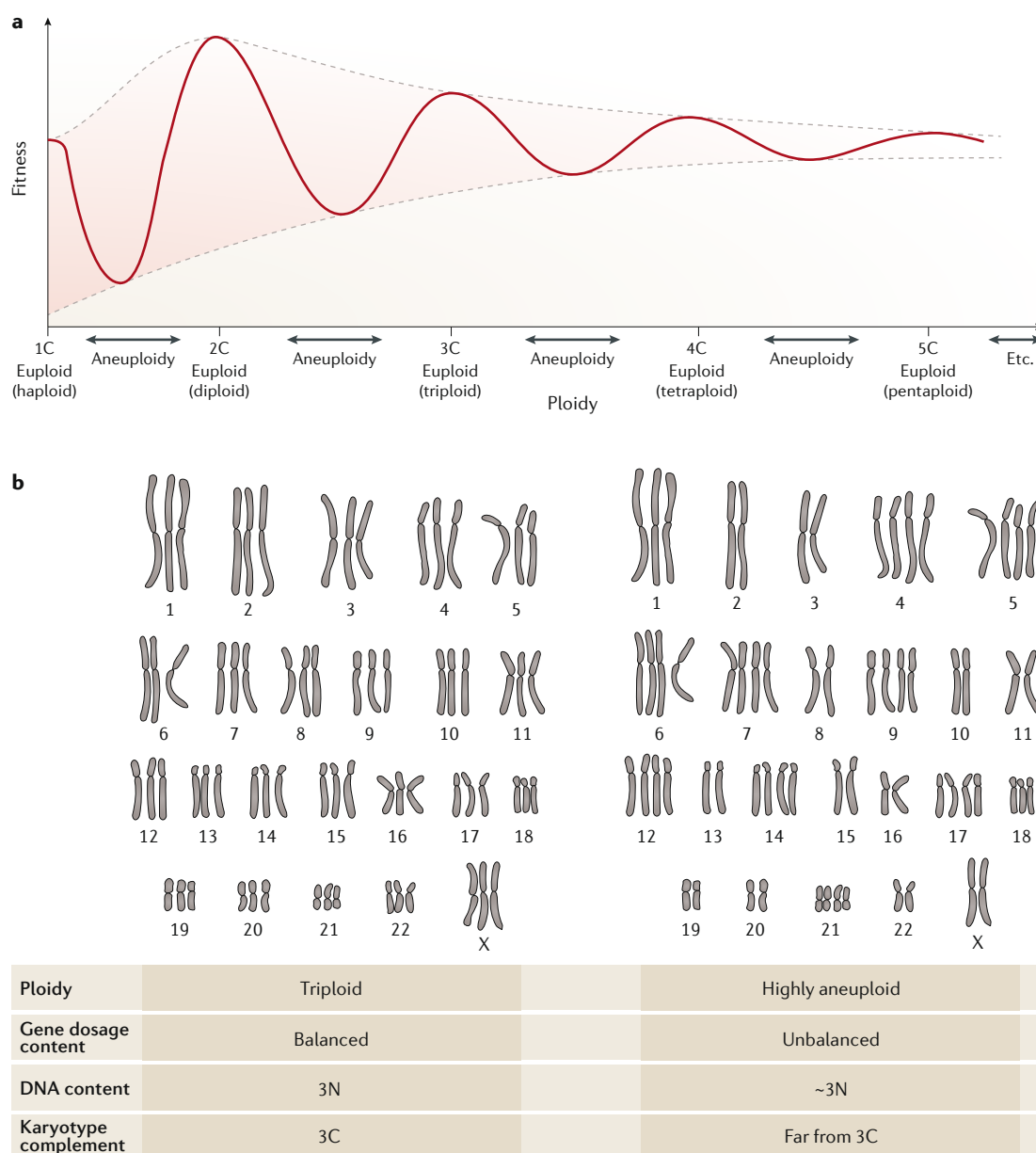
**CRISPR–Cas9 gene editing**  
A technique to introduce precise genetic alterations, ranging in size from point mutations to the deletion of entire chromosome arms.

**Prognostic value**  
The degree to which a biomarker provides information about the patients' overall survival, regardless of therapy.

**Euploidy**  
A chromosome number that is an exact multiple of the haploid complement. Diploid, triploid, tetraploid and polyploid cells are all euploid.

Equally important in the cancer aneuploidy field is the question of where to draw the line between euploidy and aneuploidy. For example, do cells with a single trisomy more closely resemble highly aneuploid cells, as they already need to survive and proliferate with an abnormal chromosome number? Or do such cells more closely resemble diploid cells, because only a small fraction of their genome is altered? The answer to such questions

is not straightforward. Single trisomies are sufficient to significantly affect cellular functions<sup>5,16,28</sup> and are, by the classical definition, aneuploid. However, when tumours with single chromosome gains or losses are classified in the 'diploid' group, the prognostic value of a high degree of aneuploidy becomes stronger<sup>29</sup>. This observation suggests that a threshold of tolerable karyotypic complexity exists (FIG. 2), potentially jeopardizing a simple



**Fig. 2 | The relationship between karyotype and fitness. a** | Normal mammalian cells are diploid; they have two chromosomal complements (2C). Changes in ploidy decrease the fitness of cells, and fitness is expected to decrease with increasing number of complements<sup>4</sup>. Nonetheless, compared to aneuploid cells, polyploid cells are still relatively fit, because their gene expression remains balanced<sup>215</sup>. The higher the degree of aneuploidy — that is, the more a karyotype deviates from a euploid state — the more imbalanced gene expression becomes, and consequently the lower cell fitness is. The relative fitness penalty of aneuploidy decreases with an increase in ploidy<sup>215</sup>. Polyploidy buffers against the adverse effects of aneuploidy, because the degree of gene expression imbalance is greater when a chromosome is gained or lost in a diploid than in a polyploid cell. Note that a high degree of polyploidy is also detrimental to most cells. **b** | Analysis of overall DNA content (denoted as 'N') does not necessarily inform as to karyotype composition. A highly aneuploid cell can have the same total DNA content as a triploid cell (3N); although the polyploid triploid cell also has a 3N DNA content, unlike the aneuploid cell, it has exactly three karyotypic complements (3C).

quantitative approach to aneuploidy. How useful, then, is the comparison of highly aneuploid tumours with near-diploid tumours using arbitrary group definitions (such as quartile comparisons)? Such considerations profoundly affect the conclusions. For example, an early study identified a gene expression signature of CIN that was associated with poor clinical outcome across human cancers<sup>30</sup>. More recent analyses called this signature into question<sup>9,20,31</sup>. It was shown that a refined view — one that considered extreme aneuploidy levels separately — was necessary to more accurately predict clinical outcome: both very high and very low levels of aneuploidy and CIN were found to be associated with response to genotoxic drugs and improved patient survival<sup>32,33</sup>.

So which convention should the field adopt? As we mentioned above, numerical aneuploidy was historically defined as whole-chromosome gains or losses<sup>6</sup>. Recent cancer genome analyses have included arm-level gains and losses — which would traditionally be called segmental or partial aneuploidies — under the broad umbrella of aneuploidy<sup>9–11</sup>. As the molecular mechanisms underlying whole-chromosome and chromosome arm alterations are different (chromosome mis-segregation and non-reciprocal translocations, respectively), we propose to adhere to the traditional definition in the context of cell biological studies. However, for quantitative genomic analyses, it does make sense to include chromosome arm-sized alterations under the definition of aneuploidy. Interestingly, large CNAs that encompass as many genes as small chromosome arms (or more) are a frequent occurrence in cancer (FIG. 1b), so a purely quantitative definition of aneuploidy would include these events as well (FIG. 1a). Nonetheless, for practical reasons we strongly encourage the field to adopt the already prevalent definition of aneuploidy as CNAs that affect either entire chromosome arms (excluding the short arms of acrocentric chromosomes) or whole chromosomes. Such a uniform definition would increase consistency and reproducibility across cancer studies.

### Effects of aneuploidy depend on context

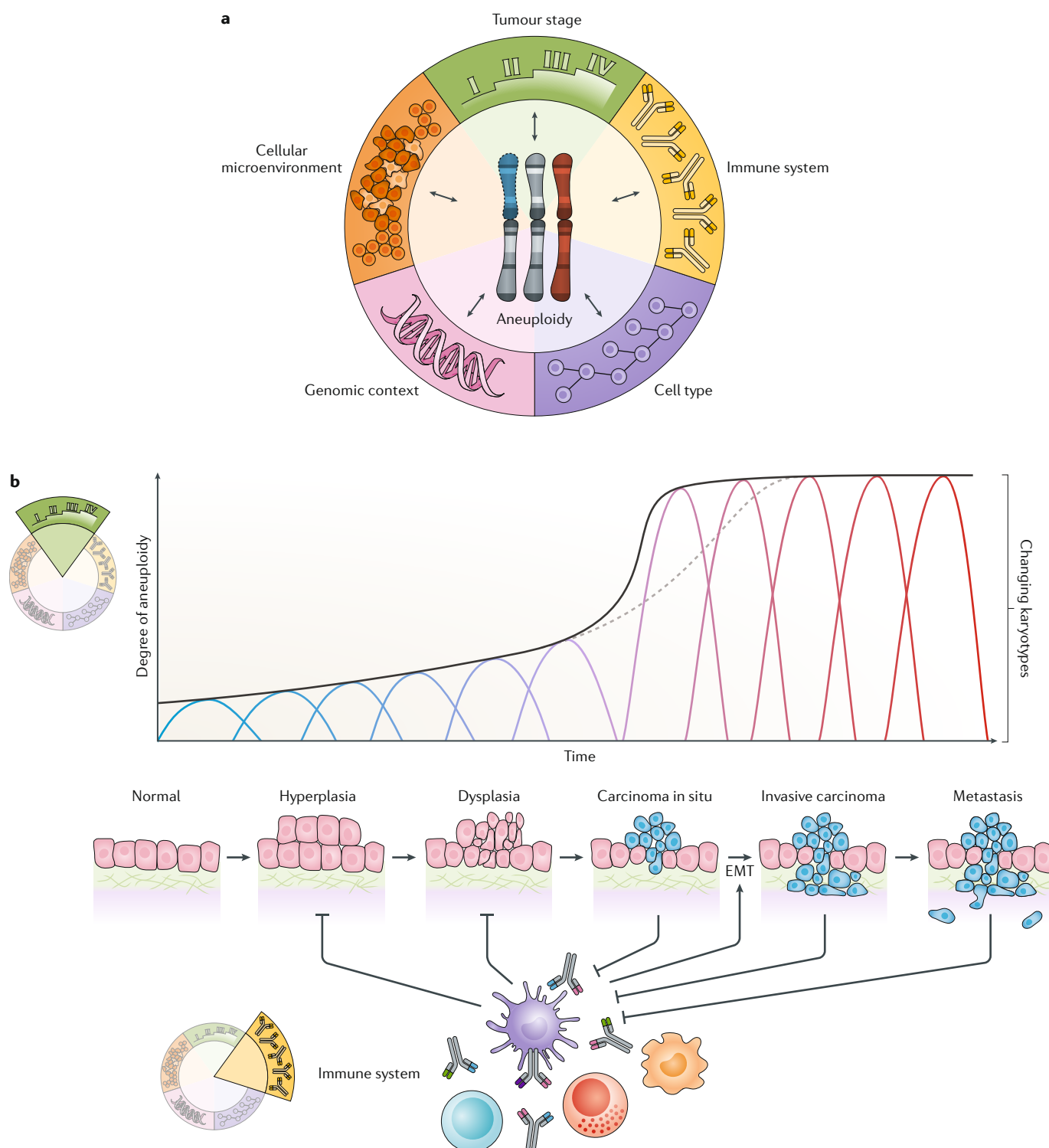
**Aneuploidy can promote or suppress tumour development.** Much like mutagenesis, CIN promotes tumour formation by inducing genetic diversity, which is the substrate for tumour evolution<sup>21</sup>. Recent findings suggest that the product of CIN — that is, aneuploidy — can both promote and suppress tumorigenesis. Systematic introduction of extra chromosomes into yeast genomes revealed that single-chromosome gains lead to slower proliferation and various detrimental metabolic and physiological consequences<sup>7</sup>. Studies in mouse cell lines and human cell lines reached similar conclusions: single-chromosome gains generally impair proliferation, alter metabolism and induce various stress responses<sup>8,16</sup>. Furthermore, oncogene-transformed trisomic cells exhibit reduced tumorigenicity compared to their diploid counterparts<sup>5</sup>. In cancer, too, a similar trend is observed: the frequency of chromosome arm gains and losses is inversely correlated with the number of coding genes on the chromosome arm<sup>10,34</sup>, suggesting that in most cases aneuploidy confers a fitness penalty. As such, aneuploidies would suppress rather than promote tumorigenesis.

