Context is everything: aneuploidy in cancer

Citation: Ben-David, Uri and Angelika Amon, "Context is everything: aneuploidy in cancer." Nature Reviews Genetics 21, 1 (January 2020): p. 44–62 doi. 10.1038/s41576-019-0171-x ©2019 Authors

As Published: https://dx.doi.org/10.1038/S41576-019-0171-X

Publisher: Springer Science and Business Media LLC

Persistent URL: https://hdl.handle.net/1721.1/126231

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

Context is everything: aneuploidy in cancer

Uri Ben-David[1] and Angelika Amon[2]

[1] Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 6997801, Israel.
Electronic address: ubendavid@tauex.tau.ac.il

[2] Koch Institute for Integrative Cancer Research, Department of Biology, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
Electronic address: angelika@mit.edu.

Correspondence should be addressed to:
ubendavid@tauex.tau.ac.il
angelika@mit.edu

Abstract

Cancer is driven by multiple types of genetic alterations, which range in size from point mutations to whole chromosome gains and losses, a condition known as aneuploidy. Chromosome instability, the process that gives rise to aneuploidy, can promote tumorigenesis by increasing genetic heterogeneity and promoting tumor evolution. However, much less is known about how aneuploidy itself contributes to tumor formation and progression. Unlike some pan-cancer oncogenes and tumor suppressor genes that drive transformation in virtually all cell types and cellular contexts, aneuploidy is not a universal promoter of tumorigenesis. Instead, a picture emerges that paints aneuploidy as a context-dependent cancer type-specific oncogenic event. In this Review, we discuss the role of aneuploidy in tumor development, and its clinical relevance as a prognostic marker and as a potential therapeutic target.

Introduction

Cancer aneuploidy is a biological enigma and a missed opportunity for cancer treatment. Aneuploidy, an imbalanced number of chromosomes, was identified as a distinct feature of cancer cells more than a century ago [1], 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. In 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 [2,3]).

The challenge to understanding the role of aneuploidy in cancer, and how this disease hallmark can be exploited clinically, stems from the "aneuploidy paradox" [4]: aneuploidy is detrimental for primary cells during organismal and tissue development and when introduced experimentally, and is associated with a substantial fitness cost under most circumstances [5-8]; at the same time, aneuploidy is well tolerated in cancer cells. ~90% of solid tumors are aneuploid (ranging from 26% to 99% across tumor types) [9]. In a typical solid tumor, ~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-arm length (or longer) per tumor [10,11]. No other genetic alterations affect cancer genomes to this extent. The existence of distinct, recurrent patterns of aneuploidy across tumor types [9,11-14] further suggests that specific aneuploidies drive tumorigenesis.

Aneuploidy is notoriously difficult to study, for several reasons. First, large chromosomal changes affect, by definition, hundreds (and sometime more) genes at once, complicating the identification of the genes that drive the recurrence of a specific aneuploidy in a particular cancer. Second, as discussed below, aneuploidy can play distinct, often opposite, roles in different contexts. Third, introducing or eliminating specific chromosomes remains technically

challenging and laborious, despite tools such as microcell-mediated chromosome transfer [15,16], Cre-Lox recombination [17] and CRISPR-Cas9 gene editing [9,18,19]. Consequently, we lack the ability to systematically characterize the consequences of aneuploidy across a wide range of chromosomes and cell types. Last but not least, it is often difficult to disentangle the effects of chromosome instability, the process that generates aneuploidy, from its product, an abnormal karyotype. While CIN is highly correlated with aneuploidy levels, some cancer cells may be highly aneuploid but chromosomally stable [20]. For example, CIN may be a transient phenomenon that is counterbalanced during tumor evolution (reviewed in [21]), but the resultant aneuploid karyotypes of cancer cells may persist long after CIN has been attenuated. Notwithstanding these challenges, recent progress in our understanding of cancer aneuploidy paves the way towards tackling them, both in the lab and in the clinic.

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

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. 1a**). Aneuploidy is classically defined as numerical aberrations of whole chromosomes and more recently in the cancer genome literature as chromosome arm gains or losses [9,11]. These definitions distinguish between aneuploidy and focal copy number alterations (CNAs), a justified distinction based on their distinct mechanistic origins and the biological differences between the two types of copy number changes. Aneuploidy usually results in small (~50%) changes in gene dosage across many genes, whereas focal CNAs frequently lead to much larger changes in gene dosage of a much smaller number of genes.

While 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, we have to ask whether there is a conceptual or functional difference between a ~16 Mbp gain/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 ~250 Mbp chromosome 2q arm – defined as a CNA. In other words, should aneuploidy be considered a quantitative trait, where 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 [9,11], 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?

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 straight-forward. Single trisomies are sufficient to significantly affect cellular functions [5,16,28] and are, by the classical definition, aneuploid. However, at the same time, when tumors with single chromosome gains or losses are classified in the "diploid" group, the prognostic value of high degree of aneuploidy becomes stronger [29]. This observation suggests that a threshold of tolerable karyotypic complexity exists, potentially jeopardizing a simple quantitative approach to aneuploidy.

How useful, then, is the comparison of highly-aneuploid tumors with near-diploid tumors using arbitrary group definitions (e.g., quartile comparisons)? Such considerations profoundly affect conclusions. For example, an early study identified a gene expression signature of CIN that was associated with poor clinical outcome across human cancers [30]. More recent analyses called this signature into question [9,20,31]. 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 [32,33].

So which convention should the field adopt? As mentioned above, historically, numerical aneuploidy was defined as whole chromosome gains or losses [6]. Recent cancer genome analyses included arm-level gains and losses – which would traditionally be called segmental or partial aneuploidies – under the broad umbrella of aneuploidy [9-11]. As the molecular mechanisms underlying whole-chromosome and chromosome-arm alterations are different (chromosome missegregation 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**), and so a pure quantitative definition of aneuploidy would include these events as well. Nonetheless, for practical reasons we strongly encourage the field to adopt the already prevalent definition of aneuploidy as CNAs that affect entire chromosomes arms (excluding the short arms of acrocentric chromosomes) or whole chromosomes. Such a uniform definition would increase consistency and reproducibility across cancer studies.

Aneuploidy and tumor development

How aneuploidy contributes to tumorigenesis is still being elucidated. In what follows we discuss the many critical questions that remain unanswered, and summarize recent work that has begun to shed light on them.

Is aneuploidy tumor-promoting or tumor-suppressive?

Much like mutagenesis, CIN promotes tumor formation by inducing genetic diversity, which is the substrate for tumor evolution [21]. Recent findings suggest that the product of CIN, 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 [7]. Studies in mouse and human cell lines reached similar conclusions: single chromosome gains generally impair proliferation, alter metabolism and induce various stress responses [8,16]. Further, oncogene-transformed trisomic cells exhibit reduced tumorigenicity compared to their diploid counterparts [5]. 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 [10,34], suggesting that in most cases aneuploidy confers a fitness penalty.

On the other hand, several analyses of clinical tumor samples found positive correlations between degree of aneuploidy and enrichment for proliferation and cell cycle-related transcriptional signatures [9,31,35]. Studies on mouse and human embryonic stem cells (ESCs) showed that specific single trisomies can be tumor-promoting as well: trisomy of mouse chromosome 8 can spontaneously arise as a sole aneuploidy in mouse ESC cultures [36,37], and confers a strong selective advantage on these cells [36,38]. Similarly, trisomy of human chromosome 12 commonly arises and spreads in cultures of human ESCs, and is associated with increased proliferation and tumorigenicity [28]. Moreover, a recent study of a near-diploid colorectal cancer cell line and aneuploid clones derived from it, found that single trisomies are able to confer a selective advantage and increase the tumorigenic behavior of human cancer cells cultured under non-standard conditions [39], consistent with previous findings from yeast [40,41]. Similarly, a study of mouse embryonic fibroblasts (MEFs) found 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 [17]. These findings are in line with studies that introduced CIN into mice, and found that CIN can promote tumorigenesis in some contexts but inhibits it in others [42-53].

It is generally thought that changes in copy number of specific chromosomes are responsible for increased fitness of cells harboring specific aneuploidies [28,39,40]. However, genetic interactions between altered chromosomes may also contribute. A key characteristic of aneuploid cells is that they often provoke genomic instability [54-56]. Cells harboring single trisomies or monosomies

often undergo spontaneous karyotype evolution, which can result in their enhanced growth [5,17]. 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 [57,58], tumors [9] and yeast cells [59,60].

Together, these studies indicate that, generally, aneuploidy is detrimental, but under specific circumstances it can confer a fitness advantage. Future studies are required to address how variables such as the cell type, the method used to generate a specific aneuploidy, and the missegregation rate, determine how a chromosome gain or loss affects the fitness of a cell. Such studies may also reveal whether any pre-existing or co-occurring (epi)genetic alterations are necessary for aneuploidy to be tolerated and to exert its tumor-promoting or tumor-inhibitory effects, potentially accounting for the different phenotypic consequences of "naturally-occurring" vs. "experimentally-induced" aneuploidies.

When does aneuploidy arise during tumorigenesis?

In genetically-engineered mouse models, aneuploidy has been observed at late stages of tumorigenesis [61-63]. For example, in mouse models of breast cancer, clonal aneuploidy was detected only during progression to invasive carcinomas [63]. Similar observations were made in human cancer. In colorectal cancer, aneuploidy is present at very low levels in early-stage tumors, but its prevalence increases in late-stage tumors [64]. In esophageal cancer, aneuploidy arises during the progression from Barrett's esophagus to esophageal adenocarcinoma [65]. In cervical cancer, the recurrent gain of chromosome arm 3q characterizes the transition from severe dysplasia to invasive carcinoma [66]. These observations indicate that in many cancers, aneuploidy increases with tumor progression, perhaps marking the transition from local to invasive disease. However, this may not be true for all cancers. Both in human breast cancer and in human lung cancer, aneuploidy has been observed already at the stage of carcinoma in situ (CIS) [67-69], suggesting that it may confer selective advantage early on. Furthermore, some tumor-specific aneuploidies tend to arise earlier in tumorigenesis than others [70]. In sum, while some specific aneuploidies can arise in pre-malignant lesions [67,69,71], the degree of aneuploidy seems to be much higher in invasive epithelial tumors than in their non-invasive precursors (**Fig. 2**).

Does aneuploidy promote metastasis?

The act of chromosome missegregation can promote metastasis by expanding karyotypic diversity or through activation of the cGAS-cGAMP-STING pathway [72], which senses cytosolic DNA and activates non-canonical NF-κB signaling, potentially triggering immune editing and immune evasion [73]. However, 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 tumor, are needed for metastasis, is supported by the fact that metastatic lesions often represent rare (or completely undetected) subclones of the primary tumor, and tend to be relatively clonal [74-77]. Some recurrent

aneuploidies become more prominent in metastases compared to primary tumors [14], whereas others are recurrent only in the metastatic context. For example, loss of chromosome arm 9p is significantly more prevalent in clear-cell renal cancer metastases than in primary tumors [78]. Recent *in vitro* studies also support the idea of specific recurrent aneuploidies promoting metastasis: while most single trisomies suppress metastatic potential in human cancer cell lines (as evaluated by *in vitro* proxies of metastasis), some promote it [79].

