ANNUAL REVIEWS

Annual Review of Cancer Biology

Mutant Allele Imbalance in Cancer

Craig M. Bielski[1,3] and Barry S. Taylor[1,2,3]

[1]Human Oncology and Pathogenesis Program and Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA; email: taylorb@mskcc.org

[2]Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

[3]Weill Cornell Medical College, New York, NY 10065, USA

Annu. Rev. Cancer Biol. 2021. 5:221–34

First published as a Review in Advance on December 1, 2020

The *Annual Review of Cancer Biology* is online at cancerbio.annualreviews.org

https://doi.org/10.1146/annurev-cancerbio-051320-124252

Keywords

cancer, genomics, oncogene, mutant allele, zygosity, selection, evolution

Abstract

The search for somatic mutations that drive the initiation and progression of human tumors has dominated recent cancer research. While much emphasis has been placed on characterizing the prevalence and function of driver mutations, comparatively less is known about their serial genetic evolution. Indeed, study of this phenomenon has largely focused on tumor-suppressor genes recessive at the cellular level or mechanisms of resistance in tumors with mutant oncogenes targeted by therapy. There is, however, a growing appreciation that despite a decades-old presumption of heterozygosity, changes in mutant oncogene zygosity are common and drive dosage and stoichiometry changes that lead to selective growth advantages. Here, we review the recent progress in understanding mutant allele imbalance and its implications for tumor biology, cancer evolution, and response to anticancer therapy.

INTRODUCTION

Over time, healthy cells acquire somatic genomic mutations that arise from cellular, organismal, and environmental processes. A small subset of these mutations cause aberrant cell growth and tumor development. These driver mutations in tumors coexist with a much larger number of passenger mutations and similar incidental genomic alterations. Population-scale sequencing of human tumor genomes over the last 15 years has revealed all but the least common of such alterations driving many cancer types. This knowledge is transforming our understanding of disease pathogenesis and has revealed novel therapeutic vulnerabilities that are changing oncology care (Garraway & Lander 2013, Hyman et al. 2017b). These new discoveries are also upending long-held assumptions in cancer biology.

Among the most enduring assumptions since the initial discovery of proto-oncogenes is that gain-of-function mutations are typically heterozygous because they are dominant acting and therefore sufficient to drive tumorigenesis (Varmus 1984). This line of reasoning has ultimately led to a new generation of therapies that inhibit mutant oncogenes, targeting these gain-of-function mutations based on their presence in affected human cancers. Multimodal genomic profiling studies have nevertheless revealed that mutant oncogenes coexist in aneuploid cancer genomes with pervasive copy number alterations (CNAs). This interplay has been studied extensively for mutant tumor-suppressor genes (TSGs), where biallelic inactivation due to loss of the remaining wild-type (WT) allele confers a distinct selective advantage to cells (Knudson 1971). There is a growing appreciation that similar serial genetic evolution of mutant oncogenes may be more common than previously thought, with the potential to alter cellular fitness and mediate key clinical phenotypes. In this review, we discuss the emerging evidence for this phenomenon and the functional, evolutionary, and clinical implications of cancer cells tuning the dosage and stoichiometry of oncogenic driver mutations in cancer.

FREQUENCY AND MECHANISMS OF MUTANT ALLELE IMBALANCE

Intersection of Multiple Somatic Alteration Types

Genomic instability, characterized by widespread CNAs and aneuploidy, is a hallmark of cancer (Hanahan & Weinberg 2000). As such, the identification and classification of CNAs have been a central challenge in cancer genomics that has driven technology innovation. Over the past 50 years, increasingly sensitive technologies—from cytogenetic techniques such as G-banding, fluorescence in situ hybridization, and comparative genomic hybridization to modern sequencing-based approaches—have revealed ever more subtle and complex chromosomal abnormalities in human cancers (Carter 2007, Meyerson et al. 2010, Speicher & Carter 2005). These include gene-specific events such as homozygous deletions of TSGs (e.g., *CDKN2A*, *PTEN*, and *RB1*) and focal amplifications of certain oncogenes (e.g., *MYC*, *CCND1*, *MDM2*, *ERBB2*, *EGFR*, and *CDK4*), as well as broader CNAs involving modest gains and losses of entire chromosomes or chromosome arms (Beroukhim et al. 2010, Zack et al. 2013). In addition, whole-genome and -transcriptome data have enabled the study of combinatorial structural rearrangements in cancer development, particularly chromothripsis and chromoplexy (Baca et al. 2013, Stephens et al. 2011). Recent work has sought to unravel the mechanistic origins and biological implications of complex genomic events (Maciejowski et al. 2015, Umbreit et al. 2020), which in certain settings can predict sensitivity to platinum-based therapies (Wang et al. 2017).

However, these CNAs have largely been studied as an isolated and distinct class of molecular alterations mediating different aspects of tumorigenesis. By contrast, aside from the genomic losses leading to biallelic inactivation of mutant TSGs or the presence of focal amplifications targeting

the mutant allele of specific oncogenes (Corcoran et al. 2010, Ercan et al. 2010, Okabe et al. 2007), little is known about the intersection between CNAs and somatic mutations in tumor genomes. Indeed, given the prevalence of CNAs in solid cancers, in many affected patients these events inevitably span the genomic loci of mutant oncogenes and will therefore lead to mutant allele imbalance, hereafter defined as an unequal number of mutant and WT copies. Many potential allelic configurations can result from allelic imbalance, of which there are several broad categories (**Figure 1*a***). These changes can result from genomic gains or losses [including copy-neutral loss of heterozygosity (CN-LOH)], focal amplifications (as described above), or complex combinatorial events in either nearly diploid tumors or those whose somatic genome has undergone whole-genome duplication (WGD), an abnormality associated with chromosomal instability (Dewhurst et al. 2014) that affects nearly one-third of all tumors pan-cancer (Bielski et al. 2018b, Carter et al. 2012, Zack et al. 2013). For example, a mutant oncogene with two mutant copies and zero WT copies could be interpreted as resulting from either CN-LOH in a diploid tumor genome or a single-copy genomic loss prior to a WGD event (**Figure 1*a***).