On the other hand, several analyses of clinical tumour samples have found positive correlations between degree of aneuploidy and enrichment for proliferation and cell cycle transcriptional signatures that are generally thought to be indicative of promoting tumorigenesis<sup>9,31,35</sup>. Studies on mouse embryonic stem cells (mESCs) and human embryonic stem cells (hESCs) showed that specific single trisomies can also be tumour-promoting. Trisomy of mouse chromosome 8 can spontaneously arise as a sole aneuploidy in mESC cultures<sup>36,37</sup>, in which it confers a strong selective advantage<sup>36,38</sup>. Similarly, trisomy of human chromosome 12 commonly arises and spreads in cultures of hESCs and is associated with increased proliferation and tumorigenicity<sup>28</sup>. Moreover, a recent study of a near-diploid colorectal cancer cell line and aneuploid clones derived from it showed that single trisomies are able to confer a selective advantage and increase the tumorigenic behaviour of human cancer cells cultured under conditions of stress (such as serum starvation, drug treatment and hypoxia)<sup>39</sup>, which is consistent with previous findings from yeast<sup>40,41</sup>. Similarly, a study of mouse embryonic fibroblasts (MEFs) demonstrated that single chromosome losses generally led to a proliferation disadvantage in vitro, but allowed tetraploid MEFs to grow better than diploid MEFs upon transplantation into immune-compromised mice<sup>17</sup>. These findings are in line with studies that introduced CIN into mice and showed that CIN can promote tumorigenesis in some contexts but inhibits it in others<sup>42–53</sup>.

It is generally thought that changes in the copy number of specific genes are responsible for the increased fitness of cells harbouring specific aneuploidies<sup>28,39,40</sup>. However, genetic interactions between altered chromosomes may also contribute. A key characteristic of aneuploid cells is that they often provoke genomic instability<sup>54–56</sup>. Cells harbouring single trisomies or monosomies often undergo spontaneous karyotype evolution, which can result in their enhanced growth<sup>5,17</sup>. Genomic evolution that generates karyotypes that are fitter than their single-aneuploidy precursors may also explain the co-occurrence of aneuploidies, which is frequently observed in stem cell cultures<sup>57,58</sup>, tumours<sup>9</sup> and yeast cells<sup>59,60</sup>.

Taken together, these studies indicate that, generally, aneuploidy is detrimental, but under specific circumstances it can confer a fitness advantage. Tumour stage, cell type, genetic make-up, tumour microenvironment and immune system interactions all determine the circumstances under which aneuploidy can drive tumorigenesis (FIG. 3a).

**The adaptive value of aneuploidy changes with tumour stage.** In genetically engineered mouse models, aneuploidy has been observed at late stages of tumorigenesis<sup>61–63</sup>. For example, in mouse models of breast cancer, clonal aneuploidy was detected only during progression to invasive carcinomas<sup>63</sup>. Similar observations have been made in human cancer. In colorectal cancer, aneuploidy is present at very low levels in early-stage tumours, but its prevalence increases in late-stage tumours<sup>64</sup>. In oesophageal cancer, aneuploidy arises during the progression from Barrett oesophagus to oesophageal



**Fig. 3 | The context-dependent role of aneuploidy during tumour development.** **a** | The major variables that determine the adaptive value of aneuploidy are presented in the circle. The interactions between aneuploidy and these variables are reciprocal. **b** | The graph and schematic show that the degree of aneuploidy increases with tumour progression. Initially, a complex (and yet to be fully elucidated) immune response limits the prevalence of aneuploid cells. For example, the cGAS–STING pathway recognizes DNA that leaks from micronuclei into the cytoplasm and activates an innate immune response. As cancer development progresses, tumours evolve mechanisms to evade immune recognition. There is evidence to suggest that this evolution occurs in bursts<sup>67</sup>, which may be

associated with the development of immune tolerance of aneuploidy. Later in tumorigenesis, the cGAS–STING pathway takes on a tumour-promoting role. The pathway activates a noncanonical nuclear factor  $\kappa$ B transcriptional response that promotes the epithelial-to-mesenchymal transition (EMT), thereby directly contributing to tumour progression. At different stages of tumorigenesis, different specific karyotypes provide a selective advantage and therefore become the dominant tumour karyotype. For example, while the degree of aneuploidy remains high in metastases, the aneuploidy landscapes of metastases would be different from that of the primary tumour, and might also be different among different metastases.



adenocarcinoma<sup>65</sup>. In cervical cancer, the recurrent gain of chromosome arm 3q characterizes the transition from severe dysplasia to invasive carcinoma<sup>66</sup>. These observations indicate that in many cancers, aneuploidy increases with tumour progression, perhaps marking the transition from local to invasive disease. However, this may not be true for all cancers. In both human breast cancer and human lung cancer, aneuploidy has been observed at the stage of carcinoma in situ (CIS)<sup>67–69</sup>, suggesting that it may confer a selective advantage early in tumorigenesis. Furthermore, some tumour-specific aneuploidies, such as trisomy 7 in glioblastoma, tend to arise earlier in tumorigenesis than others<sup>70,71</sup>. Nevertheless, although some specific aneuploidies can arise in pre-malignant lesions<sup>67,69,72</sup>, the degree of aneuploidy seems to be much higher in invasive epithelial tumours than in their non-invasive precursors (FIG. 3b).

CIN and aneuploidy not only affect primary tumour growth but also shape the metastatic process. The act of chromosome mis-segregation can promote metastasis by expanding karyotypic diversity or by activating the cGAS–cGAMP–STING pathway<sup>73</sup>. The cGAS–STING pathway is best known for sensing cytosolic DNA and triggering an innate immune response<sup>74</sup>. This pathway also induces an epithelial-to-mesenchymal transition (EMT), thereby promoting cell motility and metastatic behaviour<sup>74</sup> (FIG. 3b). Once dissemination has occurred, cells must acquire specific karyotypic compositions compatible with survival and proliferation at the distant site. This idea that specific karyotypes, distinct from those of the primary tumour, are needed for metastasis is supported by the fact that metastatic lesions often represent rare (or completely undetected) sub-clones of the primary tumour and tend to be relatively clonal<sup>75–78</sup>. Some recurrent aneuploidies become more prominent in metastases than in primary tumours<sup>14</sup>, whereas others are recurrent only in the metastatic context. For example, loss of chromosome arm 9p is considerably more prevalent in clear cell renal cancer metastases than in primary tumours<sup>79</sup>. Recent in vitro studies also support the idea that specific recurrent aneuploidies promote metastasis: although most single trisomies suppress metastatic potential in human cancer cell lines (as evaluated by in vitro proxies of metastasis), some promote it<sup>80</sup>.

The metastatic process itself consists of various unique sub-processes. Recent data obtained from cell line xenograft experiments suggest that specific karyotypes and aneuploidies promote these distinct metastatic stages. Specific aneuploidies that promote EMT were prevalent during the dissemination stages, followed by additional events that promoted the opposite state transition during metastatic colonization<sup>81</sup>. Similar adaptive mechanisms also seem to occur in earlier stages of tumorigenesis. For example, changes in the expression of metabolic genes were recently suggested to contribute to the prevalence of specific recurrent CNAs in human tumours<sup>82</sup>. As metabolic demands evolve throughout tumorigenesis (for example, as tumours grow and become more hypoxic), the fitness value of specific aneuploidies may change accordingly (FIG. 3b). Understanding karyotype dynamics will be critical for determining tumour behaviour throughout tumour

formation, progression and metastasis. However, most studies investigating this process to date have employed either advanced cancer cell lines (such as HCT116) or non-transformed cell lines (such as RPE1). Thus, novel human cell-derived model systems are needed in order to study the role of aneuploidy during distinct stages of tumorigenesis.