The metastatic process itself is comprised of various unique sub-processes. Recent data obtained from cell line xenograft experiments suggests that specific karyotypes and aneuploidies promote these distinct metastatic stages. Specific aneuploidies that promote epithelial-to-mesenchymal transition were prevalent during the dissemination stages, followed by additional events that promoted the opposite state transition during metastatic colonization [80]. Similar adaptive mechanisms also appear to occur in earlier stages of tumorigenesis. For example, metabolic genes were recently suggested to drive recurrent CNAs and contribute to their recurrence in human tumors [81]. As metabolic demands evolve throughout tumorigenesis (e.g. when tumors grow and become more hypoxic), the fitness value of specific aneuploidies may change accordingly (**Fig. 2**). Understanding karyotype dynamics will be critical for determining tumor behavior throughout tumor formation, progression and metastasis. However, most studies that have thus far been undertaken to study this process employ either advanced cancer cell lines (e.g., HCT116), or non-transformed cell lines (e.g., RPE1). Novel human cell-derived model systems to study the role of aneuploidy during distinct stages of tumorigenesis are needed to address this important question.

How does aneuploidy interact with the immune system?

Immune recognition is an important force in shaping the genomic landscape of tumors, and its association with aneuploidy is rather complicated. Recent clinical data analyses showed that the degree of tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy [9,31,35]. However, other lines of evidence suggest that aneuploidy is associated with activation of some immune responses: two recent studies demonstrated that micronuclei, which can be byproducts of chromosome missegregation, activate the innate immune response cGAS-cGAMP-STING pathway in non-transformed cells [82,83]. Another study found that aneuploid cells with complex karyotypes are cleared by natural killer cells in a co-culture experimental system where RPE-1 cells were made highly aneuploid [84]. Even cells with very low levels of aneuploidy, such as primary cells harboring discrete trisomies, express pro-inflammatory cytokines [84,85]. Furthermore, in mouse models of CIN tumors exhibit elevated expression of the autophagy marker LC3 [31], which is also elevated when aneuploidy is introduced in cell culture [86]. Given that autophagy can induce and modulate inflammation (reviewed in [87]), this may be another way by which aneuploidy elicits an immune response. It thus appears 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. 2**). Aneuploidy thus seems to be able to promote both immune detection and

immune evasion, depending on the tumorigenic stage and on the milieu of immune cells in the tumor microenvironment. The mechanism by which this transition occurs, and whether aneuploidy itself, events that correlate with high level aneuploidy (i.e. mitotic index, time of detection), or specific aneuploid karyotypes (e.g., by loss of heterozygosity of the human leukocyte antigen (HLA) [88]), play an active role in this transition remains to be elucidated.

Context matters

Recent studies of the prevalence of aneuploidy across different tumor types and experimental systems have revealed the strong context-dependence of cancer aneuploidy. It has become apparent that in order to elucidate how aneuploidy drives tumor formation and progression, and to identify vulnerabilities associated with specific recurrent aneuploidies, we have to take tumor type, genetic make-up, tumor grade, and tumor microenvironment into consideration (**Fig. 3a**).

Cell type dictates aneuploidy patterns

Aneuploidy patterns vary widely across tumor types [9,11-14]. In some instances, the same chromosome is commonly gained in one tumor type, but frequently lost in another one. For example, chromosome arm 13q is recurrently lost in lung squamous cell carcinoma and other cancer types, but commonly gained in colorectal adenocarcinoma [9,13,14]. Similarly, chromosome arm 17p loss occurs in many tumor types, but is frequently gained in kidney renal papillary cell carcinoma [9,13,14]. Similar tissue specificity is observed in mouse models of CIN. The same CIN driver gives rise to different karyotypes in different cancer types [52]. These and many other studies demonstrate that no single chromosome gain or loss universally promotes tumorigenesis. Instead, a picture emerges where the tissue of origin dictates aneuploidy patterns. Unsupervised clustering of tumors based on their aneuploidy patterns reveals that tumors that originate from the same tissue tend to cluster together [89]. Moreover, tumors of similar tissue types cluster more closely together than tumors of unrelated tissues. For example, various gynecological cancers display similar aneuploidy patterns, as do various gastrointestinal cancers [9]. Squamous cell tumors are another case in point: irrespective of tissue or organ origin, they are more related to one another than to epithelial tumors of the tissue they were isolated from [9].

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 for genes that repress proliferation [90]. The tissue-specific aneuploidy patterns in tumors indicate that these proliferation drivers function in a highly tissue-specific manner [91], a result that is highly surprising given the high degree of conservation of cell cycle control not only across tissues but across the eukaryotic kingdom. A recent study found 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 [70]. The observation that cultured stem cells tend to acquire patterns of

aneuploidy that resemble those observed in malignancies of their descendants [92] further suggests that these tissue-specific growth programs are already active well before cells undergo terminal differentiation and/or transformation (**Fig. 3b**).

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 [93]. Acquisition order of *Ras* and *Tp53* mutations defines distinct adrenocortical tumor phenotypes in mouse models [94]. Similarly, the order of occurrence of *TET2* and *JAK2* mutations affects the manifestation of human myeloproliferative neoplasms [95,96].

Given that the inherent fitness cost of aneuploidy is high and its effects are context-dependent, aneuploidy may be particularly sensitive to other genetic alterations (**Fig. 3c**). Recent evidence suggests that this is the case. Recurrent aneuploidy patterns were found to be associated with specific dysregulated pathways [97], and even with specific driver mutations [63]. Evidence for the reciprocal interaction, in which aneuploidy occurs first and dictates the acquisition of point mutations, also exists. Loss of chromosome arm 3p drives clear-cell renal cancer in >90% of patients and is an early event in tumorigenesis, decades before cancer is detected. Secondary mutations in tumor suppressors that reside on that chromosome arm are then selected for in the remaining allele, leading to cancer formation[71,78].

A genetic alteration of particular interest is whole-genome duplication (WGD). It can occur early during tumorigenesis and affects approximately one third of human cancers [11,12,98]. WGD is associated with elevated aneuploidy levels, and especially with an increased loss of chromosomes [9,12,98], presumably because the tetraploid genome buffers against the adverse consequences associated with chromosome loss. Whereas chromosome losses are rarely tolerated in diploid cells, their acquisition in tetraploid cells is frequent and can promote cancer formation [17,99]. Therefore, WGD is a common macro-evolutionary event that creates an aneuploidy-permissive condition. We conclude that both very small genetic alterations (i.e., point mutations) and very large genetic alterations (i.e., WGD) contribute to shaping the aneuploidy landscape of tumors (**Fig. 3c**).

Cellular microenvironment determines aneuploidy evolution

Aneuploidy seems to be particularly prone to genomic evolution, as 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 tumor evolution [100] (**Fig. 3d**). This importance of cellular environment on chromosome composition is highlighted by recent genomic analyses of patient-derived cancer models (reviewed in [100]). Rapid changes in the karyotype composition have been observed in patient-derived xenografts [14], in patient-derived cell lines [14], and in patient-derived organoids [101,102]. Ongoing CIN that leads to continuous selection of specific aneuploidies has also been detected in single cell-derived cultures of established human cell lines

[103,104], further demonstrating the importance of karyotype evolution and the practical challenge that it poses.

The prognostic value of aneuploidy

Aneuploidy can be readily detected using multiple technologies, including various methods of conventional and molecular cytogenetics, SNP and CGH arrays, and genome-wide DNA and RNA sequencing (reviewed in [105,106]). Some of these methods are already routinely used in the clinic [105], making aneuploidy an appealing biomarker for patient stratification, should it have a prognostic and/or a predictive value.

Despite some confounding factors that are discussed below, it is worth exploring the value of aneuploidy in diagnosis. Similar to the prognostic value of 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 degree of aneuploidy

The prognostic value of aneuploidy has long been demonstrated for several indications [107,108], with high levels of aneuploidy being associated with poorer prognosis in the vast majority of cases. A recent literature survey found cellular DNA ploidy (which served as a proxy for the degree of aneuploidy in this study) to be an independent prognostic marker in patients with invasive breast, early stage endometrial, early stage ovarian, prostate, and colorectal cancers [29]. 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 and overall survival in primary breast, endometrial, renal clear cell, thyroid, and colorectal cancers [109]. A recent TCGA analysis used more direct aneuploidy scores, that take into account only arm-level and chromosome-level alterations, and found highly-aneuploid tumors to be associated with a significantly worse prognosis in 9 out of 27 tumor types [79].

In colorectal cancer, a systematic meta-analysis of >7,000 patients revealed that later-stage tumors were more frequently aneuploid than early-stage tumors (odds ratio 1.51, p=0.0007), indicating that aneuploidy could be a marker of disease stage [64]. Importantly, over half of the studies that were analyzed in this meta-analysis reported a significant prognostic impact of aneuploidy for overall, disease-specific, and recurrence-free survival, independent of tumor stage [64]. Similar conclusions were reached in additional meta-analyses of clinical colorectal studies [29,110,111]. Of particular note are large studies that demonstrated an independent prognostic value of aneuploidy in multivariate analyses of defined cohorts of colorectal patients (mostly patients with stage II disease) [112-114]. In these studies, diploidy was found to be an even stronger marker

of favorable prognosis than microsatellite instability (MSI), a well-known favorable prognostic marker in this disease [112-114].

High degree of aneuploidy was also found to be associated with poor overall patient survival in serous ovarian cancer [34]. In multivariate analysis, aneuploidy was the strongest independent prognostic factor of recurrence-free survival in stage I ovarian carcinomas [115]. Moreover, specific copy number signatures could predict both overall survival and the probability of platinum-resistant relapse in high-grade serous ovarian cancer [116]. In breast cancer, several studies confirmed aneuploidy as a multivariate indicator of poor survival [29,109,117-119]. Aneuploidy was also associated with various clinical and histopathological parameters in squamous cell carcinomas of the tongue [120]. In lung cancer, CIN and high CNA burden were associated with progression of pre-malignant lesions to cancer [69]. Similarly, in esophageal cancer, higher levels of aneuploidy are observed in Barret's esophagus of patients that will progress to esophageal carcinoma[121], and aneuploidy can be combined with other biomarkers to identify disease that will progress to high-grade dysplasia and/or carcinoma [29,122]. In prostate cancer, aneuploidy was associated with prostate-specific antigen (PSA)-recurrence free interval [123], and prostate tumors that contain aneuploid cells are more likely to recur after resection [124,125]. Most recently, it was found that the degree of aneuploidy is associated with overall survival of prostate cancer patients [126], and is a better predictor of patient outcome than Gleason score [29,109].

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 [29,127]. Similarly, aneuploidy detection can potentially reduce erroneous diagnosis of non-small cell lung cancer (NSCLC) based on cytology findings alone [128], and improve the sensitivity of cytology in identifying early-stage NSCLC in high-risk populations, such as heavy smokers [29,129-131].