Inferring Mutant Allele Imbalance at Scale

Ultimately, and notwithstanding the precise underlying genomic mechanism, allelic imbalance can lead to subtle changes in the dosage of a given mutant allele or its stoichiometry (balance of mutant and WT copies) (Blakeslee et al. 1920). The prevalence of allelic imbalance can vary significantly depending on not only the affected cancer gene but also tumor lineage, given the established differences in the type, frequency, and overall burden of CNAs between cancer types (Beroukhim et al. 2010, Carter et al. 2012, Zack et al. 2013). Patterns of mutant allele imbalance within and across cancer types can be used to infer properties of mutant alleles at population scale, especially the relative proportion of mutant versus WT copies. Characterizing the precise mechanisms of allelic imbalance that affect oncogenic mutant alleles across cancer types can elucidate the dynamics of oncogene activity and help us to decipher the role of oncogene zygosity in tumor evolution.

However, robust estimation of such changes in tumor genomes has been notoriously challenging. Detecting mutant allele imbalance requires not only the precise inference of locus- and allele-specific integer copy number (at the resolution of single-copy changes) (Beroukhim et al. 2010, Carter et al. 2012, Shen & Seshan 2016, Van Loo et al. 2010, Zack et al. 2013), but also quantitatively accurate mutant allele frequencies (i.e., the proportion of aligned sequencing reads harboring the mutant allele at a particular locus), both of which are confounded by tumor purity and intratumoral heterogeneity. Computational approaches have started to integrate such copy number data with mutation calls to approximate the number of mutant alleles at a given locus by comparing the observed mutant allele frequency to what would be expected given the purity of the tumor specimen and the total number of copies present at the affected locus (**Figure 1*b***) (Bielski et al. 2018a, McGranahan et al. 2015). The accuracy of these estimates depends on the depth of tumor sequencing, as higher depth of coverage yields more precise estimates of integer copy number and fewer indeterminate cases for which the allelic configuration of a given driver mutation cannot be ascertained. A recent study leveraging such an analytical approach combined with prospective clinical sequencing of tumor-normal pairs found that nearly half of all oncogenic driver mutations exhibited allelic imbalance. The mechanisms of allelic imbalance varied markedly by oncogene and cancer type, with approximately one-third of cases attributable to LOH resulting in loss of the WT allele. This suggests an underlying biological basis for changes in oncogenic mutant allele zygosity (Bielski et al. 2018a), although the evolutionary origins of these changes remain unclear.