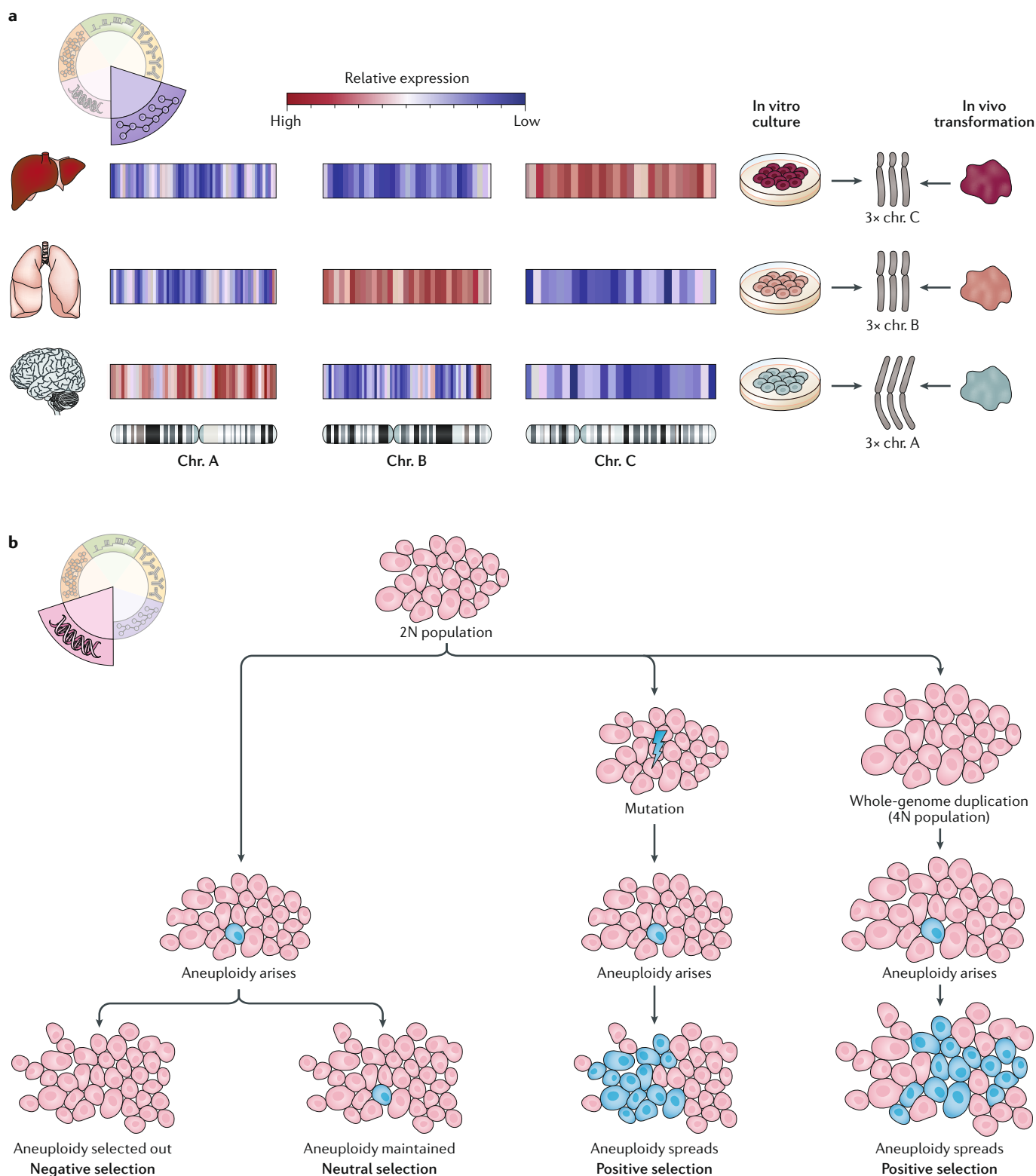
**Cell type dictates aneuploidy patterns.** Aneuploidy patterns vary widely across tumour types<sup>9,11–14</sup>. In some instances, the same chromosome is commonly gained in one tumour type, but frequently lost in another. For example, chromosome arm 13q is recurrently lost in lung squamous cell carcinoma and other cancer types, but is commonly gained in colorectal adenocarcinoma<sup>9,13,14</sup>. Similarly, chromosome arm 17p loss occurs in many tumour types, but this arm is frequently gained in kidney renal papillary cell carcinoma<sup>9,13,14</sup>. Similar tissue specificity is observed in mouse models of CIN. The same CIN driver gives rise to different karyotypes in different cancer types<sup>52</sup>. These and many other studies demonstrate that no single chromosome gain or loss universally promotes tumorigenesis. Instead, a picture emerges in which the tissue of origin dictates the aneuploidy patterns (FIG. 4a). Unsupervised clustering of tumours based on their aneuploidy patterns reveals that tumours that originate from the same tissue tend to cluster together<sup>83</sup>. Moreover, tumours of similar tissue types cluster more closely together than tumours of unrelated tissues. For example, various gynaecological cancers display similar aneuploidy patterns, as do various gastrointestinal cancers<sup>9</sup>. Squamous cell tumours are another case in point: irrespective of the tissue or organ of origin, they are more related to one another than to epithelial tumours of the tissue from which they were isolated<sup>9</sup>.

Aneuploidy patterns in cancer are thought to be driven by genes that control proliferation: chromosomes that are recurrently gained tend to be enriched for proliferation-promoting genes, and those that are recurrently lost are enriched for genes that repress proliferation<sup>84</sup>. The tissue-specific aneuploidy patterns in tumours indicate that these proliferation drivers function in a highly tissue-specific manner<sup>85</sup>, a result that is rather surprising, given the high degree of conservation of cell cycle control not only across tissues but across the eukaryotic kingdom. A recent study showed that aneuploidy recurrence patterns intensify pre-existing chromosomal gene expression differences in the respective normal tissues, thus providing another potential explanation for the tissue specificity<sup>70</sup>. The observation that cultured stem cells tend to acquire patterns of aneuploidy that resemble those observed in malignancies of their descendants<sup>86</sup> further suggests that these tissue-specific growth programs are already active well before cells undergo terminal differentiation and/or transformation (FIG. 4a).

**Genomic context shapes the aneuploidy landscape.** Genetic alterations interact with each other. This is of course also true in cancer. For example, the order in which somatic mutations occur influences cancer evolution<sup>87</sup>. The order of acquisition of *Ras* and *Tp53* mutations

#### Epithelial-to-mesenchymal transition

(EMT). A process by which epithelial cells lose their epithelial identity and adopt the properties of mesenchymal cells. They lose their ability to form cell–cell adhesion and gain migratory and invasive properties.



**Fig. 4 | The importance of cell type and genomic context in shaping the aneuploidy landscape during tumorigenesis. a** | The aneuploidy landscapes of human tumours are tissue-type-specific. Each organ (shown are the liver, lung and brain) exhibits a tissue-specific gene expression pattern; chromosome A is highly expressed in the brain, chromosome B in the lung and chromosome C in the liver. These differences in gene expression can determine aneuploidy patterns during oncogenic transformation and during culture in vitro, as cells are more likely to gain chromosomes that contain highly expressed

genes. Interestingly, the aberrations that arise frequently in a given tumour type are often similar to those that arise during the in vitro culturing of stem cells of the same lineage. **b** | The genomic context is important for determining the adaptive value of aneuploidy. A specific aneuploidy (blue) that occurs in diploid cells may be detrimental and thus may be selected against or be fitness-neutral (left). However, the same aneuploidy preceded by a specific point mutation (middle) or occurring in a tetraploid cell (right) may become advantageous and be selected for.

defines distinct adrenocortical tumour phenotypes in mouse models<sup>88</sup>. Similarly, the order of occurrence of *TET2* and *JAK2* mutations affects the manifestation of human myeloproliferative neoplasms<sup>89,90</sup>.

Given that the inherent fitness cost of aneuploidy is high and that its effects are context-dependent, aneuploidy may be particularly sensitive to other genetic alterations (FIG. 4b). Recent evidence suggests that this is the case. Recurrent aneuploidy patterns were found to be associated with specific dysregulated pathways<sup>91</sup>, and even with specific driver mutations<sup>63</sup>. For example, in breast cancer mouse models, tumours induced by *Myc* overexpression commonly gain an extra copy of mouse chromosome 15, whereas tumours induced by *Her2* overexpression frequently lose a copy of mouse chromosome 4 (REF.<sup>63</sup>). Evidence also exists for the reciprocal interaction, in which aneuploidy occurs first and dictates the acquisition of point mutations. Loss of chromosome arm 3p drives clear cell renal cancer in >90% of patients and is an early event in tumorigenesis, occurring decades before cancer is detected. Secondary mutations in tumour suppressors that reside on that chromosome arm are then selected for in the remaining allele, leading to cancer formation<sup>72,79</sup>.

A genetic alteration of particular interest is whole-genome duplication (WGD), which results in polyploidy. It can occur early during tumorigenesis and affects approximately one third of human cancers<sup>11,12,92</sup>. WGD is associated with elevated aneuploidy levels, especially with an increased loss of chromosomes<sup>9,12,92</sup>, presumably because the tetraploid genome buffers against the adverse consequences associated with chromosome loss. Whereas chromosome losses are rarely tolerated in diploid cells, they occur frequently in tetraploid cells and can promote cancer formation<sup>17,93</sup>. Therefore, WGD creates an aneuploidy-permissive condition. We conclude that both very small genetic alterations (such as point mutations) and very large genetic alterations (such as WGD) contribute to shaping the aneuploidy landscape of tumours (FIG. 4b).

**Cellular microenvironment determines aneuploidy evolution.** Aneuploidy seems to be particularly prone to genomic evolution; the inherent fitness cost associated with aneuploidy may readily shift from being advantageous to being a burden for the cell as selection pressures change during tumour evolution<sup>94</sup> (FIG. 5). This importance of cellular environment for chromosome composition is highlighted by recent genomic analyses of patient-derived cancer models (reviewed in REF.<sup>94</sup>). Rapid changes in the karyotype composition have been observed in patient-derived xenografts<sup>14</sup>, in patient-derived cell lines<sup>14</sup> and in patient-derived organoids<sup>95,96</sup>. Ongoing CIN that leads to continuous selection of specific aneuploidies has also been detected in single-cell-derived cultures of established human cell lines<sup>97,98</sup>, further demonstrating the importance of karyotype evolution and the practical challenge that it poses.