Interestingly, in multiple myeloma (MM), a plasma cell malignancy, 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 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 based on the chromosome number [132]. Hyperdiploidy is associated with a favorable prognostic value, but this association is not necessarily directly related to aneuploidy level, given the high number of other genetic alterations [133]. Hyperdiploidy has also been associated with a favorable prognosis in acute lymphoblastic lymphoma (ALL), whereas hypodiploid ALL is associated with poor prognosis [134-136]. In summary, high degree of aneuploidy has been associated with a worse clinical outcome in many different tumor types, but, curiously, it is also associated with a better prognosis under specific circumstances.

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 [137,138], patient-derived xenograft models [14] and human tumors [32]. This increased drug resistance has been attributed to heterogeneity in tumor karyotypes, which is prevalent in aneuploid cancers [32]. Similarly, high degree of aneuploidy induced by transient CIN can lead to resistance to oncogene withdrawal in genetic mouse models [42,43]. 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. 4**). In fact, extreme levels of aneuploidy/CIN were reported to render cells more sensitive – rather than more resistant – to anticancer drugs [14,32,33,139-141], in line with the notion of optimal karyotypic heterogeneity and chromosome missegregation rate [142]. Nevertheless, it is generally true that higher levels of aneuploidy are associated with resistance to chemotherapy. Thus, overall degree of aneuploidy has not only a prognostic value, but a predictive value as well.

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 hematopoietic stem cells that can progress to acute myeloid leukemia (AML)[143,144]. 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 (del5q/del7q), are highly recurrent in this hematopoietic disorder [145]. However, while patients with monosomy 5/5q have a good prognosis, patients with monosomy 7/7q are classified as being in a "poor prognosis" group[143,144]. This aneuploidy-based classification has a very strong prognostic value, as it is very significantly associated with relapse and mortality following hematopoietic stem cell transplantation [146]. 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 [147,148]).

Gliomas are another prominent example of a strong prognostic value associated with specific aneuploidies. In grade III anaplastic oligodendrogliomas in particular, the co-occurring loss of chromosome arms 1p and 19q marks a clinically distinct molecular subtype within this histologically-defined tumor type [149-151]. 1p/19p co-loss is associated with a lower rate of relapse and improved overall survival following treatment with the alkylating agent temozolomide [152], and was shown to be associated with a favorable prognosis irrespective of whether patients were receiving radiotherapy, chemotherapy, or both [153-156]. 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[155,156].

Both in MDS and in low-grade gliomas the characteristic aneuploidies exist in an otherwise quiet karyotype, indicative of low levels or no CIN. However, the occurrence of specific aneuploidies can be prognostic in highly-aneuploid CIN tumors as well [79]. For example, loss of specific chromosomes was identified as an independent prognosis factor in colorectal cancer [157]; losses and gains of specific chromosome arms are also associated with poor outcome in Multiple Myeolma (MM)[133,158]; and loss of chromosome arm 17p predicts more aggressive disease and lower drug response in Chronic Lymphocytic Leukemia (CLL; reviewed in [159]). In fact, a recent analysis of the TCGA data set identified 160 significant associations between specific aneuploidies and patient survival [79]. It thus appears that in almost any tumor type, specific aneuploidies have context-dependent prognostic value.

Factors confounding aneuploidy's prognostic value

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

Another potential caveat is that aneuploidy levels are associated with high degree of CIN, which are in turn associated with inactivation of p53 [9,98]. Recently, it was suggested that chromothripsis is another major source of aneuploidy in human cancer [160-162]. This generates an inherent challenge to disentangle these variables when attempting to analyze the prognostic value of aneuploidy *per se* [3]. The clinical relevance of CIN, of chromothripsis, and of p53 status, have been extensively reviewed [22,26,73,163,164]. It is important to bear in mind that, while these variables can be disentangled experimentally [165], 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 observed associations.

A third confounding factor is intra-tumor heterogeneity (ITH), which has been studied extensively in recent years, largely thanks to the advances in single-cell "omics" technologies. These studies revealed the importance of ITH for cancer progression and for response to therapeutics (reviewed in [166,167]). Histological ITH and tumor proliferation rates were found to reflect genetic ITH [32]. Interestingly, recent evidence suggests that numerical and structural CIN drive the development and maintenance of ITH more strongly than point mutations [32]. Furthermore, CNA heterogeneity – but not point mutation heterogeneity – is strongly associated

with clinical outcome [168]. Stratification of tumors based on ITH and CNA burden revealed that it is the interaction between these two parameters that determines clinical outcome: high CNA burden with low ITH was associated with best overall survival [32]. While this study did not examine aneuploidy specifically, CNA burden was defined as the fraction of the genome affected by CNAs, and was therefore largely determined 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 [106].

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 tumor clonality of some of the specific events and their prognostic value, leads to the conclusion that aneuploidy should be considered as a therapeutic target.

For aneuploidy, like for all other genetic lesions in cancer, such as point mutations, a fundamental distinction ought to be made between the tumorigenic role of the process – CIN and mutagenesis, and 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), are used to target genomically unstable cells that are deficient in homologous recombination and DNA repair [169], and can therefore be considered drugs targeting the mutagenic process. In contrast, inhibitors of epidermal growth factor receptor (EGFR) signaling are used to target *EGFR*-mutant tumors [170], and are thus considered therapies that target a recurrent molecular alteration. The clinical relevance and putative therapeutic value of CIN has recently been reviewed elsewhere [22,73] and will not be discussed here. Instead, we will focus on aneuploidy *per se*.

Consistent with the abovementioned definitions, 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 unique vulnerabilities induced by specific recurrent aneuploidies. The potential targeting of specific aneuploidies could be further divided into two conceptual approaches: (a) identifying and targeting drivers of recurrent aneuploidies, which might be considered a particular class of cancer genes; and (b) identifying genes linked to these drivers that do not contribute to, but are invariably associated with, the specific aneuploidy.

Targeting the aneuploid state per se

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 been recently summarized elsewhere [171,172]. They can be divided broadly into five categories: proteotoxic, metabolic, replicative, mitotic and hypo-osmotic [171,173]. These cellular stresses may induce unique vulnerabilities that are shared by many if not all highly aneuploid cells regardless of which chromosome's copy number is altered. In line with this notion, different aneuploidies were found to induce similar transcriptional programs in mammalian cell lines genetically manipulated to harbor aneuploidies [85,174].

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 appears especially wide-spread amongst aneuploid cells. Aneuploidy leads to stoichiometric imbalance among members of protein complexes, increasing aggregation and the need for protein degradation[175]. This increased burden on the protein quality control machinery leads to increased sensitivity to conditions that adversely impact cellular protein quality control. In budding yeast, aneuploid strains are uniquely sensitive to proteasome inhibition [7], and to inhibition of *Ubp3*, a deubiquitinylating enzyme involved in protein homeostasis [176]. However, the generalizability of these findings and their applicability to human cancer remains an open question. On the one hand, depletion of *USP10*, the human homolog of *Ubp3*, was detrimental to the fitness of aneuploid human cells [176]. On the other hand, trisomic mouse and human cells, although being more sensitive to HSP90 inhibitors, were not more sensitive to proteasome inhibitors compared to their diploid counterparts [177,178]. A recent analysis of TCGA data found that the agreement between DNA copy number levels and protein levels is lower than that between DNA and mRNA levels, especially for the subset of proteins that function as subunits of protein complexes [175]. In human cancer cell lines, this "protein attenuation" was regulated at least partly by proteome degradation. Surprisingly, however, this was suggested to be associated with increased resistance (rather than sensitivity) of cell lines with high CNA burden to proteasome inhibition [175]. 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 increased in aneuploid budding yeast, and genetic and chemical interventions that further upregulate ceramide levels could slow down their proliferation [179]. Elevated levels of ceramide were found in aneuploid mammalian cells as well [180]. Increasing levels of this lipid further, either genetically or pharmacologically, induced apoptosis in aneuploid mouse MEFs and in highly aneuploid human colorectal cancer cell lines [180]. Last but not least, the growth disadvantage caused by aneuploidy-induced cellular stresses could of course 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 [181] and in aneuploid human cells [178]. Inhibiting these genes may exacerbate the cellular stresses induced by aneuploidy, thereby reducing their viability and proliferation, or making them more sensitive to drugs that target these stress pathways. For example, a recent study found that p38α stress-induced MAP kinase is activated following chromosome missegregation and promotes apoptosis[182]. p38α inactivation induces aneuploidy tolerance and facilitates the expansion of aneuploid clones [182]. Moreover, p38α inhibitors can potentiate the CIN-inducing effects of taxanes [183], providing a rationale for this combination therapy. Similarly, over-expression of the anti-apoptotic protein BCL-XL was recently found to enable the survival of aneuploid human pluripotent stem cells [184]. Targeting p38α 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- and medium-scale chemical screens in isogenic model systems of diverse karyotypes [177,180]. 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 (e.g., CRISPR, CRISPRi and CRISPRa) across a large repertoire of isogenic diploid/aneuploid mammalian models, to ensure the generalizability of 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 specific aneuploidies

Targeting drivers of aneuploidy

While the successful therapeutic targeting of recurrent point mutations and specific gene amplifications should certainly inspire research aimed at targeting recurrent aneuploidies, there are critical differences between these types of genomic aberrations (**Fig. 5**). First, although cellular context always matters, it seems to be more important in the case of aneuploidy. Indeed, perturbation of specific oncogenes and tumor suppressor genes (e.g., loss of RB1) can drive tumorigenesis in a cell type specific manner [185-187]. Furthermore, many genetic alterations are cancer type-specific [89,91]. However, specific genes can be universally tumor-promoting (e.g., *KRAS*) or tumor-suppressive (e.g., *TP53*) [188], whereas no chromosome is known to be universally oncogenic or tumor-suppressive; specific chromosome gains or losses are invariably tissue-specific [9,11,13]. Second, recent analyses demonstrate that positive selection overwhelmingly outweighs negative selection during cancer development, and the vast majority (~99%) of coding mutations are tolerated and escape negative selection [189]. In contrast, aneuploidy comes with a strong fitness cost (reviewed in [4,6]), and experimentally-induced aneuploid cells are often

selected against and are outcompeted by their diploid counterparts [5,9]. Third, whereas point mutations and focal CNAs, such as multi-copy amplification or a complete deletion, can lead to drastic changes in the expression of affected genes, aneuploidy usually involves only a single copy gain/loss, thus leading to much milder changes in the expression of the affected genes [190-194]. At the same time, however, aneuploidy affects the expression of many more genes than the other aforementioned genetic alterations, thus exerting a quantitatively larger overall effect on global gene expression[190-194].

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 thus critical, but far from trivial. It has recently been suggested that aneuploidies are largely driven by the cumulative effects of oncogenes and tumor suppressors that reside within the aberrant chromosome arms [90,91]. Consistent with this idea, even when a bona-fide oncogene or tumor suppressor gene resides within a highly recurrent aneuploidy, it is likely that other genetically-linked genes contribute to the selective advantage of the aneuploidy [195,196]. For example, inactivation of *p53* is a major driver of chromosome arm 17p loss in multiple cancer types. However, even in the context of *TP53* loss, reduced dosage of neighboring tumor suppressor genes exacerbates the severity of the phenotype [195]. Therefore, identifying the sets of genes that drive recurrent aneuploidies, as well as understanding the relative importance of such aneuploidy drivers to various aspects of tumorigenesis (e.g., proliferation, migration, immune evasion, etc.), will be critical for their therapeutic exploitation.