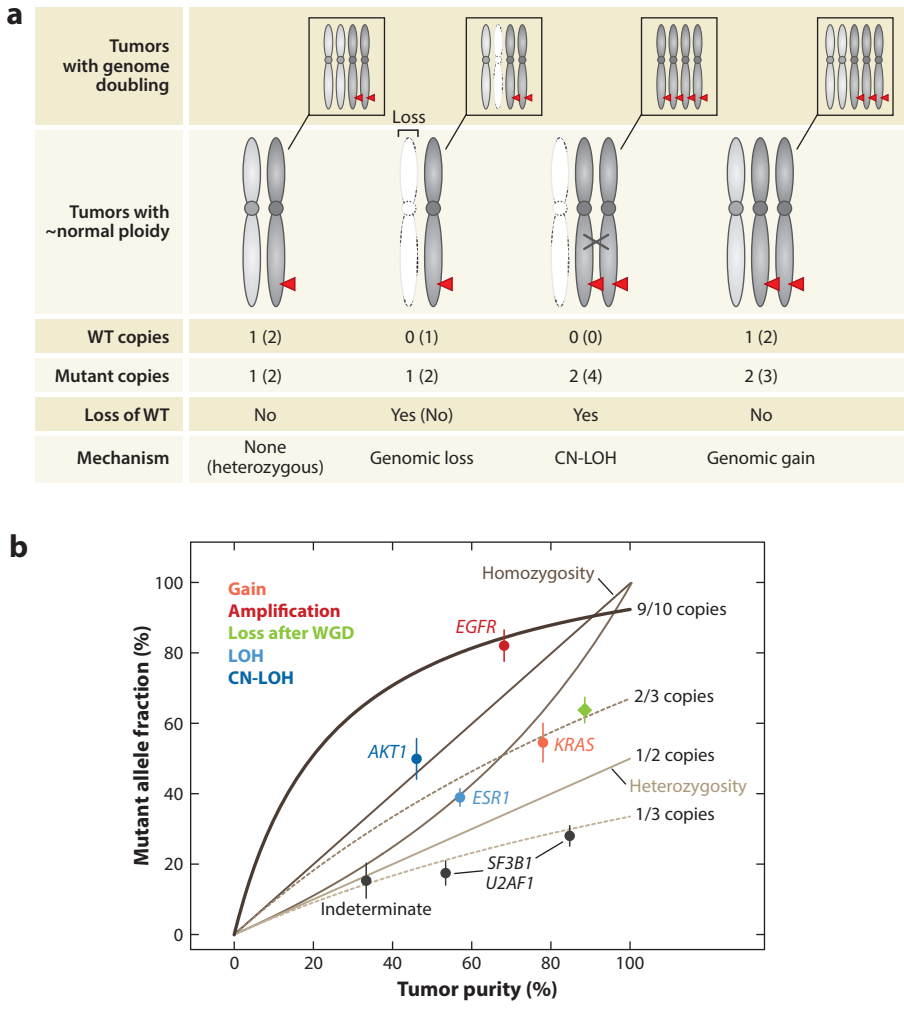

Figure 1

Identifying and characterizing mutant allele imbalance. (*a*) Classes of oncogenic mutant allele imbalance and their respective allelic configurations depending on whether the underlying tumor genome was predominantly diploid or had undergone WGD (*insets*). The red markers indicate oncogenic mutations, and the gray X for CN-LOH reflects a linkage between two chromosomes, as in the case of uniparental disomy. Not pictured are combinatorial events and focal amplifications. (*b*) Data-driven approach for determining the number of mutant copies from high-depth-of-coverage next-generation sequencing (targeted, whole-exome sequencing, or whole-genome sequencing) based on the mutant allele fraction, local copy number, and an analytical estimate of tumor purity. Points correspond to representative oncogenic mutations. Error bars represent the 95% confidence interval of the mutant allele fraction. Diagonal/curved lines indicate expected values corresponding to copy number alterations targeting the mutant or WT allele (as labeled). Figure adapted with permission from Bielski et al. (2018a); copyright 2018 Elsevier. Abbreviations: CN, copy-neutral; LOH, loss of heterozygosity; WGD, whole-genome duplication; WT, wild-type.

Evolutionary Origins of Zygosity Changes in Mutant Cancer Genes

The selective pressure for biallelic inactivation of mutant TSGs is a foundational concept in cancer biology. This establishes a key antecedent driver of allelic imbalance that links specific

mechanisms of CNAs to the underlying mutant cancer genes of affected loci. The fundamental question remains, however, whether there exists a similar relationship between CNAs and mutant oncogenes. Detecting statistically significant associations between oncogenic driver mutations and allelic states is challenging due to the high background rate of CNAs found in many solid cancer types. Nevertheless, comparing the distributions of CNAs for mutant and WT oncogenes can reveal both pan-cancer and context-specific associations between oncogenic mutations and allelic states. For the vast majority of oncogenic driver mutations (which presumably arise relatively early in the course of tumor evolution), CNAs consistently give rise to allelic configurations with more mutant than WT copies, indicating selection for the mutant allele. In contrast, CNAs tend to target randomly the mutant or WT alleles at loci of common single-nucleotide polymorphisms or variants of uncertain significance, reflecting the absence of selective pressure for additional copies of mutant alleles not associated with a fitness gain. Selection for oncogenic mutant alleles persists even in cases where allelic imbalance is not dependent on the presence of a mutant allele. In these cancers, CNAs appear to be co-opted during the course of tumor evolution to produce a mutant allele dosage that confers a fitness advantage. Elucidating how two independently evolving alteration types in tumor genomes interact to produce mutant allele imbalance will therefore require the application of evolutionary concepts not conventionally applied in cancer research (Bielski et al. 2018a, Gould & Vrba 1982).

Allelic Imbalance and Negative Selection

Selection for the mutant allele is not a universal property of oncogenes. Mutations in genes that encode components of the human spliceosome such as *SF3B1*, *U2AF1*, and *SRSF2* (Dvinge et al. 2016, Yoshida et al. 2011) are often spanned by low-level genomic gains, but these selectively target the WT allele rather than the mutant allele (Bielski et al. 2018a). This finding underscores the mutual exclusivity of splicing mutations in human cancer (Lee et al. 2018) and is consistent with functional studies in animal models of splicing-deficient tumors that exhibit dependence on WT splicing factors (Lee et al. 2016, Zhou et al. 2015). Multiple spliceosome components are therefore haplo-essential cancer genes. Hence, while strong positive selective pressure for dosage increases in mutant oncogenes is common during cancer evolution, even the absence of such an effect reflects a selective pressure to maintain a precise configuration of mutant and WT alleles that can enhance fitness and contribute to clonal outgrowth.

FUNCTIONAL AND BIOLOGICAL CONSEQUENCES

Dosage Versus Stoichiometry

The biological implications of changes in gene dosage due to CNAs have been extensively studied in evolutionary genetics (Pires & Conant 2016). Transcriptional regulation is critical for genes encoded by the sex chromosomes, where biological processes such as X-chromosome inactivation and transcriptional upregulation help to maintain homeostasis and achieve dosage compensation (Avner & Heard 2001, Nguyen & Disteche 2006, Raznahan et al. 2018). The effects of modest changes in gene dosage are less clear for autosomes, although stoichiometric changes in the components of regulatory complexes have been shown to alter gene expression levels, which in turn can have phenotypic ramifications (Birchler et al. 2001, 2005). Proteomic analysis of trisomic and tetrasomic human cell lines indicates that ploidy generally correlates with transcriptional output, although changes in gene dosage do not necessarily result in concomitant changes in protein abundance for certain subunits of protein complexes (Stingele et al. 2012). Similar posttranscriptional

attenuation has been observed in aneuploid human cancers (Gonçalves et al. 2017). Despite this progress, the functional consequences of dosage imbalance for mutant oncogenes remain poorly understood.