**The immune system governs aneuploidy tolerance.** Immune recognition is an important force in shaping the genomic landscape of tumours, and its association with aneuploidy is rather complicated. Recent clinical data analyses showed that the degree of tumour aneuploidy

correlates with markers of immune evasion and with reduced response to immunotherapy<sup>9,31,35</sup>. However, other lines of evidence suggest that aneuploidy is associated with the activation of some immune responses: two recent studies demonstrated that micronuclei, which can be by-products of chromosome mis-segregation, activate the innate immune response cGAS–cGAMP–STING pathway in non-transformed cells<sup>99,100</sup>. In another study, RPE-1 cells were made highly aneuploid by chemical perturbation of their mitotic checkpoint. These cells died when they were co-cultured with natural killer cells<sup>101</sup>. Even cells with very low levels of aneuploidy, such as primary cells harbouring discrete trisomies, express pro-inflammatory cytokines<sup>101,102</sup>. Furthermore, in mouse models of CIN, tumours exhibit elevated expression of the autophagy marker LC3 (also known as microtubule-associated proteins 1A/1B light chain 3A)<sup>31</sup>, which is also elevated when aneuploidy is introduced in cell culture<sup>103</sup>. Given that autophagy can induce and modulate inflammation (reviewed in REF.<sup>104</sup>), this may be another way through which aneuploidy elicits an immune response. It thus seems that aneuploidy induces immune recognition of cancer cells during the early stages of tumorigenesis, but at some point the aneuploid cancer cells successfully evade the immune system (FIG. 3b). Aneuploidy thus seems to be able to promote both immune detection and immune evasion, depending on the tumorigenic stage and the milieu of immune cells in the tumour microenvironment. The mechanism by which this transition occurs — and whether aneuploidy itself, events that correlate with high levels of aneuploidy (such as mitotic index or time of detection) or specific aneuploid karyotypes (for example, loss of heterozygosity of genes encoding components of the human leukocyte antigen complex)<sup>105</sup> play an active role in this transition — remains to be elucidated.

### The prognostic value of aneuploidy

Aneuploidy can readily be detected using multiple technologies, including various conventional and molecular cytogenetic methods, single-nucleotide polymorphism arrays, comparative genomic hybridization arrays and genome-wide DNA and RNA sequencing (reviewed in REFS<sup>106,107</sup>). Some of these methods are already routinely used in the clinic<sup>106</sup>, making aneuploidy an appealing biomarker for patient stratification, should it have a prognostic and/or a predictive value. Despite some confounding factors, it is worth exploring the value of aneuploidy in diagnosis. Similar to point mutations, aneuploidy could inform prognosis in a quantitative manner — that is, through overall aneuploidy burden or through specific recurrent alterations. An extensive body of evidence supports both types of associations in multiple cancer types (TABLE 1).

**The prognostic value of the degree of aneuploidy.** The prognostic value of aneuploidy has long been demonstrated for several indications<sup>108,109</sup>, with high levels of aneuploidy being associated with poorer prognosis in the vast majority of cases. A recent literature survey showed cellular DNA ploidy (which served as a proxy for the degree of aneuploidy in this study) to be an independent

#### Polyploidy

A euploid genome comprising more than two sets of chromosomes.

#### Human leukocyte antigen complex

A gene complex that encodes the major histocompatibility complex proteins and is responsible for regulation of the immune system.

#### Single-nucleotide polymorphism arrays

A DNA microarray that is used to detect genetic variation (including copy number alterations) on a genome-wide scale.

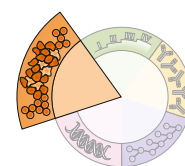
#### Comparative genomic hybridization arrays

A molecular technique to detect copy number alterations on a genome-wide scale and with high resolution.

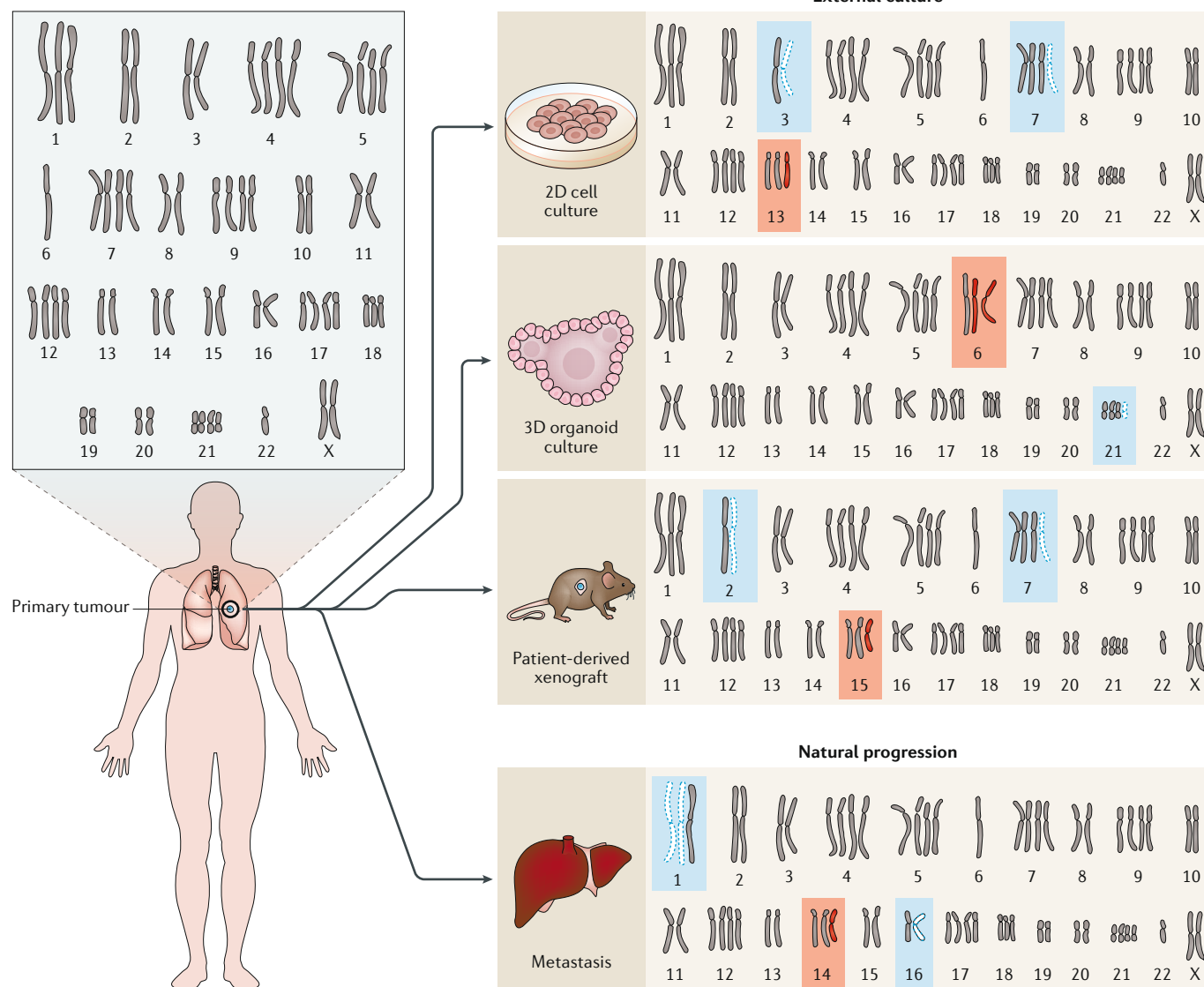
#### Predictive value

The degree to which a biomarker provides information about the effect of a therapeutic intervention.





External culture



**Fig. 5 | The cellular microenvironment shapes the cancer karyotype.** The environmental context shapes the aneuploidy landscape. When cancers are removed from their natural environment and are cultured as cell lines, organoids or patient-derived xenografts, the selection pressures change. As a result, karyotypes evolve, and different chromosomes are gained (red) or lost (blue) in different culture types. This process is conceptually similar to the aneuploidy evolution seen in metastases, where tumour cells also need to cope with selection pressures that are different from those of the primary tumour environment.

**The Cancer Genome Atlas (TCGA).** A cancer genomics repository that contains sequence information for over 20,000 primary cancers and matched normal samples across 33 cancer types.

#### CNA burden

The prevalence of copy number alterations (CNAs) within a tumour, commonly defined by the proportion of the genome that is affected by CNAs.

prognostic marker in patients with invasive breast, early-stage endometrial, early-stage ovarian, prostate and colorectal cancers<sup>29</sup>. Congruently, a recent analysis of data from The Cancer Genome Atlas (TCGA) revealed that CNA burden (to which aneuploidy is the major contributor) is significantly associated with disease-free survival and overall survival in primary breast, endometrial, renal clear cell, thyroid and colorectal cancers<sup>110</sup>. A recent TCGA analysis used more direct aneuploidy scores that

take into account only arm-level and chromosome-level alterations and revealed highly aneuploid tumours to be associated with a significantly worse prognosis in 9 out of 27 tumour types<sup>80</sup>.

In colorectal cancer, a systematic meta-analysis of >7,000 patients revealed that later-stage tumours were more frequently aneuploid than early-stage tumours (odds ratio 1.51,  $p=0.0007$ ), indicating that aneuploidy could be a marker of disease stage<sup>64</sup>. Importantly, more

**Table 1 | The prognostic value of aneuploidy**

| Biomarker type            | Specific biomarker                     | Tumour type                           | Association with clinical outcome |   | Refs              |
|---------------------------|--|---------------------------------------|-----------------------------------|---|-------------------|
|                           |  |                                       | Directionality                    | Associated features   |                   |
| High degree of aneuploidy | Various estimates of aneuploidy levels | Colorectal cancer                     | Adverse                           | OS, DSS, RFS  | 29,64,110–115     |
|                           |  | Serous ovarian cancer                 | Adverse                           | RFS   | 29,34,80,116      |
|                           |  | Breast cancer                         | Adverse                           | OS, RFS   | 29,80,110,118–120 |
|                           |  | Squamous cell carcinoma of the tongue | Adverse                           | OS  | 121               |
|                           |  | Oesophageal carcinoma                 | Adverse                           | Disease progression   | 29,122,123        |
|                           |  | Prostate cancer                       | Adverse                           | OS, PSA recurrence, RFS   | 29,80,110,124–126 |
|                           |  | Cervical cancer                       | Adverse                           | Disease progression   | 29,128            |
|                           |  | Non-small-cell lung cancer            | Adverse                           | Disease progression   | 29,129–132        |
|                           | Hyperdiploid subgroup                  | Multiple myeloma                      | Favourable                        | PFS, OS   | 134               |
|                           | Hypodiploid subgroup                   | Acute lymphoblastic lymphoma          | Adverse                           | OS, RFS   | 135–137           |
| Specific aneuploidy       | Hyperdiploid subgroup                  |                                       | Favourable                        |   |                   |
|                           | 5 or 5q loss                           | Myelodysplastic syndrome              | Favourable                        | Disease progression, relapse, mortality following stem cell transplantation | 144–149           |
|                           | 7 or 7q loss                           |                                       | Adverse                           |   |                   |
|                           | 1p and 9p loss                         | Gliomas                               | Favourable                        | RFS, OS   | 153–157           |
|                           | 4 loss                                 | Colorectal cancer                     | Adverse                           | RFS   | 158               |
|                           | 1q gain or 1p or 12p or 17p loss       | Multiple myeloma                      | Adverse                           | PFS, OS   | 134,159           |
|                           | 17p loss                               | Chronic lymphocytic leukaemia         | Adverse                           | PFS, OS   | 160               |

DSS, disease-specific survival; OS, overall survival; PFS, progression-free survival; PSA, prostate-specific antigen; RFS, recurrence-free survival.