How can we identify drivers of recurrent aneuploidies? Several complementary strategies could be combined (**Fig. 5a**). First, driver genes are expected to reside within the minimal recurrent aberrant region (**Fig. 5a**; I; [10,97,197]). Second, driver genes may be altered in additional ways, such as focal CNAs, point mutations, and/or epigenetic alterations (**Fig. 5a**; II). For example, the most common *TP53* configuration involves a missense mutation in one allele and loss of the other through a 17p chromosome arm loss[147]. 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 [150,198,199]. Third, as coding genes typically exert their impact via gene expression, drivers are expected to be differentially expressed when genetically altered (**Fig. 5a**; III). 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 [63,97]. Fourth, cross-species comparative oncogenomic approaches can be used to identify evolutionarily-conserved drivers within syntenic chromosomal regions (**Fig. 5a**; IV). Aneuploidy landscapes of genetically-engineered mouse models have been shown to be similar to those that characterize human cancer [150], and the incomplete synteny between the mouse and human genomes could thus help to focus the regions of interest within recurrent aneuploidies [63,200-202]. Fifth, 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 (**Fig. 5a**; V) [203,204].

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

Targeting passengers of aneuploidy

The genetic linkage that is inherent to chromosomes presents a unique opportunity to eliminate aneuploid cells (**Fig. 5b**). Genes that are linked to genes that drive a particular aneuploidy may enable the targeting of cells that harbor that aneuploidy. Such targetable passenger genes could be identified by unbiased genetic and chemical screens of isogenic cell models (e.g., cell lines with and without an aneuploidy that is characteristic of that particular tumor type). Unlike screens to identify general aneuploidy-induced vulnerabilities [177], 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 the chromosome arm 8p is associated with increased sensitivity to autophagy inhibitors, potentially due to the downregulation of the acid ceramidase gene ASAH1 [205]. A smaller-scale chemical screen suggested that pluripotent stem cells and germ cell tumor cells with trisomy 12 may be more sensitive to replication inhibitors [28].

Haploinsufficient genes within recurrent chromosomal losses are of particular interest in this context. Between 27% to 45% of essential genes are estimated to be haploinsufficient [90]. Copy-number loss, such as occurs in monosomies, renders cells more sensitive to further suppression of these genes [206]. For example, the splicing factor SF3B1is partially lost in 11% of human cancers, most often (in 81% of cases) due to a loss of a chromosome arm 2q [207]. Breast and hematopoietic cell lines with this particular aneuploidy are consequently more sensitive to SF3B1 inhibition [207]. Importantly, this type of vulnerability has been recently predicted to be common in human cancer [207]. Interestingly, the opposite of haploinsufficiency – overexpression toxicity – may also be targetable. Overexpression of many genes reduces cell viability and proliferation [91,208]. Not surprisingly, copy number landscapes in cancer evolve to avoid the gain of such genes [209]. When dosage-sensitive genes reside within a recurrent trisomy, their genetic or epigenetic silencing (e.g., by promoter hypermethylation [210]) may be required for the tolerance or positive selection of this trisomy. Reversing these inactivation mechanisms (e.g., by demethylation) will antagonize the fitness advantage conferred by a particular trisomy. In budding yeast, most, perhaps all haploinsufficient genes are also toxic when overexpressed [202]. 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 abovementioned example of *TP53*). Such focal deletions could encompass genes that are irrelevant for tumorigenesis but provide cancer-cell specific synthetic lethality. For example, deletion of the enzyme MTAP, which is a common event in multiple cancers due to its genetic proximity to the tumor suppressor *CDKN2A*, increases the sensitivity of cells to PRMT5 inhibition [211,212].

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[203,204]. As 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 [148,203,213]. Loss of some of these genes, e.g. *RPS14*, likely drives the disease [203], whereas loss of others, e.g. *CSNK1A1*, is merely a passenger event [206]. The case of lenalidomide and chromosome arm 5q loss demonstrates that identification of selective vulnerabilities of recurrent aneuploidies can be exploited therapeutically – importantly, even without a precise understanding of the mechanism that underlies this selectivity.

Concluding remarks / Future perspective

The last 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 arm-level gains and losses challenging to dissect, we face (unnecessary) hurdles because as a field we have yet to decide on how we define aneuploidy, its causes and its consequences.

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 chromosome instability as its underlying mechanism. Third, when describing the phenotypic consequences of the phenomenon or its therapeutic relevance, a clear distinction between high degree of aneuploidy and specific recurrent aneuploidies is warranted. We believe that clarity in terminology is important to facilitate a fruitful scientific discussion and avoid 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. Due to the general fitness penalty of aneuploidy, tumor aneuploidy landscapes are likely the product of both positive and

negative selection, determined by the cell type, the genomic context, and the microenvironment. It is therefore not surprising that both the degree of aneuploidy and the presence of specific aneuploidies have been associated both with adverse and with favorable clinical outcomes. These recent discoveries argue that we need to be cautious not to over-generalize 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 tumor 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, have all been demonstrated 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, make this approach increasingly feasible.

700 **Table 1: The prognostic value of aneuploidy**

Biomarker type	Specific biomarker	Tumor type	Association with clinical outcome		References
			Directionality	Associated feature	
High degree of aneuploidy	Various estimates of aneuploidy levels	Colorectal cancer	Adverse	OS, DSS, RFS	29,64,109-114
		Serous ovarian cancer	Adverse	RFS	29,34,79,115
		Breast cancer	Adverse	OS, RFS	29,79,109,117-119
		Squamous cell carcinoma of the tongue	Adverse	OS	120
		Esophageal carcinoma	Adverse	Disease progression	29,121,122
		Prostate cancer	Adverse	OS, PSA-recurrence, RFS	29,79,109,123-125
		Cervical cancer	Adverse	Disease progression	29,127
		Non-small cell lung cancer	Adverse	Disease progression	29,128-131
	Hyperdiploid subgroup	Multiple myeloma	Favorable	PFS, OS	133
	Hypodiploid subgroup	Acute lymphoblastic lymphoma	Adverse	OS, RFS	134-136
	Hyperdiploid subgroup		Favorable		
Specific aneuploidy	5 or 5q loss	Myelodysplastic syndrome	Favorable	Disease progression, relapse, mortality following stem cell transplantation	143-148
	7 or 7q loss		Adverse		
	1p and 9p loss	Gliomas	Favorable	RFS, OS	152-156
	4 loss	Colorectal cancer	Adverse	RFS	157
	1q gain or 1p or 12p or 17p loss	Multiple myeloma	Adverse	PFS, OS	133,158
	17p loss	Chronic lymphocytic leukemia	Adverse	PFS, OS	159

701

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

703

704

Figure Legends

Figure 1: Definitions of aneuploidy

(**a**) The classic definition of aneuploidy refers to changes in the copy number of whole chromosomes. Recent genomic analyses of aneuploidy in cancer have extended this definition to include chromosome arm gains and losses. 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. 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**) Bar plots showing the number of recurrent DNA copy number gains (left) and losses (right) that encompass ≥ 104 genes, the number of genes residing on chromosome arm 18p, across 12 cancer types. ~1/3 of these recurrent alterations are not chromosome arm-level events. These CNAs are expected to have similar effects on cellular fitness as chromosome arm alterations in the size range of chromosome 18p, demonstrating the limitation of an arm-focused definition of aneuploidy. Data were extracted from the GISTIC 2.0 analysis of TCGA data, provided by the GDAC portal (http://fire- browse.org/).

Figure 2: Aneuploidy during tumor development

(**a**) The degree of aneuploidy increases with tumor 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, tumors evolve mechanisms to evade immune recognition. There is evidence to suggest that this evolution occurs in bursts [67], which may be associated with the development of aneuploidy immune-tolerance. Later in tumorigenesis the cGAS-STING pathway takes on a tumor-promoting role. The pathway activates a noncanonical NF-κB transcriptional response that promotes the epithelial-to-mesenchymal transition (EMT), thereby directly contributing to tumor progression.

(**b**) At different stages of tumorigenesis, different specific karyotypes provide a selective advantage and therefore become the dominant tumor 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 tumor, and might also be different from one another.

Figure 3: The importance of context for shaping aneuploidy landscapes

(**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 aneuploidy landscapes of human tumors are tissue type-specific. Each organ (shown here are liver, lung and brain) exhibits a tissue-specific gene expression pattern. These differences in

gene expression can determine aneuploidy patterns during oncogenic transformation and during culture *in vitro*. Interestingly, the aberrations that arise frequently in a given tumor type are often similar to those that arise during the *in vitro* culturing of stem cells of the same lineage.

(**c**) The genomic context is important for determining the adaptive value of aneuploidy. A specific aneuploidy that occurs in diploid cells may be detrimental and thus be selected against or be fitness neutral (top). However, the same aneuploidy occurring in a tetraploid cell (middle), or preceded by a specific point mutation (bottom), may become advantageous and be selected for.

(**d**) The environmental context shapes the aneuploidy landscape. When cancers are removed from their natural environment and are cultured as cell lines, organoids or PDXs, the selection pressures change. As a result, karyotypes evolve. This is conceptually similar to the aneuploidy evolution seen in metastases, where tumor cells also need to cope with selection pressures that are different from those of the primary tumor environment.

Figure 4: 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 [4]. Nonetheless, compared to aneuploid cells, polyploid cells are still relatively fit, because their gene expression remains balanced [214]. The higher the degree of aneuploidy, that is the more a karyotype deviates from a euploid state, the more imbalanced their gene expression is, and consequently the lower their fitness is. The relative fitness penalty of aneuploidy decreases with increase in ploidy[214]. 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 cell than in a polyploid cell.

(**b**) DNA content analysis does not necessarily inform karyotype composition. A highly aneuploid cell can have a 3N DNA content just like a triploid cell with exactly three complements.

Figure 5: Comparison between aneuploidy and gene-focused genetic changes

Gene-focused genetic alterations, such as point mutations and focal CNAs, differ from aneuploidy in their effects on cellular fitness. In both cases, context matters. However, some oncogenes and tumor 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 be selected against in most contexts.

Figure 6: Strategies to target recurrent aneuploidies in cancer

(**a**) Several strategies can be combined to identify driver genes that underlie recurrent aneuploidies. These include: I) minimal recurrence analysis, II) integrative analysis with alternative modes of gene activation/inactivation (e.g., point mutations, focal CNAs and promoter methylation), III) gene expression analysis, IV) cross-species synteny comparison, and V) loss-of-function and gain-of-function genetic screens.

(**b**) Recurrent aneuploidies can be exploited therapeutically either by targeting the driver CNAs or genetically-linked passenger CNAs. For example, monosomy 10 is extremely common in glioblastomas. The loss of the tumor suppressor *PTEN* is thought to be a major driver of this monosomy [215]. Cells that harbor this monosomy could be targeted either by exploiting vulnerabilities caused by PTEN loss (e.g., using PI3K inhibitors) [216] or by haploinsufficiency of other chromosome 10 encoded genes. Due to the large number of mis-regulated genes in specific aneuploidies, opportunities to target "passenger CNAs" might be greater than of targeting driver CNAs.