Early studies of oncogene dosage primarily focused on aberrant expression due to structural rearrangements and focal amplifications of otherwise WT genes identified in cell line models (Collins & Groudine 1982, Dalla-Favera et al. 1982, Klein 1981). In the case of mutant oncogenes, evidence that subtle changes in allelic balance could give rise to significant biological effects first began to emerge from studies of cultured cell lines and murine models of human cancer. The crucible in which these discoveries emerged was mutant Ras. Recurrent allelic imbalance of oncogenic *Ras* mutants due to loss of the WT allele was first observed in mouse model systems of skin carcinomas, suggesting that WT *Ras* can function as a tumor suppressor in *Ras*-mutant cells (Bremner & Balmain 1990). Subsequent studies found that mice bearing either heterozygous or homozygous *Kras*-mutant tumors developed very different spectrums of disease; the former were typified by a limited number of well-differentiated adenomas, while the latter had more numerous and larger, poorly differentiated adenocarcinomas, although expressing WT *Kras* could inhibit cell growth and colony formation (Zhang et al. 2001).

Tumor-Suppressive Effects of the Wild-Type Allele

Recent data have emerged supporting a biological role for the WT allele of *RAS* in tumors with heterozygous *RAS* mutations (Bentley et al. 2013, Mueller et al. 2018, To et al. 2013), suggesting a model whereby WT RAS exerts tumor-suppressive effects in RAS-mutant disease. However, the mechanism by which WT RAS suppresses tumor growth remains elusive. One model posits that WT RAS inhibits mutant RAS through the formation of a heterodimer. In genetically inducible murine models of *Kras*-mutant lung adenocarcinoma, a dimerization-incompetent $Kras^{G12}$ mutant disrupted MAPK activation and cellular transformation (Ambrogio et al. 2018). These results corroborate earlier work showing that mutant Ras must dimerize to activate the MAPK pathway (Nan et al. 2015). Indeed, the dimerization hypothesis might explain the high rate of allelic imbalance associated with mutant *RAS* (Westcott et al. 2015), as well as the nearly universal selection for the mutant *RAS* allele observed across human cancer types (Bielski et al. 2018a). Allelic imbalance of mutant *RAS*, whether through low-level genomic gains or loss of the corresponding WT allele, could thus represent a crucial evolutionary step in *RAS*-mutant tumors that enables cells to overcome the tumor-suppressive effects of WT *RAS*.

Beyond mutant RAS, distinct biological properties conditioned on zygosity have also been observed for oncogenic mutations in *PIK3CA*, which encodes the p110a catalytic subunit of phosphoinositide 3-kinase (PI3K). *PIK3CA* mutations are among the most common mutations in human cancer (Vivanco & Sawyers 2002) and also underlie a family of noncancerous overgrowth disorders referred to as *PIK3CA*-related overgrowth spectrum (PROS) (Keppler-Noreuil et al. 2015). In patients with PROS, somatic activating *PIK3CA* mutations are mosaic and arise heterozygously (Madsen et al. 2018). Endogenously expressed heterozygous activating *PIK3CA* mutations are indeed insufficient to initiate tumorigenesis in most model systems (Kinross et al. 2012, Tikoo et al. 2012, Van Keymeulen et al. 2015, Yuan et al. 2013). Tellingly, only the overexpression of mutant *PIK3CA* or the presence of additional oncogenic drivers will precipitate cellular transformation (Gustin et al. 2009, Isakoff et al. 2005, Koren et al. 2015, Liu et al. 2011, Meyer et al. 2011, Yueh et al. 2016). This is consistent with the observation that secondary PI3K pathway alterations often arise in *PIK3CA*-mutant tumors across a wide range of diverse human cancer types (Yuan & Cantley 2008). Moreover, in a study testing directly the impact of mutant *PIK3CA* zygosity,

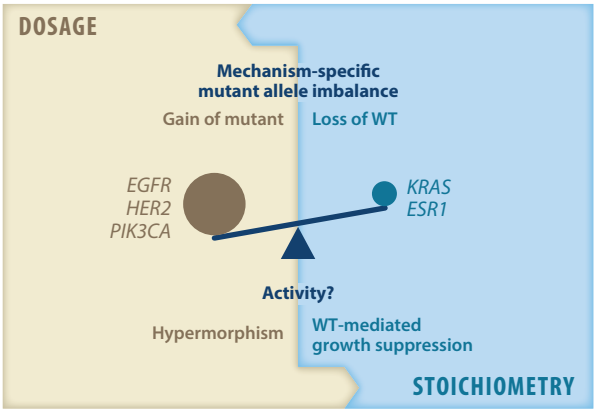

Figure 2

Linking the mechanisms of mutant allele imbalance with their functional consequences. Different mutant oncogenes select for different mechanisms of allelic imbalance that reflect unique selective pressures. Mutant allele imbalance often leads to modest dosage gains of an oncogenic driver mutation (*left*). These likely target dosage-dependent oncogenes such as *EGFR*, *HER2*, and *PIK3CA*, among others, and lead predominantly to hypermorphism. By contrast, mutant allele imbalance indicative of a stoichiometric relationship between alleles (*right*) appears to be mediated predominantly by loss of heterozygosity and may abrogate the tumor-suppressive effect of the wild-type (WT) allele by selecting for its loss.

induced pluripotent stem cells expressing heterozygous *PIK3CA*^{H1047R} underwent normal differentiation with transcriptional profiles and morphologies similar to those of WT cells. By contrast, expressing homozygous *PIK3CA*^{H1047R} led to dramatic transcriptional remodeling and impaired differentiation both in vitro and in vivo (Madsen et al. 2019). Collectively, these results suggest that mutant *PIK3CA* allele imbalance can differentiate benign overgrowths such as those seen in PROS from malignant lesions. Therefore, mutant *PIK3CA* is likely a weak oncogene (Berenjeno et al. 2017, Kinross et al. 2012, Madsen et al. 2019) whose function is conditioned in part on its mutant zygosity in a dosage-dependent manner (**Figure 2**).