## Overall survival

The length of time from diagnosis or start of treatment during which patients remain alive.

## Disease-specific survival

The length of time from diagnosis or start of treatment during which patients have not died from that specific disease.

## Recurrence-free survival

The length of time from treatment during which no sign of cancer is found.

## Progression-free survival

The length of time from treatment during which patients live with a disease but it does not get worse.

## Microsatellite instability

Predisposition of a cell to mutations (hypermutability) due to impaired DNA mismatch repair.

## Prostate-specific antigen (PSA)

A protein produced by prostate cells. Its levels in the blood are elevated in prostate cancer. PSA is therefore used as a prostate cancer screening tool.

## Gleason score

A commonly used system to stage prostate cancers, based on their pathological features.

than half of the studies that were analysed in this meta-analysis reported a significant prognostic impact of aneuploidy for overall survival, disease-specific survival and recurrence-free survival, independent of tumour stage<sup>64</sup>. Similar conclusions were reached in additional meta-analyses of clinical colorectal studies<sup>29,111,112</sup>. Of particular note are large studies that have demonstrated an independent prognostic value of aneuploidy in multivariate analyses of defined cohorts of colorectal patients (mostly patients with stage II disease)<sup>113–115</sup>. In these studies, diploidy was found to be an even stronger marker of favourable prognosis than microsatellite instability (MSI), a well-known favourable prognostic marker in this disease<sup>113–115</sup>.

A high degree of aneuploidy was also found to be associated with poor overall patient survival in serous ovarian cancer<sup>34</sup>. In multivariate analysis, aneuploidy was the strongest independent prognostic factor of recurrence-free survival in stage I ovarian carcinomas<sup>116</sup>. Moreover, specific copy number signatures could predict both overall survival and the probability of platinum-resistant relapse in high-grade serous ovarian cancer<sup>117</sup>. In breast cancer, several studies confirmed aneuploidy as a multivariate indicator of poor survival<sup>29,110,118–120</sup>. Aneuploidy was also associated with various clinical and histopathological parameters in squamous cell carcinomas of the tongue<sup>121</sup>. In lung cancer, CIN and high CNA burden were associated with progression of pre-malignant lesions to cancer<sup>69</sup>. Similarly, in oesophageal cancer, higher levels of aneuploidy are observed in Barrett oesophagus of patients who will progress to oesophageal carcinoma<sup>122</sup>, and aneuploidy can be combined with other biomarkers to identify disease that will progress to high-grade dysplasia and/or carcinoma<sup>29,123</sup>.

In prostate cancer, aneuploidy was associated with prostate-specific antigen (PSA) recurrence-free interval<sup>124</sup>, and prostate tumours that contain aneuploid cells are more likely to recur after resection<sup>125,126</sup>. Most recently, it was found that the degree of aneuploidy is associated with overall survival of prostate cancer patients<sup>127</sup> and is a better predictor of patient outcome than Gleason score<sup>29,110</sup>. Assessment of the degree of aneuploidy has also been shown to augment traditional diagnostic tools. In cervical cancer, the detection of aneuploid cells can improve the sensitivity and the positive predictive value of the cytological analysis of Pap smears, making it a reliable, cost-effective indicator of the early stages of cancer progression<sup>29,128</sup>. Similarly, aneuploidy detection potentially can reduce erroneous diagnosis of non-small-cell lung cancer (NSCLC) based on cytology findings alone<sup>129</sup> and can improve the sensitivity of cytology in identifying early-stage NSCLC in high-risk populations, such as heavy smokers<sup>29,130–132</sup>.

Interestingly, in multiple myeloma (MM), a plasma cell malignancy, a high degree of aneuploidy predicts positive patient outcome and is, in fact, among the most important prognostic factors in this disease. MM is divided into two major subgroups based on aneuploidy: hyperdiploid MM is characterized by a high degree of aneuploidy, whereas non-hyperdiploid MM is characterized by smaller deviations from a diploid or a tetraploid karyotype and can be further sub-divided on the basis of chromosome number<sup>133</sup>. Hyperdiploidy is associated with a favourable prognostic value, but this association is not necessarily directly related to aneuploidy level, given the high number of other genetic alterations<sup>134</sup>. A similar association has been observed in acute lymphoblastic lymphoma (ALL), where hyperdiploid ALL is associated

### Pap smears

The Papanicolaou test, a commonly used histological method to screen for cervical cancer.

### Hyperdiploid MM

A subtype of multiple myeloma (MM) that is characterized by trisomy of eight specific chromosomes (3, 5, 7, 9, 11, 15, 19 and 21).

### Non-hyperdiploid MM

A subtype of multiple myeloma (MM) that can be further subdivided into hypodiploid ( $\leq 44$  chromosomes), pseudodiploid (45–46 chromosomes) and near-tetraploid ( $> 75$  chromosomes) subtypes.

### Hyperdiploid ALL

A subtype of acute lymphoblastic lymphoma (ALL) that is characterized by a chromosome count of 51–65, often involving one additional copy of chromosomes X, 4, 6, 10, 14, 17 and 18, and two additional copies of chromosome 21.

### Hypodiploid ALL

A subtype of acute lymphoblastic lymphoma (ALL) that can be further divided into near-haploid (24–31 chromosomes), low-hypodiploid (32–39 chromosomes) and high-hypodiploid (40–43 chromosomes) subtypes.

### Chromothripsis

The shattering of an individual chromosome into many pieces and its religation in random order, with amplification of some segments (those that provide a growth advantage, including oncogenes) and loss of others (for example, tumour suppressors).

### Intratour heterogeneity

(ITH). Genomic and/or phenotypic cell-to-cell variability within a tumour.

with favourable prognosis, whereas hypodiploid ALL is associated with poor prognosis<sup>135–137</sup>.

In summary, a high degree of aneuploidy has been associated with a worse clinical outcome in many different tumour types, but, curiously, it is also associated with a better prognosis in specific haematopoietic malignancies. An important question that is not yet fully answered is why aneuploidy is generally associated with adverse prognosis. One reason is that highly aneuploid cancer cells are generally less sensitive to chemotherapies. Decreased sensitivity of aneuploid cancer cells to genotoxic agents has been reported in cancer cell lines<sup>138,139</sup>, patient-derived xenograft models<sup>14</sup> and human tumours<sup>32</sup>. This increased drug resistance has been attributed to heterogeneity in tumour karyotypes, which is prevalent in aneuploid cancers<sup>32</sup>. Similarly, high degree of aneuploidy induced by transient CIN can lead to resistance to oncogene withdrawal in genetic mouse models<sup>42,43</sup>. Karyotype heterogeneity is of course caused by CIN, so it is possible that it is CIN rather than aneuploidy that causes drug resistance. Importantly, the relationship between aneuploidy levels and drug resistance is not a simple linear relationship, as there is a limit to the karyotypic complexities that cells can tolerate (FIG. 2). In fact, extreme levels of aneuploidy and/or CIN were reported to render cells more sensitive — rather than more resistant — to anticancer drugs<sup>14,32,33,140–142</sup>, in line with the notion of optimal karyotypic heterogeneity and chromosome mis-segregation rate<sup>143</sup>. Nevertheless, it is generally true that higher levels of aneuploidy are associated with resistance to chemotherapy. Thus, the overall degree of aneuploidy has not only a prognostic value, but also a predictive value.

**The prognostic value of specific recurrent aneuploidies.** In some cancers, specific recurrent aneuploidies have long been recognized to be of prognostic value. Moreover, specific aneuploidies can, in some cases, inform clinical patient management. The best example for this is myelodysplastic syndrome (MDS), a clonal disorder of haematopoietic stem cells that can progress to acute myeloid leukaemia<sup>144,145</sup>. The current risk classification of MDS patients defines five risk groups based on specific aneuploidies. For example, monosomy of chromosomes 5 and 7 or loss of the long arms of one of these chromosomes (monosomy 5/5q or 7/7q) is highly recurrent in this haematopoietic disorder<sup>146</sup>. However, whereas patients with monosomy 5/5q have a good prognosis, patients with monosomy 7/7q are classified as being in a ‘poor prognosis’ group<sup>144,145</sup>. This aneuploidy-based classification has a very strong prognostic value, as it is very significantly associated with relapse and mortality following haematopoietic stem cell transplantation<sup>147</sup>. Moreover, this cytogenetic classification determines the course of treatment of MDS patients: most notably, the apoptosis-inducing drug lenalidomide is specifically indicated for the treatment of MDS patients with a loss of chromosome arm 5q (reviewed in REFS<sup>148,149</sup>).

Gliomas are another prominent example of a strong prognostic value associated with specific aneuploidies. In grade III anaplastic oligodendrogliomas, the co-occurring loss of chromosome arms 1p and 19q marks

a clinically distinct molecular subtype within this histologically defined tumour type<sup>150–152</sup>. Co-loss of 1p/19p is associated with a lower rate of relapse and improved overall survival following treatment with the alkylating agent temozolomide<sup>153</sup> and has been shown to be associated with a favourable prognosis, irrespective of whether patients were receiving radiotherapy, chemotherapy or both<sup>154–157</sup>. Furthermore, the status of these co-occurring aneuploidies directs treatment: 1p/19p co-loss predicts benefit from the addition of a chemotherapy regimen to radiotherapy<sup>156,157</sup>.

Both in MDS and in low-grade gliomas, the characteristic aneuploidies exist in an otherwise largely normal karyotype, indicative of low levels of, or no, CIN. However, the occurrence of specific aneuploidies can be prognostic in highly aneuploid CIN tumours as well<sup>80</sup>. For example, loss of specific chromosomes was identified as an independent prognostic factor in colorectal cancer<sup>158</sup>; losses and gains of specific chromosome arms are associated with a poor outcome in MM<sup>134,159</sup>; and loss of chromosome arm 17p predicts a more aggressive disease and lower drug response in chronic lymphocytic leukaemia (reviewed in REF<sup>160</sup>). In fact, a recent analysis of the TCGA data set identified 160 significant associations between specific aneuploidies and patient survival<sup>80</sup>. It thus seems that in almost any tumour type, specific aneuploidies have context-dependent prognostic value.