Glossary

Complement (C): Set of all chromosomes. The haploid complement consists of one chromosome each, the diploid of two, and so forth.

Aneuploidy: 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.

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

Polyploidy: A euploid genome comprising more than two sets of chromosomes.

Chromosome instability: High rate of chromosome mis-segregation that gives rise to aneuploidy.

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 (e.g., tumor suppressors).

Whole-genome duplication (WGD): A duplication of the entire genome, which results in polyploidy.

Microcell-mediated chromosome transfer: A technique to transfer a chromosome from a donor cell line into 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 deletion of entire chromosome arms.

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

Copy number alteration (CNA) burden: The prevalence of CNAs within a tumor, commonly defined by the proportion of the genome that is affected by CNAs.

Microsatellite instability (MSI): Predisposition to mutations (hypermutability) due to impaired DNA mismatch repair.

cGAS-cGAMP-STING pathway: An immune response pathway that is activated by cytoplasmic DNA.

Human leukocyte antigen (HLA): A gene complex encoding the major histocompatibility complex (MHC) proteins, responsible for the regulation of the immune system.

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 the disease but it does not get worse.

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.

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

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

Non-hyperdiploid multiple myeloma: A subtype of multiple myeloma that can be further subdivided into hypodiploid (≤44 chromosomes), pseudodiploid (45–46 chromosomes) and near tetraploid (>75 chromosomes) subtypes.

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

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

Intra-tumor heterogeneity (ITH): Genomic and/or phenotypic cell-to-cell variability within a tumor.

Synteny: The conservation of chromosomal regions between two species.

Haploinsufficiency: A state where deletion of one copy of a gene in a diploid organism results in a phenotype.

Acknowledgements

The authors thank Iris Fung for her assistance with figure design. 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. 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 Center for Biology of Aging Research at MIT. The authors apologize to the authors of many important publications not cited due to space limitations.

Author contributions

Both authors researched data, discussed content, wrote, reviewed and edited the manuscript.

Competing interests

The authors declare no competing financial interests.

References

1. Boveri, T. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. *J Cell Sci* **121 Suppl 1**, 1-84 (2008).
2. Gordon, D.J., Resio, B. & Pellman, D. Causes and consequences of aneuploidy in cancer. *Nat Rev Genet* **13**, 189-203 (2012).
3. van Jaarsveld, R.H. & Kops, G. Difference Makers: Chromosomal Instability versus Aneuploidy in Cancer. *Trends Cancer* **2**, 561-571 (2016).
4. Sheltzer, J.M. & Amon, A. The aneuploidy paradox: costs and benefits of an incorrect karyotype. *Trends Genet* **27**, 446-53 (2011).
5. Sheltzer, J.M. *et al.* Single-chromosome Gains Commonly Function as Tumor Suppressors. *Cancer Cell* **31**, 240-255 (2017).
   This paper shows that experimental introduction of extra chromosomes into mammalian cells is tumor-suppressive.
6. Santaguida, S. & Amon, A. Short- and long-term effects of chromosome mis-segregation and aneuploidy. *Nat Rev Mol Cell Biol* **16**, 473-85 (2015).
7. Torres, E.M. *et al.* Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science* **317**, 916-24 (2007).
8. Williams, B.R. *et al.* Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. *Science* **322**, 703-9 (2008).
9. Taylor, A.M. *et al.* Genomic and Functional Approaches to Understanding Cancer Aneuploidy. *Cancer Cell* **33**, 676-689 e3 (2018).
   This paper presents a comprehensive pancancer analysis of aneuploidy across over 10,000 human tumors.
10. Beroukhim, R. *et al.* The landscape of somatic copy-number alteration across human cancers. *Nature* **463**, 899-905 (2010).
11. Zack, T.I. *et al.* Pan-cancer patterns of somatic copy number alteration. *Nat Genet* **45**, 1134-40 (2013).
12. Carter, S.L. *et al.* Absolute quantification of somatic DNA alterations in human cancer. *Nat Biotechnol* **30**, 413-21 (2012).
13. Knouse, K.A., Davoli, T., Elledge, S.J. & Amon, A. Aneuploidy in Cancer: Seq-ing Answers to Old Questions. *Annual Review of Cancer Biology* **1**, 335-354 (2017).
14. Ben-David, U. *et al.* Patient-derived xenografts undergo mouse-specific tumor evolution. *Nat Genet* **49**, 1567-1575 (2017).
    This paper shows that aneuploidies that are highly recurrent in human tumors can be slected against when human tumors are transplanted into recipient mice.
15. Upender, M.B. *et al.* Chromosome transfer induced aneuploidy results in complex dysregulation of the cellular transcriptome in immortalized and cancer cells. *Cancer Res* **64**, 6941-9 (2004).
16. Stingele, S. *et al.* Global analysis of genome, transcriptome and proteome reveals the response to aneuploidy in human cells. *Mol Syst Biol* **8**, 608 (2012).
17. Thomas, R., Marks, D.H., Chin, Y. & Benezra, R. Whole chromosome loss and associated breakage-fusion-bridge cycles transform mouse tetraploid cells. *EMBO J* **37**, 201-218 (2018).
    This manuscript shows that aneuploidy can cause transformation in polyploid cells.
18. Essletzbichler, P. *et al.* Megabase-scale deletion using CRISPR/Cas9 to generate a fully haploid human cell line. *Genome Res* **24**, 2059-65 (2014).
19. Adikusuma, F., Williams, N., Grutzner, F., Hughes, J. & Thomas, P. Targeted Deletion of an Entire Chromosome Using CRISPR/Cas9. *Mol Ther* **25**, 1736-1738 (2017).

20. Sheltzer, J.M. A transcriptional and metabolic signature of primary aneuploidy is present in chromosomally unstable cancer cells and informs clinical prognosis. *Cancer Res* **73**, 6401-12 (2013).
21. Sansregret, L. & Swanton, C. The Role of Aneuploidy in Cancer Evolution. *Cold Spring Harb Perspect Med* **7**(2017).
22. Sansregret, L., Vanhaesebroeck, B. & Swanton, C. Determinants and clinical implications of chromosomal instability in cancer. *Nat Rev Clin Oncol* **15**, 139-150 (2018).
This recent review summarizes the clinical implications of CIN.
23. Simonetti, G., Bruno, S., Padella, A., Tenti, E. & Martinelli, G. Aneuploidy: Cancer strength or vulnerability? *Int J Cancer* **144**, 8-25 (2019).
24. Targa, A. & Rancati, G. Cancer: a CINful evolution. *Curr Opin Cell Biol* **52**, 136-144 (2018).
25. Lens, S.M.A. & Medema, R.H. Cytokinesis defects and cancer. *Nat Rev Cancer* **19**, 32-45 (2019).
26. Luijten, M.N.H., Lee, J.X.T. & Crasta, K.C. Mutational game changer: Chromothripsis and its emerging relevance to cancer. *Mutat Res* **777**, 29-51 (2018).
27. Bakhoum, S.F. & Landau, D.A. Chromosomal Instability as a Driver of Tumor Heterogeneity and Evolution. *Cold Spring Harb Perspect Med* **7**(2017).
28. Ben-David, U. *et al.* Aneuploidy induces profound changes in gene expression, proliferation and tumorigenicity of human pluripotent stem cells. *Nat Commun* **5**, 4825 (2014).
29. Danielsen, H.E., Pradhan, M. & Novelli, M. Revisiting tumour aneuploidy - the place of ploidy assessment in the molecular era. *Nat Rev Clin Oncol* **13**, 291-304 (2016).
30. Carter, S.L., Eklund, A.C., Kohane, I.S., Harris, L.N. & Szallasi, Z. A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. *Nat Genet* **38**, 1043-8 (2006).
31. Buccitelli, C. *et al.* Pan-cancer analysis distinguishes transcriptional changes of aneuploidy from proliferation. *Genome Res* **27**, 501-511 (2017).
This paper demonstrates that high degree of aneuploidy and CIN are not directly associated with gene expression programs of proliferation.
32. Andor, N. *et al.* Pan-cancer analysis of the extent and consequences of intratumor heterogeneity. *Nat Med* **22**, 105-13 (2016).
33. Birkbak, N.J. *et al.* Paradoxical relationship between chromosomal instability and survival outcome in cancer. *Cancer Res* **71**, 3447-52 (2011).
34. Duijf, P.H., Schultz, N. & Benezra, R. Cancer cells preferentially lose small chromosomes. *Int J Cancer* **132**, 2316-26 (2013).
35. Davoli, T., Uno, H., Wooten, E.C. & Elledge, S.J. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. *Science* **355**(2017).
This paper shows that immune evasion is correlated with aneuploid in human cancers.
36. Liu, X. *et al.* Trisomy eight in ES cells is a common potential problem in gene targeting and interferes with germ line transmission. *Dev Dyn* **209**, 85-91 (1997).
37. Ben-David, U. & Benvenisty, N. High prevalence of evolutionarily conserved and species-specific genomic aberrations in mouse pluripotent stem cells. *Stem Cells* **30**, 612-22 (2012).
38. Zhang, M. *et al.* Aneuploid embryonic stem cells exhibit impaired differentiation and increased neoplastic potential. *EMBO J* **35**, 2285-2300 (2016).
39. Rutledge, S.D. *et al.* Selective advantage of trisomic human cells cultured in non-standard conditions. *Sci Rep* **6**, 22828 (2016).
This study demonstrates that aneuploidies that inhibit proliferation under normal culture conditions can promote proliferation under conditions of stress.
40. Pavelka, N. *et al.* Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. *Nature* **468**, 321-5 (2010).