Allelic Imbalance Through Composite Mutations

PIK3CA has also emerged as a model of a new and previously occult mechanism of mutant allele imbalance. Indeed, beyond changes in mutant *PIK3CA* zygosity mediated by mutant-allele specific CNAs, composite mutations (i.e., two somatic nonsynonymous mutations in the same gene and tumor specimen) define a subset of *PIK3CA*-mutant tumors. Most commonly found in breast cancers, composite *PIK3CA* mutations promote enhanced PI3K activity, hyperactivate downstream signaling, increase cellular proliferation, and exhibit greater sensitivity to PI3K inhibitors than do conventional singleton *PIK3CA* driver mutations (Vasan et al. 2019). Moreover, composite mutations appear to arise more frequently than expected in several key oncogenes, raising the possibility that composite mutations represent an alternative non–copy number mechanism of mutant allele imbalance. Notably, composite mutations are enriched for rare, weakly activating alleles, and genes beyond *PIK3CA* are also hypermorphic, for example, in *TERT* (Saito et al. 2020, Gorelick et al. 2020). Much like secondary loss-of-function mutations targeting mutant TSGs, which constitute an uncommon mechanism of biallelic inactivation, composite mutations in oncogenes may thus represent a rare class of mutant allele imbalance with context-specific properties.

Competitive Fitness as a Driver of Mechanism-Specific Mutant Allele Imbalance

Finally, mutant allele imbalance appears to mediate functional differences beyond key signaling pathways. For instance, LOH appears to be a common mechanism for loss of the WT allele of mutant *ESR1*, which encodes the estrogen receptor (ER) (Bielski et al. 2018a). Diverse mutations in the ligand binding domain of *ESR1* arise in hormone receptor–positive breast cancers that become refractory to antihormonal therapy (Toy et al. 2013). Curiously, loss of WT ER accounts for the majority of *ESR1* mutant allele imbalance in this subset of breast cancers, suggesting that competitive fitness between the mutant and WT alleles underpins selection for LOH. Indeed, coexpression of both WT and mutant ER in different proportions (modeling ratios corresponding to amplifications, gains, and balanced heterozygosity) has revealed dose-dependent attenuation of mutant ER activity with increasing proportions of WT ER, confirming that loss of WT ER provides a fitness advantage in tumors with *ESR1* mutations (Bielski et al. 2018a). Taken together, these findings suggest that driver mutations in diverse oncogenes are often accompanied by mutant allele imbalance, and the widespread loss of the corresponding WT allele supports a broader growth-inhibitory effect of the WT allele on oncogenic mutations than previously appreciated across multiple cancer types (**Figure 2**).

MUTANT ONCOGENE ZYGOSITY AS A THERAPEUTIC BIOMARKER

The prevalence of allelic imbalance affecting mutant oncogenes that are established or emerging targets of pharmacological inhibition raises the possibility that mutant oncogene zygosity may represent a novel biomarker of therapeutic sensitivity. Preclinical studies of *Kras*-mutant mouse models have found that mutant allele zygosity can play a key role in shaping clonal dynamics and modulating therapeutic responses to MAPK pathway inhibition. For example, in murine models of *Kras*-mutant lung adenocarcinomas, tumors lacking the WT *Kras* allele exhibited increased cellular proliferation and sensitivity to MEK inhibition compared to tumors with an intact WT *Kras* allele (Ambrogio et al. 2018). In a preclinical trial of MEK inhibition in $Kras^{G12D}$-driven mouse models of acute myeloid leukemia, one exceptional responder resulted from the duplication of $Kras^{G12D}$ and loss of WT *Kras* via somatic CN-LOH (Burgess et al. 2017). Here, a subclone with duplication of mutant *Kras* and a single copy of WT *Kras* existed prior to therapy and expanded to become dominant at the time of relapse. Subsequent functional studies confirmed that WT *Kras* overexpression was sufficient to overcome the fitness advantage conferred by gain of *Kras*-mutant dosage and render the pretreatment tumor resistant to MEK inhibition. Furthermore, CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 editing of the WT allele to G13D in a $KRAS^{G13D}$-heterozygous colorectal cancer similarly increased RAS-GTPase activity, growth, and MEK inhibitor sensitivity (Burgess et al. 2017). These studies established that small changes in the balance between mutant and WT alleles of *KRAS* can impact pathway dependence and drug sensitivity. Of course, it remains to be seen if the zygosity of mutant *KRAS* will prove to be a robust biomarker of therapeutic sensitivity in cancer patients. The advent of a new generation of mutant allele–specific inhibitors of *KRAS* (Canon et al. 2019, Hallin et al. 2020, Ostrem et al. 2013, Patricelli et al. 2016), after decades of futility in developing effective RAS inhibitors (Downward 2003, Haigis 2017, McCormick 2015, Stephen et al. 2014), will facilitate correlative studies of *KRAS*-mutant zygosity as a predictive biomarker in a manner that acknowledges their distinct mechanisms of action (**Figure 3**).