**Factors that confound the prognostic value of aneuploidy.** Because aneuploidy is most pervasive in the late stages of tumorigenesis, its detection would be associated with more advanced stages of disease. This in turn could generate an apparent association between aneuploidy and clinical outcome, simply because more advanced tumours would tend to be both more aneuploid and more aggressive. Therefore, it is extremely challenging to interpret the relationship between aneuploidy and patient prognosis on the basis of studies that do not stratify patients according to the clinical stage or grade of their tumours. To establish a direct link between aneuploidy and aggressiveness, the timing of diagnosis and the proliferation rate should be controlled.

Another potential caveat is that aneuploidy levels are associated with a high degree of CIN, which in turn is associated with inactivation of p53 (REFS<sup>9,92</sup>). Recently, it was suggested that chromothripsis is another major source of aneuploidy in human cancer<sup>161–163</sup>. The associations between these variables make it inherently challenging to disentangle their effects when attempting to analyse the prognostic value of aneuploidy per se<sup>3</sup>. The clinical relevance of CIN, chromothripsis and p53 status has been reviewed extensively<sup>22,26,74,164,165</sup>. It is important to bear in mind that, although these variables can be disentangled experimentally<sup>166</sup>, it is often impossible to entirely control for them when studying aneuploidy in a clinical context, rendering some of the literature ambiguous with respect to the causal relationships underlying the observed associations.

A third confounding factor is intratumour heterogeneity (ITH), which has been studied extensively in recent years, largely thanks to the advances in single-cell ‘omics’ technologies. These studies have revealed

the importance of ITH for cancer progression and for response to therapeutics (reviewed in REFS<sup>167,168</sup>). Histological ITH and tumour proliferation rates were found to reflect genetic ITH<sup>32</sup>. Interestingly, recent evidence suggests that numerical and structural CIN drives the development and maintenance of ITH more strongly than point mutations<sup>32</sup>. Furthermore, CNA heterogeneity, but not point mutation heterogeneity, is strongly associated with clinical outcome<sup>169</sup>. Stratification of tumours based on ITH and CNA burden revealed that it is the interaction between these two parameters that determines the clinical outcome: high CNA burden with low ITH was associated with the best overall survival<sup>32</sup>. Although this study did not examine aneuploidy specifically, CNA burden was defined as the fraction of the genome affected by CNAs, and was therefore determined largely by aneuploidy. These findings highlight the importance of controlling for ITH when assessing the association between aneuploidy and clinical outcome. Recent developments in single-cell sequencing now enable more comprehensive analyses of ITH and its association with aneuploidy<sup>107</sup>.

It is impressive that despite the inherent challenges, both the degree of aneuploidy and specific aneuploidies have been successfully and convincingly associated with clinical outcome, to the point that they can inform clinical management in some specific cases. Accounting and controlling for potentially confounding factors is expected to further improve our understanding of the prognostic and predictive value of cancer aneuploidy.

### Aneuploidy as a therapeutic target

The overwhelming prevalence of aneuploidy in human cancer, along with the tumour clonality of some of the specific events and their prognostic value, leads to the conclusion that aneuploidy should be considered as a therapeutic target. As for all other genetic lesions in cancer (such as point mutations), a fundamental distinction ought to be made between the tumorigenic roles of the process — CIN and mutagenesis — and those of its outcomes — aneuploidy and mutations. Both the process and its outcomes may present therapeutic opportunities. For example, inhibitors of DNA damage response proteins, such as poly(ADP-ribose) polymerase (PARP) inhibitors, are used to target genomically unstable cells that are deficient in homologous recombination and DNA repair<sup>170</sup>, and therefore can be considered drugs that target the mutagenic process. By contrast, inhibitors of epidermal growth factor receptor (EGFR) signalling are used to target *EGFR*-mutant tumours<sup>171</sup>, and thus are considered therapies that target a recurrent molecular alteration. The clinical relevance and putative therapeutic value of CIN has recently been reviewed elsewhere<sup>22,74</sup> and will not be discussed here. Instead, we will focus on aneuploidy per se. Consistent with the definitions above, exploiting aneuploidy for cancer therapy merits consideration in two distinct ways: targeting the cellular consequences induced by a high degree of aneuploidy (independently of CIN) and targeting the unique vulnerabilities induced by specific recurrent aneuploidies. The potential targeting of specific aneuploidies could be further divided into two conceptual approaches: identifying and targeting the

drivers of recurrent aneuploidies, which might be considered a particular class of cancer genes, and identifying genes linked to these drivers that do not contribute to, but are invariably associated with, the specific aneuploidy.

**Targeting the aneuploid state.** High levels of aneuploidy elicit cellular stress, as cells need to rewire their basic physiological functions to cope with the broad consequences of an imbalanced karyotype. The cellular stresses induced by aneuploidy have recently been summarized elsewhere<sup>172,173</sup>. They can be divided broadly into five categories: proteotoxic, metabolic, replicative, mitotic and hypo-osmotic<sup>172,174</sup>. These cellular stresses may induce unique vulnerabilities that are shared by many, if not all, highly aneuploid cells, regardless of which chromosome has an altered copy number. In line with this notion, different aneuploidies were found to induce similar transcriptional programs in mammalian cell lines genetically manipulated to harbour aneuploidies<sup>102,175</sup>.

The cellular stresses of aneuploidy could be exploited therapeutically by identifying genetic alterations or compounds that are synthetic lethal with the condition. For example, proteotoxic stress seems to be especially widespread amongst aneuploid cells. Aneuploidy leads to stoichiometric imbalance among the members of protein complexes, thereby increasing aggregation and the need for protein degradation<sup>176</sup>. Thus, the protein quality control machinery is limiting in aneuploid cells, which causes them to be more sensitive to conditions that require increased protein folding and degradation capacity. For example, in budding yeast, aneuploid strains are uniquely sensitive to proteasome inhibition<sup>7</sup> and to deletion of the gene encoding ubiquitin carboxyl-terminal hydrolase 3 (Ubp3), a deubiquitylating enzyme involved in protein homeostasis<sup>177</sup>. However, the generalizability of these findings and their applicability to human cancer remains an open question. On the one hand, knockdown of the gene encoding ubiquitin carboxyl-terminal hydrolase 10 (USP10), the human homologue of Ubp3, was detrimental to the fitness of aneuploid human cells<sup>177</sup>. On the other hand, although trisomic mouse cells and human cells were more sensitive than their diploid counterparts to inhibitors of heat shock protein 90 (HSP90), which is a major regulator of proteostasis, they were not more sensitive to proteasome inhibitors<sup>178,179</sup>. A recent analysis of TCGA data revealed that the agreement between DNA copy number levels and protein levels was lower than that between DNA and mRNA levels, especially for the subset of proteins that function as subunits of protein complexes<sup>176</sup>. In human cancer cell lines, this 'protein attenuation' was regulated, at least partly, by proteome degradation. Surprisingly, however, increased protein attenuation was suggested to be associated with increased resistance (rather than sensitivity) of cell lines with high CNA burden to proteasome inhibition<sup>176</sup>. Therefore, the potential vulnerability of aneuploid human cancer cells to different classes of antagonists of protein homeostasis, and the specific contexts in which such dependence might be therapeutically relevant, remains to be elucidated.

Dysregulated sphingolipid metabolism is another example of a potentially actionable aneuploidy-induced vulnerability. Ceramide levels are higher in aneuploid



budding yeast than in diploids, and genetic and chemical interventions that further upregulate ceramide levels slow down their proliferation<sup>180</sup>. Elevated levels of ceramide were also found in aneuploid mammalian cells<sup>181</sup>. Further increasing the levels of this lipid, either genetically or pharmacologically, induced apoptosis in aneuploid MEFs and in highly aneuploid human colorectal cancer cell lines<sup>181</sup>. Last but not least, the growth disadvantage caused by aneuploidy-induced cellular stresses could also lend itself to therapeutic exploitation.

In addition to vulnerabilities associated with the stress response to aneuploidy, genes that enable aneuploid cells to tolerate such stress comprise another class of potential targets. Such genes have been identified in aneuploid yeast<sup>182</sup> and in aneuploid human cells<sup>179</sup>. Inhibiting these genes may exacerbate the cellular stresses induced by aneuploidy, thereby reducing the viability and proliferation of aneuploid cells or making them more sensitive to drugs that target these stress pathways. For example, a recent study showed that the stress-induced mitogen-activated protein kinase (MAPK) p38 $\alpha$  (also known as MAPK14) is activated following chromosome mis-segregation and promotes apoptosis<sup>183</sup>. MAPK p38 $\alpha$  inactivation induces aneuploidy tolerance and facilitates the expansion of aneuploid clones<sup>183</sup>. Moreover, MAPK p38 $\alpha$  inhibitors can potentiate the CIN-inducing effects of taxanes<sup>184</sup>, which provides a rationale for combining these drugs for cancer therapy. Similarly, overexpression of the anti-apoptotic protein BCL-X<sub>L</sub> was recently found to enable the survival of aneuploid human pluripotent stem cells<sup>185</sup>. Targeting MAPK p38 $\alpha$  or anti-apoptotic proteins in aneuploid cells could therefore suppress aneuploidy tolerance.

The identification of cellular dependencies induced by aneuploidy itself, by the general stresses caused by aneuploidy or by the cellular changes that enable aneuploidy tolerance has so far been based mostly on small-scale and medium-scale chemical screens in isogenic model systems of diverse karyotypes<sup>178,181</sup>. These proof-of-concept efforts should now be expanded to include large-scale chemical screens and genome-wide loss-of-function and gain-of-function screens (such as CRISPR–Cas9, CRISPR interference and CRISPR activation screens) across a large repertoire of isogenic diploid and aneuploid mammalian models, to ensure the generalizability of the identified differential vulnerabilities. Importantly, it is unlikely that any single drug could kill aneuploid cells selectively and potently across all cancer contexts, so even ‘general’ dependencies should not be expected to be universal. It therefore remains crucial to dissect the molecular mechanisms underlying such dependencies, in order to elucidate the most promising cellular contexts for their targeting.