41. Yona, A.H. *et al.* Chromosomal duplication is a transient evolutionary solution to stress. *Proc Natl Acad Sci U S A* **109**, 21010-5 (2012).
42. Sotillo, R., Schvartzman, J.M., Socci, N.D. & Benezra, R. Mad2-induced chromosome instability leads to lung tumour relapse after oncogene withdrawal. *Nature* **464**, 436-40 (2010).
43. Rowald, K. *et al.* Negative Selection and Chromosome Instability Induced by Mad2 Overexpression Delay Breast Cancer but Facilitate Oncogene-Independent Outgrowth. *Cell Rep* **15**, 2679-91 (2016).
This study shows that complex karyotypes that suppress tumorigenesis can also promote resistance to oncogene withdrawal.
44. de Carcer, G. *et al.* Plk1 overexpression induces chromosomal instability and suppresses tumor development. *Nat Commun* **9**, 3012 (2018).
45. Baker, D.J., Jin, F., Jeganathan, K.B. & van Deursen, J.M. Whole chromosome instability caused by Bub1 insufficiency drives tumorigenesis through tumor suppressor gene loss of heterozygosity. *Cancer Cell* **16**, 475-86 (2009).
46. Ricke, R.M., Jeganathan, K.B. & van Deursen, J.M. Bub1 overexpression induces aneuploidy and tumor formation through Aurora B kinase hyperactivation. *J Cell Biol* **193**, 1049-64 (2011).
47. Wijshake, T. *et al.* Reduced life- and healthspan in mice carrying a mono-allelic BubR1 MVA mutation. *PLoS Genet* **8**, e1003138 (2012).
48. Levine, M.S. *et al.* Centrosome Amplification Is Sufficient to Promote Spontaneous Tumorigenesis in Mammals. *Dev Cell* **40**, 313-322 e5 (2017).
49. Hoevenaar, W.H.M. *et al.* Degree and site of chromosomal instability define its oncogenic potential. 638460 (2019).
50. Weaver, B.A., Silk, A.D., Montagna, C., Verdier-Pinard, P. & Cleveland, D.W. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell* **11**, 25-36 (2007).
51. Foijer, F. *et al.* Chromosome instability induced by Mps1 and p53 mutation generates aggressive lymphomas exhibiting aneuploidy-induced stress. *Proc Natl Acad Sci U S A* **111**, 13427-32 (2014).
52. Foijer, F. *et al.* Deletion of the MAD2L1 spindle assembly checkpoint gene is tolerated in mouse models of acute T-cell lymphoma and hepatocellular carcinoma. *Elife* **6**(2017).
53. Laucius, C.D., Orr, B. & Compton, D.A. Chromosomal Instability Suppresses the Growth of K-Ras-induced Lung Adenomas. *Cell Cycle* (2019).
54. Burrell, R.A. *et al.* Replication stress links structural and numerical cancer chromosomal instability. *Nature* **494**, 492-496 (2013).
55. Lamm, N. *et al.* Genomic Instability in Human Pluripotent Stem Cells Arises from Replicative Stress and Chromosome Condensation Defects. *Cell Stem Cell* **18**, 253-61 (2016).
56. Ly, P. *et al.* Chromosome segregation errors generate a diverse spectrum of simple and complex genomic rearrangements. *Nat Genet* (2019).
57. Mayshar, Y. *et al.* Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. *Cell Stem Cell* **7**, 521-31 (2010).
58. International Stem Cell, I. *et al.* Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. *Nat Biotechnol* **29**, 1132-44 (2011).
59. Anders, K.R. *et al.* A strategy for constructing aneuploid yeast strains by transient nondisjunction of a target chromosome. *BMC Genet* **10**, 36 (2009).
60. Ravichandran, M.C., Fink, S., Clarke, M.N., Hofer, F.C. & Campbell, C.S. Genetic interactions between specific chromosome copy number alterations dictate complex aneuploidy patterns. *Genes Dev* **32**, 1485-1498 (2018).
61. Westcott, P.M. *et al.* The mutational landscapes of genetic and chemical models of Kras-driven lung cancer. *Nature* **517**, 489-92 (2015).

62. Nassar, D., Latil, M., Boeckx, B., Lambrechts, D. & Blanpain, C. Genomic landscape of carcinogen-induced and genetically induced mouse skin squamous cell carcinoma. *Nat Med* **21**, 946-54 (2015).
63. Ben-David, U. *et al.* The landscape of chromosomal aberrations in breast cancer mouse models reveals driver-specific routes to tumorigenesis. *Nat Commun* **7**, 12160 (2016).
64. Laubert, T. *et al.* Stage-specific frequency and prognostic significance of aneuploidy in patients with sporadic colorectal cancer--a meta-analysis and current overview. *Int J Colorectal Dis* **30**, 1015-28 (2015).
65. Ross-Innes, C.S. *et al.* Whole-genome sequencing provides new insights into the clonal architecture of Barrett's esophagus and esophageal adenocarcinoma. *Nat Genet* **47**, 1038-1046 (2015).
66. Heselmeyer, K. *et al.* Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix. *Proc Natl Acad Sci U S A* **93**, 479-84 (1996).
67. Gao, R. *et al.* Punctuated copy number evolution and clonal stasis in triple-negative breast cancer. *Nat Genet* **48**, 1119-30 (2016).
This paper shows that aneuploidy develops in punctuated bursts during breast cancer tumorigenesis.
68. Eriksson, E.T., Schimmelpenning, H., Aspenblad, U., Zetterberg, A. & Auer, G.U. Immunohistochemical expression of the mutant p53 protein and nuclear DNA content during the transition from benign to malignant breast disease. *Hum Pathol* **25**, 1228-33 (1994).
69. Teixeira, V.H. *et al.* Deciphering the genomic, epigenomic, and transcriptomic landscapes of pre-invasive lung cancer lesions. *Nat Med* **25**, 517-525 (2019).
70. Auslander, N. *et al.* Cancer-type specific aneuploidies hard-wire chromosome-wide gene expression patterns of their tissue of origin. *bioRxiv*, 563858 (2019).
71. Mitchell, T.J. *et al.* Timing the Landmark Events in the Evolution of Clear Cell Renal Cell Cancer: TRACERx Renal. *Cell* **173**, 611-623 e17 (2018).
This whole-genome analysis of renal tumors reeveals that chromosome arm 3p loss is often the initiating driver of this tumor type.
72. Bakhoum, S.F. *et al.* Chromosomal instability drives metastasis through a cytosolic DNA response. *Nature* **553**, 467-472 (2018).
This study shows that chromosomally unstable tumor cells activate innate immune pathways to spread into distant organs.
73. Bakhoum, S.F. & Cantley, L.C. The Multifaceted Role of Chromosomal Instability in Cancer and Its Microenvironment. *Cell* **174**, 1347-1360 (2018).
74. Liu, W. *et al.* Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer. *Nat Med* **15**, 559-65 (2009).
75. Brastianos, P.K. *et al.* Genomic Characterization of Brain Metastases Reveals Branched Evolution and Potential Therapeutic Targets. *Cancer Discov* **5**, 1164-1177 (2015).
76. Gibson, W.J. *et al.* The genomic landscape and evolution of endometrial carcinoma progression and abdominopelvic metastasis. *Nat Genet* **48**, 848-55 (2016).
77. Reiter, J.G. *et al.* Minimal functional driver gene heterogeneity among untreated metastases. *Science* **361**, 1033-1037 (2018).
78. Turajlic, S. *et al.* Tracking Cancer Evolution Reveals Constrained Routes to Metastases: TRACERx Renal. *Cell* **173**, 581-594 e12 (2018).
79. Vasudevan, A. *et al.* Single chromosome gains can function as metastasis suppressors and metastasis promoters. *bioRxiv*, 590547 (2019).
80. Gao, C. *et al.* Chromosome instability drives phenotypic switching to metastasis. *Proc Natl Acad Sci U S A* **113**, 14793-14798 (2016).

This study suggests that epithelial-to-mesenchymal and mesenchymal-to-epithelia transitions select for specific distinct karyotypes

81. Graham, N.A. *et al.* Recurrent patterns of DNA copy number alterations in tumors reflect metabolic selection pressures. *Mol Syst Biol* **13**, 914 (2017).
82. Harding, S.M. *et al.* Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature* **548**, 466-470 (2017).
This paper shows that micronucei activate the cGAS-STING pathway.
83. Mackenzie, K.J. *et al.* cGAS surveillance of micronuclei links genome instability to innate immunity. *Nature* **548**, 461-465 (2017).
This paper shows that micronucei activate the cGAS-STING pathway.
84. Santaguida, S. *et al.* Chromosome Mis-segregation Generates Cell-Cycle-Arrested Cells with Complex Karyotypes that Are Eliminated by the Immune System. *Dev Cell* **41**, 638-651 e5 (2017).
This paper shows that cells with highly abberant karyotypes are recognized by Natural Killer cells.
85. Sheltzer, J.M., Torres, E.M., Dunham, M.J. & Amon, A. Transcriptional consequences of aneuploidy. *Proc Natl Acad Sci U S A* **109**, 12644-9 (2012).
86. Santaguida, S., Vasile, E., White, E. & Amon, A. Aneuploidy-induced cellular stresses limit autophagic degradation. *Genes Dev* **29**, 2010-21 (2015).
87. Netea-Maier, R.T., Plantinga, T.S., van de Veerdonk, F.L., Smit, J.W. & Netea, M.G. Modulation of inflammation by autophagy: Consequences for human disease. *Autophagy* **12**, 245-60 (2016).
88. McGranahan, N. *et al.* Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution. *Cell* **171**, 1259-1271 e11 (2017).
89. Hoadley, K.A. *et al.* Cell-of-Origin Patterns Dominate the Molecular Classification of 10,000 Tumors from 33 Types of Cancer. *Cell* **173**, 291-304 e6 (2018).
90. Davoli, T. *et al.* Cumulative haploinsufficiency and triplosensitivity drive aneuploidy patterns and shape the cancer genome. *Cell* **155**, 948-62 (2013).
91. Sack, L.M. *et al.* Profound Tissue Specificity in Proliferation Control Underlies Cancer Drivers and Aneuploidy Patterns. *Cell* **173**, 499-514 e23 (2018).
This TCGA analysis shows that tissue-specific gene expresion underlies the tissue specificity of aneuploidy patterns.
92. Ben-David, U., Mayshar, Y. & Benvenisty, N. Large-scale analysis reveals acquisition of lineage-specific chromosomal aberrations in human adult stem cells. *Cell Stem Cell* **9**, 97-102 (2011).
93. Levine, A.J., Jenkins, N.A. & Copeland, N.G. The Roles of Initiating Truncal Mutations in Human Cancers: The Order of Mutations and Tumor Cell Type Matters. *Cancer Cell* **35**, 10-15 (2019).
94. Herbet, M., Salomon, A., Feige, J.J. & Thomas, M. Acquisition order of Ras and p53 gene alterations defines distinct adrenocortical tumor phenotypes. *PLoS Genet* **8**, e1002700 (2012).
95. Ortmann, C.A. *et al.* Effect of mutation order on myeloproliferative neoplasms. *N Engl J Med* **372**, 601-612 (2015).
96. Kent, D.G. & Green, A.R. Order Matters: The Order of Somatic Mutations Influences Cancer Evolution. *Cold Spring Harb Perspect Med* **7**(2017).
97. Gatza, M.L., Silva, G.O., Parker, J.S., Fan, C. & Perou, C.M. An integrated genomics approach identifies drivers of proliferation in luminal-subtype human breast cancer. *Nat Genet* **46**, 1051-9 (2014).
98. Bielski, C.M. *et al.* Genome doubling shapes the evolution and prognosis of advanced cancers. *Nat Genet* **50**, 1189-1195 (2018).
This TCGA analysis demonstrates that whole-genome doubling increases aneuploidy tolerance of tumor cells.