The potential clinical relevance of mutant allele imbalance extends to the PI3K/AKT pathway. Clinical drug development for this pathway has largely focused on inhibiting the PI3K isoforms (Liu et al. 2009, Rodon et al. 2013), although recent efforts have targeted other effectors of PI3K signaling such as the AKT family of kinases. A recent study of a pan-AKT kinase inhibitor (Davies

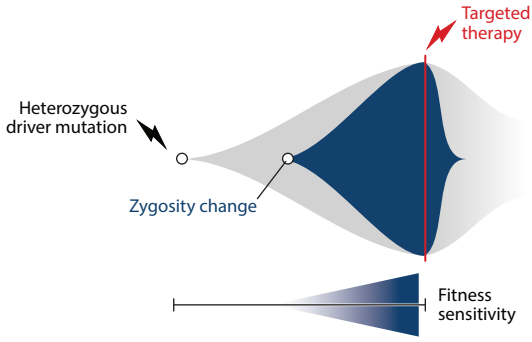

Figure 3

Tradeoff between fitness and therapeutic sensitivity. Schematic representation of a tumor in which a heterozygous oncogenic driver mutation is followed by a zygosity change that produces mutant allele imbalance, leading to a tradeoff between a greater fitness advantage and subsequent clonal outgrowth and increased therapeutic susceptibility (*red*). This phenomenon raises the possibility that therapy resistance could emerge via restoration of the wild-type allele.

et al. 2012) in *AKT1*-mutant tumors found that mutant allele imbalance of *AKT1*^{E17K}, typically via CN-LOH resulting in loss of the WT allele, was associated with improved progression-free survival (PFS) (Hyman et al. 2017a). Notably, this study employed a unique basket design, which combined *AKT1*^{E17K}-mutant solid tumors from different lineages to overcome the fact that AKT mutations are relatively uncommon in any individual cancer type and thus not amenable to traditional clinical trial designs. The clinical outcomes associated with *AKT1* mutant allele imbalance are therefore more likely to reflect fundamental properties of PI3K/AKT biology than cancer type–specific effects.

The possibility that the depth and durability of treatment responses can be influenced by the zygosity of sensitizing mutant oncogenes has significant implications for precision oncology. As a greater number of these therapies exploiting diverse mechanisms of action become standard of care in more indications (Chakravarty et al. 2017), it will be imperative that modern clinical sequencing initiatives move beyond detecting the presence or absence of individual driver mutations to profile even subtle mutant allele–specific changes thereof. For example, exploratory data indicate that patients with homozygous *BRAF*^{V600E}-mutant melanomas treated with RAF inhibitor therapy have markedly improved PFS compared to patients with either heterozygous mutations or mutations targeted by genomic gains (Bielski et al. 2018a). Subtle dosage effects may also mediate therapeutic resistance, especially for dosage-dependent actionable oncogenes such as mutant *HER2* (Smyth et al. 2020). Identifying the effect of similarly predictive allelically imbalanced genotypes on therapeutic sensitivity is a critical need that will be complicated by the rapid evolution of next-generation technologies such as tumor-derived cell-free DNA sequencing, platforms that necessitate improved and sophisticated analytical and data-driven methods. Ultimately, population-scale cohorts of prospectively sequenced cancer patients with this level of rich molecular annotation, integrated with clinical outcomes and therapeutic phenotypes, will likely drive the discovery and validation of such novel predictive biomarkers and accelerate their clinical translation more efficiently than smaller-sample-size, albeit homogeneously treated, clinical trial populations.

OUTLOOK

Mutant allele zygosity is emerging as a hallmark of oncogene biology, with significant functional and clinical ramifications. There is still much to be learned, however. As current tumor sequencing

initiatives expand to population scale, so too will our statistical power to analyze oncogene zygosity at the level of individual mutant alleles, which can have distinct biochemical, mechanistic, and therapeutic consequences even within the same actionable cancer gene (Brenan et al. 2016; Gao et al. 2018; Yao et al. 2015, 2017). Unraveling the functional impact of subtle dosage changes of oncogenic mutations will necessitate new experimental strategies. Decades of overexpression experiments will require reconsideration, as even modest allelic imbalance can drive clonal outgrowth. Even with the advent of CRISPR/Cas9, the mechanisms of allelic imbalance and their effects on function must be considered during study design and cell type selection. From a clinical perspective, the prevalence and selective pressure for zygosity changes in mutant oncogenes raise the question of whether a viable therapeutic window exists for modulating WT expression (**Figure 3**). These issues are relevant to more than just molecularly targeted therapies, with implications for a newer generation of immunotherapies such as personalized vaccines tailored to exploit the presence or absence of particular neoepitopes. Ultimately, characterizing mutant oncogene zygosity across human cancers will help to advance the fields of cancer biology and clinical oncology.

DISCLOSURE STATEMENT

B.S.T. reports honoraria and research funding from Genentech and scientific advisory board activities for Boehringer Ingelheim and Loxo Oncology, a wholly owned subsidiary of Eli Lilly. C.M.B. is not aware of any factors that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank the members of the Taylor laboratory and Marie-Josée and Henry R. Kravis Center for Molecular Oncology for their comments and feedback, in particular M.T.A. Donoghue. This work was supported by National Institutes of Health awards P30 CA008748, U54 OD020355, R01 CA207244, R01 CA204749, and R01 CA245069.