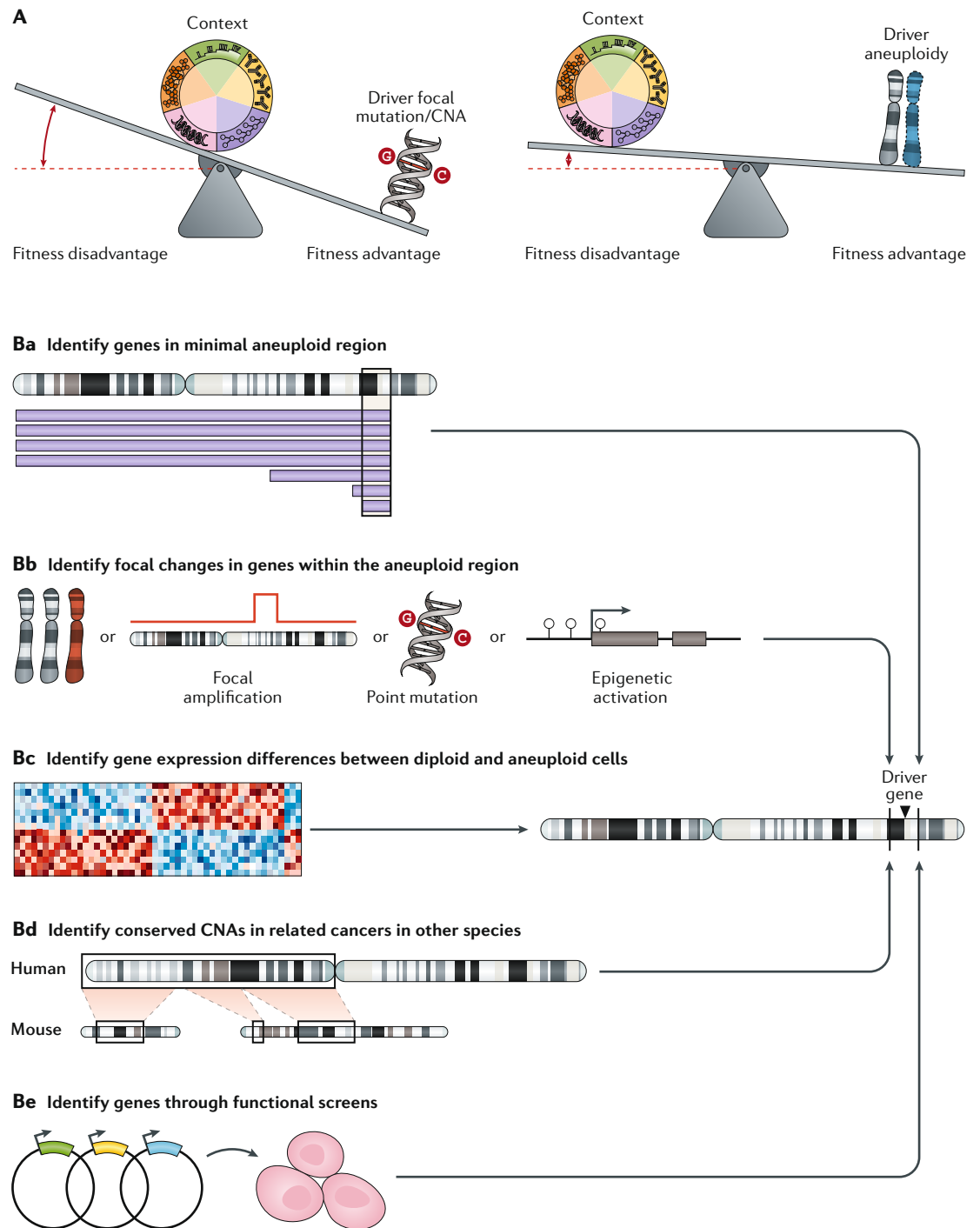
**Targeting drivers of specific aneuploidies.** Although the successful therapeutic targeting of recurrent point mutations and specific gene amplifications should certainly inspire research aimed at targeting recurrent aneuploidies, critical differences between these types of genomic aberrations are likely to affect how they are targeted (FIG. 6A). Firstly, although cellular context always matters, it seems to be more important in the case of aneuploidy. Perturbation of specific oncogenes and tumour suppressor genes (such as loss of the retinoblastoma-associated protein RB1) can

drive tumorigenesis in a cell-type-specific manner<sup>186–188</sup>, and many genetic alterations are cancer-type-specific<sup>83,85</sup>, but specific genes can be universally tumour-promoting (such as *KRAS*) or tumour-suppressive (such as *TP53*)<sup>189</sup>. By contrast, no chromosome is known to be universally oncogenic or tumour-suppressive; specific chromosome gains or losses are invariably tissue-specific<sup>9,11,13</sup>, and their targeting would likely be tissue-specific as well. Secondly, recent analyses have demonstrated that positive selection overwhelmingly outweighs negative selection during cancer development, and the vast majority (~99%) of coding mutations are tolerated and escape negative selection<sup>190</sup>. By contrast, aneuploidy comes with a strong fitness cost (reviewed in REFS<sup>4,6</sup>) (FIG. 6A), and experimentally induced aneuploid cells are often selected against and outcompeted by their diploid counterparts<sup>5,9</sup>. Thirdly, whereas point mutations and focal CNAs, such as multi-copy amplification or a complete deletion, can lead to drastic changes in expression of the affected genes, aneuploidy usually involves only a single-copy gain or loss, thus leading to much milder changes in the expression of the affected genes<sup>191–195</sup>. The small difference in gene expression between euploid and aneuploid cells may make it more difficult to target aneuploid genes. At the same time, however, aneuploidy affects the expression of many more genes than do the other aforementioned genetic alterations, thus exerting a quantitatively larger overall effect on global gene expression<sup>191–195</sup>. Taken together, these considerations suggest that targeted therapeutics should focus on the genes that drive the gain or loss of a specific chromosome.

Identifying these driver genes is critical but far from trivial. It has recently been suggested that aneuploidies are largely driven by the cumulative effects of oncogenes and tumour suppressor genes that reside within the aberrant chromosome<sup>84,85</sup>. Consistent with this idea, even when a bona fide oncogene or tumour suppressor gene resides within a highly recurrent aneuploidy, it is likely that other genetically linked genes contribute to the selective advantage of the aneuploidy<sup>196,197</sup>. For example, inactivation of the *TP53* gene is a major driver of chromosome arm 17p loss in multiple cancer types. However, even in the context of *TP53* loss, reduced dosage of neighbouring tumour suppressor genes exacerbates the severity of the phenotype<sup>196</sup>. Therefore, identifying the genes that drive recurrent aneuploidies and understanding the relative importance of such aneuploidy drivers to various aspects of tumorigenesis (such as proliferation, migration and immune evasion) will be critical for their therapeutic exploitation.

How can we identify the drivers of recurrent aneuploidies? Several complementary strategies could be combined (FIG. 6B). Firstly, driver genes are expected to reside within the minimal recurrent aberrant region<sup>10,91,198</sup> (FIG. 6Ba). Secondly, driver genes may be disrupted by alternative mechanisms, such as focal CNAs, point mutations and/or epigenetic alterations (FIG. 6Bb). For example, the most common loss of the *TP53* gene in cancer involves a missense mutation in one allele and loss of the other through a 17p chromosome arm loss<sup>147</sup>. Similarly, mutations in the genes *FUBP1* and *CIC*, which reside on chromosome arms 1p and 19q, respectively, are very





common in a subtype of low-grade gliomas with 1p/19q co-loss, implicating them as drivers of these chromosome arm losses<sup>151,199,200</sup>. Thirdly, because coding genes typically exert their effect through gene expression, drivers are expected to be differentially expressed when genetically altered (FIG. 6Bc). Differential gene expression analyses can therefore help prioritize candidate driver genes within aneuploid chromosomes, as has been recently shown in luminal and HER2-enriched breast cancer subtypes<sup>63,91</sup>. Fourthly, cross-species comparative oncogenomic approaches can be used to identify evolutionarily conserved drivers within syntenic chromosomal

regions (FIG. 6Bd). The aneuploidy landscapes of genetically engineered mouse models have been shown to be similar to those that characterize human cancer<sup>150</sup>, and the incomplete synteny between the mouse and human genomes could thus help focus the regions of interest within recurrent aneuploidies<sup>63,201–203</sup>. Finally, systematic loss-of-function and gain-of-function genetic screens can reveal genes whose perturbation phenocopies the aneuploidy, or that can rescue the disease phenotype, thus implicating them as drivers of these events<sup>204,205</sup> (FIG. 6Be).

Identifying drivers of specific aneuploidies will be important for revealing their functional role in the

**Syntenic**  
Chromosomal regions that are conserved between two species.

◀ **Fig. 6 | Strategies to identify drivers of recurrent aneuploidies.** **A** | Gene-focused genetic alterations (left), such as point mutations and focal copy number alterations (CNAs), differ from aneuploidy (right) in their effects on cellular fitness. In both cases, context matters. However, some oncogenes and tumour suppressor genes are universal, whereas the adaptive value of aneuploidy is always context-dependent. The advantage conferred by aneuploidy drivers is counterbalanced by the fitness penalty associated with the simultaneous dysregulation of the many other genes located on the aneuploid chromosome. Consequently, most passenger point mutations are tolerated and escape negative selection, whereas most aneuploidies are expected to be selected against in most contexts. Recurrent aneuploidies must therefore include driver genes that counterbalance rather strong negative selection pressures. The seesaw analogy illustrates this point. Driver mutations induce a strong positive selection, pushing one side of the seesaw all the way down. Driver aneuploidies induce a weaker overall positive selection due to the fitness penalties associated with changing the copy number of many other genes located on the aneuploid chromosome, and the seesaw is therefore pushed down to a lesser degree. **B** | Several strategies can be combined in order to identify the driver genes that underlie recurrent aneuploidies. **Ba** | Minimal recurrence analysis can identify smaller regions of interest within a recurrent aneuploidy. **Bb** | Integrative analysis with alternative modes of gene activation or inactivation (such as point mutations, focal CNAs and epigenetic regulation) can identify genes that are under strong selection within a recurrent aneuploidy. **Bc** | Gene expression analysis can focus the search on expressed genes. **Bd** | Cross-species synteny comparison can identify synteny blocks and orthologous genes of interest. **Be** | Loss-of-function and gain-of-function genetic screens can functionally confirm the contributions of specific candidate genes to phenotypes associated with a recurrent aneuploidy.

particular context of their prevalence. It may also spark efforts to target these aneuploidy drivers. Encouragingly, because these cancer drivers function through single-copy number gain or loss, they may be especially susceptible to subtle manipulations of their expression levels. However, such fine-tuning of gene expression levels is likely to be a challenging task.