99. Davoli, T. & de Lange, T. Telomere-driven tetraploidization occurs in human cells undergoing crisis and promotes transformation of mouse cells. *Cancer Cell* **21**, 765-76 (2012).
100. Ben-David, U., Beroukhim, R. & Golub, T.R. Genomic evolution of cancer models: perils and opportunities. *Nat Rev Cancer* **19**, 97-109 (2019).
101. Li, X. *et al.* Organoid cultures recapitulate esophageal adenocarcinoma heterogeneity providing a model for clonality studies and precision therapeutics. *Nat Commun* **9**, 2983 (2018).
102. Bolhaqueiro, A.C.F. *et al.* Ongoing chromosomal instability and karyotype evolution in human colorectal cancer organoids. *Nat Genet* **51**, 824-834 (2019).
103. Ben-David, U. *et al.* Genetic and transcriptional evolution alters cancer cell line drug response. *Nature* **560**, 325-330 (2018).
104. Wangsa, D. *et al.* The Evolution of Single Cell-derived Colorectal Cancer Cell Lines is Dominated by the Continued Selection of Tumor Specific Genomic Imbalances, Despite Random Chromosomal Instability. *Carcinogenesis* (2018).
105. Das, K. & Tan, P. Molecular cytogenetics: recent developments and applications in cancer. *Clin Genet* **84**, 315-25 (2013).
106. van den Bos, H., Bakker, B., Spierings, D.C.J., Lansdorp, P.M. & Foijer, F. Single-cell sequencing to quantify genomic integrity in cancer. *Int J Biochem Cell Biol* **94**, 146-150 (2018).
107. Auer, G.U., Caspersson, T.O. & Wallgren, A.S. DNA content and survival in mammary carcinoma. *Anal Quant Cytol* **2**, 161-5 (1980).
108. Steinbeck, R.G., Heselmeyer, K.M. & Auer, G.U. DNA ploidy in human colorectal adenomas. *Anal Quant Cytol Histol* **16**, 196-202 (1994).
109. Hieronymus, H. *et al.* Tumor copy number alteration burden is a pan-cancer prognostic factor associated with recurrence and death. *Elife* **7**(2018).
This study identifies a strong association between high CNA levels, largely driven by aneuploidy, and adverse prognosis across multiple tumor types.
110. Walther, A., Houlston, R. & Tomlinson, I. Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis. *Gut* **57**, 941-50 (2008).
111. Araujo, J.P., Lourenco, P., Rocha-Goncalves, F., Ferreira, A. & Bettencourt, P. Nutritional markers and prognosis in cardiac cachexia. *Int J Cardiol* **146**, 359-63 (2011).
112. Sinicrope, F.A. *et al.* Prognostic impact of microsatellite instability and DNA ploidy in human colon carcinoma patients. *Gastroenterology* **131**, 729-37 (2006).
113. Mouradov, D. *et al.* Survival in stage II/III colorectal cancer is independently predicted by chromosomal and microsatellite instability, but not by specific driver mutations. *Am J Gastroenterol* **108**, 1785-93 (2013).
114. Hveem, T.S. *et al.* Prognostic impact of genomic instability in colorectal cancer. *Br J Cancer* **110**, 2159-64 (2014).
115. Kristensen, G.B. *et al.* Large-scale genomic instability predicts long-term outcome for women with invasive stage I ovarian cancer. *Ann Oncol* **14**, 1494-500 (2003).
116. Macintyre, G. *et al.* Copy number signatures and mutational processes in ovarian carcinoma. *Nat Genet* **50**, 1262-1270 (2018).
117. Gazic, B. *et al.* S-phase fraction determined on fine needle aspirates is an independent prognostic factor in breast cancer - a multivariate study of 770 patients. *Cytopathology* **19**, 294-302 (2008).
118. Karra, H. *et al.* Securin predicts aneuploidy and survival in breast cancer. *Histopathology* **60**, 586-96 (2012).
119. Pinto, A.E. *et al.* DNA ploidy is an independent predictor of survival in breast invasive ductal carcinoma: a long-term multivariate analysis of 393 patients. *Ann Surg Oncol* **20**, 1530-7 (2013).

120. Hemmer, J., Schon, E., Kreidler, J. & Haase, S. Prognostic implications of DNA ploidy in squamous cell carcinomas of the tongue assessed by flow cytometry. *J Cancer Res Clin Oncol* **116**, 83-6 (1990).
121. Martinez, P. *et al.* Evolution of Barrett's esophagus through space and time at single-crypt and whole-biopsy levels. *Nat Commun* **9**, 794 (2018).
This study tracks the evolution of Barretts's esophagus to esophageal carcinoma, and finds that aneuploidy is associated with the likelihood of malignant progression.
122. Bird-Lieberman, E.L. *et al.* Population-based study reveals new risk-stratification biomarker panel for Barrett's esophagus. *Gastroenterology* **143**, 927-35 e3 (2012).
123. Lennartz, M. *et al.* The Combination of DNA Ploidy Status and PTEN/6q15 Deletions Provides Strong and Independent Prognostic Information in Prostate Cancer. *Clin Cancer Res* **22**, 2802-11 (2016).
124. Deliveliotis, C. *et al.* The prognostic value of p53 and DNA ploidy following radical prostatectomy. *World J Urol* **21**, 171-6 (2003).
125. Pretorius, M.E. *et al.* Large scale genomic instability as an additive prognostic marker in early prostate cancer. *Cell Oncol* **31**, 251-9 (2009).
126. Stopsack, K.H. *et al.* Aneuploidy drives lethal progression in prostate cancer. *Proc Natl Acad Sci U S A* (2019).
127. Garner, D. Clinical application of DNA ploidy to cervical cancer screening: A review. *World J Clin Oncol* **5**, 931-65 (2014).
128. Schramm, M. *et al.* Equivocal cytology in lung cancer diagnosis: improvement of diagnostic accuracy using adjuvant multicolor FISH, DNA-image cytometry, and quantitative promoter hypermethylation analysis. *Cancer Cytopathol* **119**, 177-92 (2011).
129. Choma, D., Daures, J.P., Quantin, X. & Pujol, J.L. Aneuploidy and prognosis of non-small-cell lung cancer: a meta-analysis of published data. *Br J Cancer* **85**, 14-22 (2001).
130. Yang, J. & Zhou, Y. Detection of DNA aneuploidy in exfoliated airway epithelia cells of sputum specimens by the automated image cytometry and its clinical value in the identification of lung cancer. *J Huazhong Univ Sci Technolog Med Sci* **24**, 407-10 (2004).
131. Xing, S. *et al.* Predictive value of image cytometry for diagnosis of lung cancer in heavy smokers. *Eur Respir J* **25**, 956-63 (2005).
132. Fonseca, R. *et al.* Genetics and cytogenetics of multiple myeloma: a workshop report. *Cancer Res* **64**, 1546-58 (2004).
133. Manier, S. *et al.* Genomic complexity of multiple myeloma and its clinical implications. *Nat Rev Clin Oncol* **14**, 100-113 (2017).
134. Secker-Walker, L.M., Lawler, S.D. & Hardisty, R.M. Prognostic implications of chromosomal findings in acute lymphoblastic leukaemia at diagnosis. *Br Med J* **2**, 1529-30 (1978).
135. Pui, C.H. *et al.* Hypodiploidy is associated with a poor prognosis in childhood acute lymphoblastic leukemia. *Blood* **70**, 247-53 (1987).
136. Shago, M. Recurrent Cytogenetic Abnormalities in Acute Lymphoblastic Leukemia. *Methods Mol Biol* **1541**, 257-278 (2017).
137. Lee, A.J. *et al.* Chromosomal instability confers intrinsic multidrug resistance. *Cancer Res* **71**, 1858-70 (2011).
138. Kuznetsova, A.Y. *et al.* Chromosomal instability, tolerance of mitotic errors and multidrug resistance are promoted by tetraploidization in human cells. *Cell Cycle* **14**, 2810-20 (2015).
139. Silk, A.D. *et al.* Chromosome missegregation rate predicts whether aneuploidy will promote or suppress tumors. *Proc Natl Acad Sci U S A* **110**, E4134-41 (2013).

140. Roylance, R. *et al.* Relationship of extreme chromosomal instability with long-term survival in a retrospective analysis of primary breast cancer. *Cancer Epidemiol Biomarkers Prev* **20**, 2183-94 (2011).
141. Jamal-Hanjani, M. *et al.* Extreme chromosomal instability forecasts improved outcome in ER-negative breast cancer: a prospective validation cohort study from the TACT trial. *Ann Oncol* **26**, 1340-6 (2015).
142. Laughney, A.M., Elizalde, S., Genovese, G. & Bakhoum, S.F. Dynamics of Tumor Heterogeneity Derived from Clonal Karyotypic Evolution. *Cell Rep* **12**, 809-20 (2015).
143. Greenberg, P. *et al.* International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood* **89**, 2079-88 (1997).
144. Schanz, J. *et al.* Coalesced multicentric analysis of 2,351 patients with myelodysplastic syndromes indicates an underestimation of poor-risk cytogenetics of myelodysplastic syndromes in the international prognostic scoring system. *J Clin Oncol* **29**, 1963-70 (2011).
145. Kawankar, N. & Vundinti, B.R. Cytogenetic abnormalities in myelodysplastic syndrome: an overview. *Hematology* **16**, 131-8 (2011).
146. Deeg, H.J. *et al.* Five-group cytogenetic risk classification, monosomal karyotype, and outcome after hematopoietic cell transplantation for MDS or acute leukemia evolving from MDS. *Blood* **120**, 1398-408 (2012).
147. Giagounidis, A.A. Lenalidomide for del(5q) and non-del(5q) myelodysplastic syndromes. *Semin Hematol* **49**, 312-22 (2012).
148. List, A., Ebert, B.L. & Fenaux, P. A decade of progress in myelodysplastic syndrome with chromosome 5q deletion. *Leukemia* **32**, 1493-1499 (2018).
This review summarizes strategies to target MDS with 5q loss, the first clinical targeting of a recurrent cancer aneuploidy.
149. Idbaih, A. *et al.* BAC array CGH distinguishes mutually exclusive alterations that define clinicogenetic subtypes of gliomas. *Int J Cancer* **122**, 1778-86 (2008).
150. Cancer Genome Atlas Research, N. *et al.* Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med* **372**, 2481-98 (2015).
151. Wiestler, B. *et al.* Integrated DNA methylation and copy-number profiling identify three clinically and biologically relevant groups of anaplastic glioma. *Acta Neuropathol* **128**, 561-71 (2014).
152. Wahl, M. *et al.* Chemotherapy for adult low-grade gliomas: clinical outcomes by molecular subtype in a phase II study of adjuvant temozolomide. *Neuro Oncol* **19**, 242-251 (2017).
153. Weller, M. *et al.* Personalized care in neuro-oncology coming of age: why we need MGMT and 1p/19q testing for malignant glioma patients in clinical practice. *Neuro Oncol* **14 Suppl 4**, iv100-8 (2012).
154. Wick, W. *et al.* NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *J Clin Oncol* **27**, 5874-80 (2009).
155. Cairncross, G. *et al.* Phase III trial of chemoradiotherapy for anaplastic oligodendroglioma: long-term results of RTOG 9402. *J Clin Oncol* **31**, 337-43 (2013).
156. van den Bent, M.J. *et al.* Adjuvant procarbazine, lomustine, and vincristine chemotherapy in newly diagnosed anaplastic oligodendroglioma: long-term follow-up of EORTC brain tumor group study 26951. *J Clin Oncol* **31**, 344-50 (2013).
157. Bardi, G., Fenger, C., Johansson, B., Mitelman, F. & Heim, S. Tumor karyotype predicts clinical outcome in colorectal cancer patients. *J Clin Oncol* **22**, 2623-34 (2004).
158. Fonseca, R. *et al.* Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood* **101**, 4569-75 (2003).