LITERATURE CITED

Ambrogio C, Köhler J, Zhou Z-W, Wang H, Paranal R, et al. 2018. KRAS dimerization impacts MEK inhibitor sensitivity and oncogenic activity of mutant KRAS. *Cell* 172(4):857–68.e15

Avner P, Heard E. 2001. X-chromosome inactivation: counting, choice and initiation. *Nat. Rev. Genet.* 2(1):59–67

Baca SC, Prandi D, Lawrence MS, Mosquera JM, Romanel A, et al. 2013. Punctuated evolution of prostate cancer genomes. *Cell* 153(3):666–77

Bentley C, Jurinka SS, Kljavin NM, Vartanian S, Ramani SR, et al. 2013. A requirement for wild-type Ras isoforms in mutant KRas-driven signalling and transformation. *Biochem. J.* 452(2):313–20

Berenjeno IM, Piñeiro R, Castillo SD, Pearce W, McGranahan N, et al. 2017. Oncogenic *PIK3CA* induces centrosome amplification and tolerance to genome doubling. *Nat. Commun.* 8(1):1773

Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, et al. 2010. The landscape of somatic copy-number alteration across human cancers. *Nature* 463(7283):899–905

Bielski CM, Donoghue MTA, Gadiya M, Hanrahan AJ, Won HH, et al. 2018a. Widespread selection for oncogenic mutant allele imbalance in cancer. *Cancer Cell* 34(5):852–62.e4

Bielski CM, Zehir A, Penson AV, Donoghue MTA, Chatila W, et al. 2018b. Genome doubling shapes the evolution and prognosis of advanced cancers. *Nat. Genet.* 50(8):1189–95

Birchler JA, Bhadra U, Bhadra MP, Auger DL. 2001. Dosage-dependent gene regulation in multicellular eukaryotes: implications for dosage compensation, aneuploid syndromes, and quantitative traits. *Dev. Biol.* 234(2):275–88

Birchler JA, Riddle NC, Auger DL, Veitia RA. 2005. Dosage balance in gene regulation: biological implications. *Trends Genet.* 21(4):219–26

Blakeslee AF, Belling J, Farnham ME. 1920. Chromosomal duplication and mendelian phenomena in *Datura* mutants. *Science* 52(1347):388–90

Bremner R, Balmain A. 1990. Genetic changes in skin tumor progression: correlation between presence of a mutant *ras* gene and loss of heterozygosity on mouse chromosome 7. *Cell* 61(3):407–17

Brenan L, Andreev A, Cohen O, Pantel S, Kamburov A, et al. 2016. Phenotypic characterization of a comprehensive set of MAPK1/ERK2 missense mutants. *Cell Rep*. 17(4):1171–83

Burgess MR, Hwang E, Mroue R, Bielski CM, Wandler AM, et al. 2017. *KRAS* allelic imbalance enhances fitness and modulates MAP kinase dependence in cancer. *Cell* 168(5):817–29.e15

Canon J, Rex K, Saiki AY, Mohr C, Cooke K, et al. 2019. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature* 575(7781):217–23

Carter NP. 2007. Methods and strategies for analyzing copy number variation using DNA microarrays. *Nat. Genet.* 39:S16–21

Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, et al. 2012. Absolute quantification of somatic DNA alterations in human cancer. *Nat. Biotechnol.* 30(5):413–21

Chakravarty D, Gao J, Phillips SM, Kundra R, Zhang H, et al. 2017. OncoKB: a precision oncology knowledge base. *JCO Precis. Oncol.* 2017:PO.17.00011

Collins S, Groudine M. 1982. Amplification of endogenous *myc*-related DNA sequences in a human myeloid leukaemia cell line. *Nature* 298(5875):679–81

Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA. 2010. *BRAF* gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. *Sci. Signal.* 3(149):ra84

Dalla-Favera R, Wong-Staal F, Gallo RC. 1982. *onc* gene amplification in promyelocytic leukaemia cell line HL-60 and primary leukaemic cells of the same patient. *Nature* 299(5878):61–63

Davies BR, Greenwood H, Dudley P, Crafter C, Yu D-H, et al. 2012. Preclinical pharmacology of AZD5363, an inhibitor of AKT: pharmacodynamics, antitumor activity, and correlation of monotherapy activity with genetic background. *Mol. Cancer Ther.* 11(4):873–87

Dewhurst SM, McGranahan N, Burrell RA, Rowan AJ, Grönroos E, et al. 2014. Tolerance of whole-genome doubling propagates chromosomal instability and accelerates cancer genome evolution. *Cancer Discov*. 4(2):175–85

Downward J. 2003. Targeting RAS signalling pathways in cancer therapy. *Nat. Rev. Cancer* 3(1):11–22

Dvinge H, Kim E, Abdel-Wahab O, Bradley RK. 2016. RNA splicing factors as oncoproteins and tumour suppressors. *Nat. Rev. Cancer* 16(7):413–30

Ercan D, Zejnullahu K, Yonesaka K, Xiao Y, Capelletti M, et al. 2010. Amplification of *EGFR* T790M causes resistance to an irreversible EGFR inhibitor. *Oncogene* 29(16):2346–56

Gao Y, Chang MT, McKay D, Na N, Zhou B, et al. 2018. Allele-specific mechanisms of activation of MEK1 mutants determine their properties. *Cancer Discov*. 8(5):648–61

Garraway LA, Lander ES. 2013. Lessons from the cancer genome. *Cell* 153(1):17–37

Gonçalves E, Fragoulis A, Garcia-Alonso L, Cramer T, Saez-Rodriguez J, Beltrao P. 2017. Widespread post-transcriptional attenuation of genomic copy-number variation in cancer. *Cell Syst*. 5(4):386–98.e4