**Targeting passengers of specific aneuploidies.** The genetic linkage that is inherent to chromosomes presents a unique opportunity to eliminate aneuploid cells (FIG. 7). Genes that are linked to genes that drive a particular aneuploidy may enable the targeting of cells that harbour that aneuploidy. Such targetable passenger genes could be identified by unbiased genetic and chemical screens of isogenic cell models (such as cell lines with and without an aneuploidy that is characteristic of that particular tumour type). Unlike in screens to identify general aneuploidy-induced vulnerabilities<sup>178</sup>, the identified liabilities would be unique to a specific karyotypic composition of interest. For example, a chemical screen of isogenic cell lines against 4,000 compounds revealed that loss of chromosome arm 8p is associated with increased sensitivity to autophagy inhibitors, potentially owing to down-regulation of the acid ceramidase gene, *ASAH1* (REF.<sup>206</sup>). A smaller-scale chemical screen suggested that pluripotent stem cells and germ cell tumour cells with trisomy 12 may be more sensitive to replication inhibitors<sup>28</sup>.

Haploinsufficient genes within recurrent chromosomal losses are of particular interest in this context. Between 27% and 45% of essential genes are estimated to be haploinsufficient<sup>84</sup>. Copy number loss, such as occurs in monosomies, renders cells more sensitive to further suppression of these genes<sup>207</sup>. For example, one copy of the gene encoding the splicing factor SF3B1 is lost in 11% of human cancers, most often (in 81% of cases) because of the loss of chromosome arm 2q<sup>208</sup>. Breast and haematopoietic cell lines with this particular aneuploidy are consequently

more sensitive to SF3B1 inhibition<sup>208</sup>. Importantly, this type of vulnerability has been recently predicted to be common in human cancer<sup>208</sup>. Interestingly, the opposite of haploinsufficiency — overexpression toxicity — may also be targetable. Many genes, when overexpressed, reduce cell viability and proliferation<sup>85,209</sup>. Not surprisingly, copy number landscapes in cancer evolve to avoid the gain of such genes<sup>210</sup>. When dosage-sensitive genes reside within a recurrent trisomy, their genetic or epigenetic silencing (for example, by promoter hypermethylation)<sup>211</sup> may be required for the tolerance or positive selection of this trisomy. Reversing these inactivation mechanisms (for example, by demethylation) will antagonize the fitness advantage conferred by a particular trisomy. In budding yeast, most or perhaps all haploinsufficient genes are also toxic when overexpressed<sup>202</sup>. If this finding holds true in human cancer cells, it would raise the intriguing possibility that some dosage-sensitive cancer genes could be targeted through both inhibition and activation.

Homozygous deletions of passenger genes may represent additional therapeutic opportunities. Loss of both copies of an autosome or autosome arm is rare, but monosomies can contribute to the complete inactivation of genes whose other allele is mutated or focally deleted (such as in the example of *TP53* above). Such focal deletions could encompass genes that are irrelevant for tumorigenesis but that provide cancer-cell-specific synthetic lethality. For example, deletion of the *MTAP* gene is a common event in multiple cancers, owing to its genetic proximity to the tumour-suppressor gene *CDKN2A*. *MTAP*-deleted cells accumulate the metabolite methylthioadenosine (MTA), which inhibits protein arginine *N*-methyltransferase 5 (PRMT5) methyltransferase activity, rendering cells more sensitive to further PRMT5 inhibition<sup>212,213</sup>.

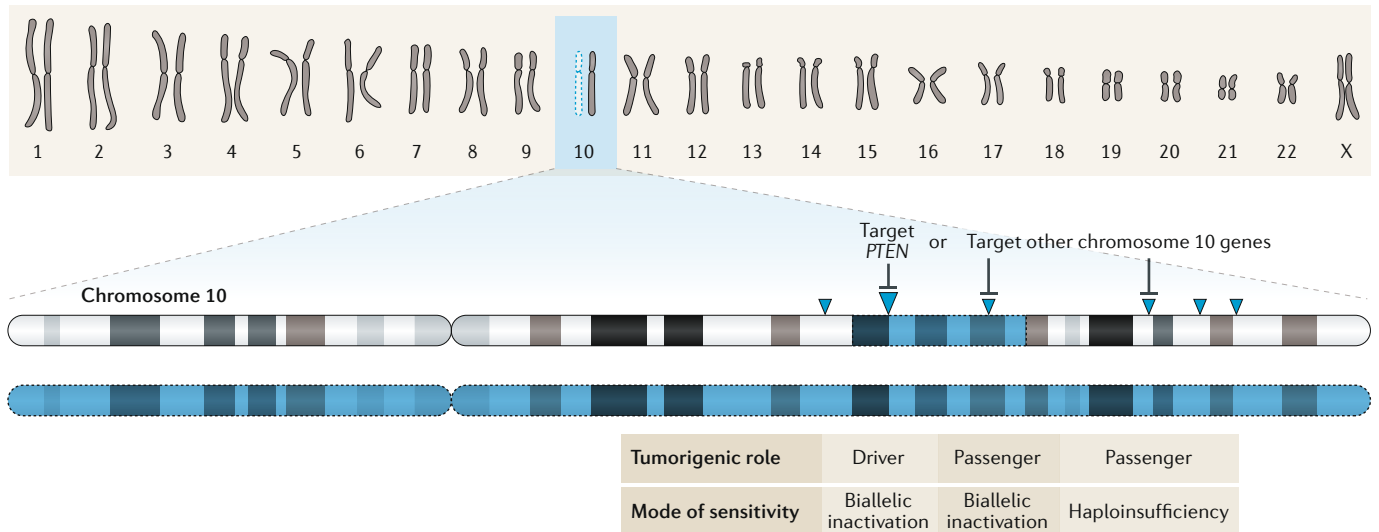
Given the importance of the loss of chromosome arms 5q and 7q in the pathogenesis of MDS, many attempts were made to identify vulnerabilities conferred by these chromosome arm losses<sup>204,205</sup>. As we mentioned above, lenalidomide is specifically used for the treatment of MDS with chromosome arm 5q loss. Haploinsufficiency of several genes within chromosome arm 5q — in particular, *CSNK1A1*, *RPS14*, *EGR1*, *miR-145* and *miR-146a* — was suggested to underlie this increased lenalidomide sensitivity<sup>149,204,214</sup>. Loss of some of these genes, such as *RPS14*, likely drives the disease<sup>204</sup>, whereas loss of others, such as *CSNK1A1*, is merely a passenger event<sup>207</sup>. The case of lenalidomide and chromosome arm 5q loss demonstrates that the identification of selective vulnerabilities of recurrent aneuploidies can be exploited therapeutically — importantly, even without a precise understanding of the mechanism that underlies this selectivity.

## Conclusions and future perspectives

The past five years have seen substantial progress towards understanding how aneuploidy influences and shapes tumorigenesis. Yet many questions remain unanswered. Not only is the biology of chromosome and chromosome arm gains and losses challenging to dissect, but the field faces (unnecessary) hurdles because it has yet to decide on how to define aneuploidy, its causes and its consequences.

### Haploinsufficient

A state in which deletion of one copy of a gene in a diploid organism results in a phenotype.



**Fig. 7 | Strategies to target recurrent aneuploidies in cancer.** Recurrent aneuploidies can be exploited therapeutically either by targeting the driver genes or by targeting genetically linked passenger genes. Passenger genes could be targeted if they are haploinsufficient or biallelically inactivated. For example, monosomy 10 is extremely common in glioblastomas. The loss of the tumour suppressor gene *PTEN* is thought to be a major driver of this monosomy<sup>216</sup>. Cells that harbour this monosomy could be targeted either by exploiting vulnerabilities caused by *PTEN* loss (for example, using phosphoinositide 3-kinase (PI3K) inhibitors<sup>217</sup>, by targeting other genes on chromosome 10 that are biallelically inactivated (for example, due to their proximity to *PTEN*) or by targeting haploinsufficient genes encoded on chromosome 10. Due to the large number of misregulated genes in specific aneuploidies, opportunities to target passenger genes might be greater than opportunities to target driver genes.

A generally accepted convention of defining aneuploidy would greatly facilitate the comparison of studies, especially those that investigate aneuploidy in cancer genomes. Many recent publications have adopted a chromosome arm definition of aneuploidy. We urge the field to adopt this convention. A clear distinction must also be made between the aneuploid state of a cell and CIN as its underlying mechanism. Furthermore, when describing the phenotypic consequences of the phenomenon or its therapeutic relevance, a clear distinction between a high degree of aneuploidy and specific recurrent aneuploidies is warranted. We believe that clarity in terminology is important for facilitating a fruitful scientific discussion and avoiding unnecessary ambiguities.

A major conceptual advance in the field is the realization that aneuploidy plays a context-dependent and dynamic role in cancer initiation and progression. Owing to the general fitness penalty of aneuploidy, tumour aneuploidy landscapes are likely the product of both positive and negative forms of selection, which are determined by tumour stage, cell type, genomic context, microenvironment and immune system interactions. It is therefore not surprising that the degree of aneuploidy and the presence of specific aneuploidies have been associated both with

adverse and with favourable clinical outcomes. These recent discoveries argue that we need to be cautious not to overgeneralize context-dependent experimental and clinical observations.

A refined view of cancer aneuploidy, which considers the complex relationship between aneuploidy and various spatial, temporal and context-dependent variables, is more likely to expose therapeutic vulnerabilities of this hallmark of cancer. Given the prevalence and recurrence patterns of aneuploidy across tumour types, tapping the potential of aneuploidy for cancer prognosis and treatment is urgently needed. Targeting the aneuploid state, specific aneuploidy drivers or specific aneuploidy passengers has been demonstrated to be useful in selectively killing aneuploid cells. However, translation of such approaches into the clinical care of cancer patients has so far been very limited. Thanks to the conceptual, methodological and technical advances that the field of cancer aneuploidy has recently seen, we predict that the uniquely large ‘attack surface’ inherent to large chromosomal alterations will make the clinical targeting of aneuploidy increasingly feasible.

Published online: 23 September 2019

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# Acknowledgements

The authors thank Iris Fung for her assistance with figure design. The work by U.B.-D. described in this Review was supported by the European Molecular Biology Organization Long-Term Fellowship and by the Human Frontier Science Program Postdoctoral Fellowship. U.B.-D. is an Azrieli Faculty Fellow. The work by the Amon lab described in this Review was supported by NIH grants CA206157 and GM118066. A.A. is an investigator of the Howard Hughes Medical Institute and the Paul F. Glenn Centre for Biology of Ageing Research at MIT. The authors apologize to the authors of many important publications not cited due to space limitations.

# Author contributions

The authors contributed equally to all aspects of the article.

# Competing interests

The authors declare no competing interests.

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*Nature Reviews Genetics* thanks F. Fojer, T. Ried and other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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