159. Buccheri, V. *et al.* Prognostic and therapeutic stratification in CLL: focus on 17p deletion and p53 mutation. *Ann Hematol* **97**, 2269-2278 (2018).
160. Zhang, C.Z. *et al.* Chromothripsis from DNA damage in micronuclei. *Nature* **522**, 179-84 (2015).
161. Soto, M., Garcia-Santisteban, I., Krenning, L., Medema, R.H. & Raaijmakers, J.A. Chromosomes trapped in micronuclei are liable to segregation errors. *J Cell Sci* **131**(2018).
162. He, B. *et al.* Chromosomes missegregated into micronuclei contribute to chromosomal instability by missegregating at the next division. *Oncotarget* **10**, 2660-2674 (2019).
163. Olivier, M., Hollstein, M. & Hainaut, P. TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol* **2**, a001008 (2010).
164. Kastenhuber, E.R. & Lowe, S.W. Putting p53 in Context. *Cell* **170**, 1062-1078 (2017).
165. Bakhoum, S.F., Thompson, S.L., Manning, A.L. & Compton, D.A. Genome stability is ensured by temporal control of kinetochore-microtubule dynamics. *Nat Cell Biol* **11**, 27-35 (2009).
166. Jamal-Hanjani, M., Quezada, S.A., Larkin, J. & Swanton, C. Translational implications of tumor heterogeneity. *Clin Cancer Res* **21**, 1258-66 (2015).
167. Dagogo-Jack, I. & Shaw, A.T. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* **15**, 81-94 (2018).
168. Jamal-Hanjani, M. *et al.* Tracking the Evolution of Non-Small-Cell Lung Cancer. *N Engl J Med* **376**, 2109-2121 (2017).
This study finds that CNA heterogeneity, but not point mutation heterogeneity, is strongly associated with clinical outcome.
169. Pilie, P.G., Tang, C., Mills, G.B. & Yap, T.A. State-of-the-art strategies for targeting the DNA damage response in cancer. *Nat Rev Clin Oncol* **16**, 81-104 (2019).
170. Pao, W. & Chmielecki, J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. *Nat Rev Cancer* **10**, 760-74 (2010).
171. Zhu, J., Tsai, H.J., Gordon, M.R. & Li, R. Cellular Stress Associated with Aneuploidy. *Dev Cell* **44**, 420-431 (2018).
172. Chunduri, N.K. & Storchova, Z. The diverse consequences of aneuploidy. *Nat Cell Biol* **21**, 54-62 (2019).
This review summarizes current understanding of the cellular stresses induced by aneuploidy.
173. Tsai, H.J. *et al.* Hypo-osmotic-like stress underlies general cellular defects of aneuploidy. *Nature* **570**, 117-121 (2019).
174. Durrbaum, M. *et al.* Unique features of the transcriptional response to model aneuploidy in human cells. *BMC Genomics* **15**, 139 (2014).
175. Goncalves, E. *et al.* Widespread Post-transcriptional Attenuation of Genomic Copy-Number Variation in Cancer. *Cell Syst* **5**, 386-398 e4 (2017).
176. Dodgson, S.E., Santaguida, S., Kim, S., Sheltzer, J. & Amon, A. The pleiotropic deubiquitinase Ubp3 confers aneuploidy tolerance. *Genes Dev* **30**, 2259-2271 (2016).
177. Tang, Y.C., Williams, B.R., Siegel, J.J. & Amon, A. Identification of aneuploidy-selective antiproliferation compounds. *Cell* **144**, 499-512 (2011).
This paper provides a proof-of-concept that highly aneuploid cancer cells can be targeted by exploiting non-chromosome-specific vulnerabilities of aneuploid cells.
178. Donnelly, N., Passerini, V., Durrbaum, M., Stingele, S. & Storchova, Z. HSF1 deficiency and impaired HSP90-dependent protein folding are hallmarks of aneuploid human cells. *EMBO J* **33**, 2374-87 (2014).
179. Hwang, S. *et al.* Serine-Dependent Sphingolipid Synthesis Is a Metabolic Liability of Aneuploid Cells. *Cell Rep* **21**, 3807-3818 (2017).
180. Tang, Y.C. *et al.* Aneuploid Cell Survival Relies upon Sphingolipid Homeostasis. *Cancer Res* **77**, 5272-5286 (2017).

181. Torres, E.M. *et al.* Identification of aneuploidy-tolerating mutations. *Cell* **143**, 71-83 (2010).
182. Simoes-Sousa, S. *et al.* The p38alpha Stress Kinase Suppresses Aneuploidy Tolerance by Inhibiting Hif-1alpha. *Cell Rep* **25**, 749-760 e6 (2018).
This manuscript demonstrates that the p38 pathway regulates the cellular response to aneuploidy.
183. Canovas, B. *et al.* Targeting p38alpha Increases DNA Damage, Chromosome Instability, and the Anti-tumoral Response to Taxanes in Breast Cancer Cells. *Cancer Cell* **33**, 1094-1110 e8 (2018).
This study demonstrates the therapeutic value of targeting mechanisms of aneuploidy tolerance.

184. Zhang, J. *et al.* Anti-apoptotic Mutations Desensitize Human Pluripotent Stem Cells to Mitotic Stress and Enable Aneuploid Cell Survival. *Stem Cell Reports* (2019).
185. Knudsen, E.S. & Knudsen, K.E. Tailoring to RB: tumour suppressor status and therapeutic response. *Nat Rev Cancer* **8**, 714-24 (2008).
186. Sieber, O.M., Tomlinson, S.R. & Tomlinson, I.P. Tissue, cell and stage specificity of (epi)mutations in cancers. *Nat Rev Cancer* **5**, 649-55 (2005).
187. Schaefer, M.H. & Serrano, L. Cell type-specific properties and environment shape tissue specificity of cancer genes. *Sci Rep* **6**, 20707 (2016).
188. Bailey, M.H. *et al.* Comprehensive Characterization of Cancer Driver Genes and Mutations. *Cell* **174**, 1034-1035 (2018).
189. Martincorena, I. *et al.* Universal Patterns of Selection in Cancer and Somatic Tissues. *Cell* **173**, 1823 (2018).
190. Henrichsen, C.N. *et al.* Segmental copy number variation shapes tissue transcriptomes. *Nat Genet* **41**, 424-9 (2009).
191. Pollack, J.R. *et al.* Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. *Proc Natl Acad Sci U S A* **99**, 12963-8 (2002).
192. Schoch, C. *et al.* Genomic gains and losses influence expression levels of genes located within the affected regions: a study on acute myeloid leukemias with trisomy 8, 11, or 13, monosomy 7, or deletion 5q. *Leukemia* **19**, 1224-8 (2005).
193. Tsafrir, D. *et al.* Relationship of gene expression and chromosomal abnormalities in colorectal cancer. *Cancer Res* **66**, 2129-37 (2006).
194. Ben-David, U., Mayshar, Y. & Benvenisty, N. Virtual karyotyping of pluripotent stem cells on the basis of their global gene expression profiles. *Nat Protoc* **8**, 989-97 (2013).
195. Liu, Y. *et al.* Deletions linked to TP53 loss drive cancer through p53-independent mechanisms. *Nature* **531**, 471-475 (2016).
This paper demonstrates that large CNAs are often driven by multiple genes, even when a strong tumor suppresses or oncogene resides on the affected chromosomes.
196. Xue, W. *et al.* A cluster of cooperating tumor-suppressor gene candidates in chromosomal deletions. *Proc Natl Acad Sci U S A* **109**, 8212-7 (2012).
197. Curtis, C. *et al.* The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* **486**, 346-52 (2012).
198. Bettegowda, C. *et al.* Mutations in CIC and FUBP1 contribute to human oligodendroglioma. *Science* **333**, 1453-5 (2011).
199. Suzuki, H. *et al.* Mutational landscape and clonal architecture in grade II and III gliomas. *Nat Genet* **47**, 458-68 (2015).
200. Maser, R.S. *et al.* Chromosomally unstable mouse tumours have genomic alterations similar to diverse human cancers. *Nature* **447**, 966-71 (2007).

201. Herschkowitz, J.I. *et al.* Comparative oncogenomics identifies breast tumors enriched in functional tumor-initiating cells. *Proc Natl Acad Sci U S A* **109**, 2778-83 (2012).
202. Weaver, Z.A. *et al.* A recurring pattern of chromosomal aberrations in mammary gland tumors of MMTV-cmyc transgenic mice. *Genes Chromosomes Cancer* **25**, 251-60 (1999).
203. Ebert, B.L. *et al.* Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. *Nature* **451**, 335-9 (2008).
204. Kotini, A.G. *et al.* Functional analysis of a chromosomal deletion associated with myelodysplastic syndromes using isogenic human induced pluripotent stem cells. *Nat Biotechnol* **33**, 646-55 (2015).
205. Cai, Y. *et al.* Loss of Chromosome 8p Governs Tumor Progression and Drug Response by Altering Lipid Metabolism. *Cancer Cell* **29**, 751-766 (2016).
This paper provides a proof-of-concept that bystander genes can be exploited to target recurrent aneuploidies.
206. Nijhawan, D. *et al.* Cancer vulnerabilities unveiled by genomic loss. *Cell* **150**, 842-54 (2012).
207. Paolella, B.R. *et al.* Copy-number and gene dependency analysis reveals partial copy loss of wild-type SF3B1 as a novel cancer vulnerability. *Elife* **6**(2017).
208. Morrill, S.A. & Amon, A. Why haploinsufficiency persists. *Proc Natl Acad Sci U S A* **116**, 11866-11871 (2019).
209. Inaki, K. *et al.* Systems consequences of amplicon formation in human breast cancer. *Genome Res* **24**, 1559-71 (2014).
210. Mohanty, V., Akmamedova, O. & Komurov, K. Selective DNA methylation in cancers controls collateral damage induced by large structural variations. *Oncotarget* **8**, 71385-71392 (2017).
211. Kryukov, G.V. *et al.* MTAP deletion confers enhanced dependency on the PRMT5 arginine methyltransferase in cancer cells. *Science* **351**, 1214-8 (2016).
212. Mavrakis, K.J. *et al.* Disordered methionine metabolism in MTAP/CDKN2A-deleted cancers leads to dependence on PRMT5. *Science* **351**, 1208-13 (2016).
213. Hosono, N. *et al.* Recurrent genetic defects on chromosome 5q in myeloid neoplasms. *Oncotarget* **8**, 6483-6495 (2017).
214. Storchova, Z. & Kuffer, C. The consequences of tetraploidy and aneuploidy. *J Cell Sci* **121**, 3859-66 (2008).
215. Bostrom, J. *et al.* Mutation of the PTEN (MMAC1) tumor suppressor gene in a subset of glioblastomas but not in meningiomas with loss of chromosome arm 10q. *Cancer Res* **58**, 29-33 (1998).
216. Dillon, L.M. & Miller, T.W. Therapeutic targeting of cancers with loss of PTEN function. *Curr Drug Targets* **15**, 65-79 (2014).