Gorelick AN, Sánchez-Rivera FJ, Cai Y, Bielski CM, Biederstedt E, et al. 2020. Phase and context shape the function of composite oncogenic mutations. *Nature* 582(7810):100–3

Gould SJ, Vrba ES. 1982. Exaptation—a missing term in the science of form. *Paleobiology* 8(01):4–15

Gustin JP, Karakas B, Weiss MB, Abukhdeir AM, Lauring J, et al. 2009. Knockin of mutant PIK3CA activates multiple oncogenic pathways. *PNAS* 106(8):2835–40

Haigis KM. 2017. *KRAS* alleles: The devil is in the detail. *Trends Cancer* 3(10):686–97

Hallin J, Engstrom LD, Hargis L, Calinisan A, Aranda R, et al. 2020. The $KRAS^{G12C}$ inhibitor MRTX849 provides insight toward therapeutic susceptibility of KRAS-mutant cancers in mouse models and patients. *Cancer Discov*. 10(1):54–71

Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. *Cell* 100(1):57–70

Hyman DM, Smyth LM, Donoghue MTA, Westin SN, Bedard PL, et al. 2017a. AKT inhibition in solid tumors with AKT1 mutations. *J. Clin. Oncol.* 35(20):2251–59

Hyman DM, Taylor BS, Baselga J. 2017b. Implementing genome-driven oncology. *Cell* 168(4):584–99

Isakoff SJ, Engelman JA, Irie HY, Luo J, Brachmann SM, et al. 2005. Breast cancer–associated *PIK3CA* mutations are oncogenic in mammary epithelial cells. *Cancer Res.* 65(23):10992–11000

Keppler-Noreuil KM, Rios JJ, Parker VER, Semple RK, Lindhurst MJ, et al. 2015. *PIK3CA*-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. *Am. J. Med. Genet. A.* 167(2):287–95

Kinross KM, Montgomery KG, Kleinschmidt M, Waring P, Ivetac I, et al. 2012. An activating *Pik3ca* mutation coupled with *Pten* loss is sufficient to initiate ovarian tumorigenesis in mice. *J. Clin. Investig.* 122(2):553–57

Klein G. 1981. The role of gene dosage and genetic transpositions in carcinogenesis. *Nature* 294(5839):313–18

Knudson AG. 1971. Mutation and cancer: statistical study of retinoblastoma. *PNAS* 68(4):820–23

Koren S, Reavie L, Couto JP, De Silva D, Stadler MB, et al. 2015. *PIK3CA*^{H1047R} induces multipotency and multi-lineage mammary tumours. *Nature* 525(7567):114–18

Lee SC-W, Dvinge H, Kim E, Cho H, Micol J-B, et al. 2016. Modulation of splicing catalysis for therapeutic targeting of leukemia with mutations in genes encoding spliceosomal proteins. *Nat. Med.* 22(6):672–78

Lee SC-W, North K, Kim E, Jang E, Obeng E, et al. 2018. Synthetic lethal and convergent biological effects of cancer-associated spliceosomal gene mutations. *Cancer Cell* 34(2):225–41.e8

Liu P, Cheng H, Roberts TM, Zhao JJ. 2009. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat. Rev. Drug Discov.* 8(8):627–44

Liu P, Cheng H, Santiago S, Raeder M, Zhang F, et al. 2011. Oncogenic *PIK3CA*-driven mammary tumors frequently recur via PI3K pathway–dependent and PI3K pathway–independent mechanisms. *Nat. Med.* 17(9):1116–20

Maciejowski J, Li Y, Bosco N, Campbell PJ, de Lange T. 2015. Chromothripsis and kataegis induced by telomere crisis. *Cell* 163(7):1641–54

Madsen RR, Knox RG, Pearce W, Lopez S, Mahler-Araujo B, et al. 2019. Oncogenic *PIK3CA* promotes cellular stemness in an allele dose-dependent manner. *PNAS* 116(17):8380–89

Madsen RR, Vanhaesebroeck B, Semple RK. 2018. Cancer-associated *PIK3CA* mutations in overgrowth disorders. *Trends Mol. Med.* 24(10):856–70

McCormick F. 2015. KRAS as a therapeutic target. *Clin. Cancer Res.* 21(8):1797–1801

McGranahan N, Favero F, de Bruin EC, Birkbak NJ, Szallasi Z, Swanton C. 2015. Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. *Sci. Transl. Med.* 7(283):283ra54

Meyer DS, Brinkhaus H, Müller U, Müller M, Cardiff RD, Bentires-Alj M. 2011. Luminal expression of *PIK3CA* mutant H1047R in the mammary gland induces heterogeneous tumors. *Cancer Res.* 71(13):4344–51

Meyerson M, Gabriel S, Getz G. 2010. Advances in understanding cancer genomes through second-generation sequencing. *Nat. Rev. Genet.* 11(10):685–96

Mueller S, Engleitner T, Maresch R, Zukowska M, Lange S, et al. 2018. Evolutionary routes and *KRAS* dosage define pancreatic cancer phenotypes. *Nature* 554(7690):62–68

Nan X, Tamgüney TM, Collisson EA, Lin L-J, Pitt C, et al. 2015. Ras-GTP dimers activate the mitogen-activated protein kinase (MAPK) pathway. *PNAS* 112(26):7996–8001

Nguyen DK, Disteche CM. 2006. Dosage compensation of the active X chromosome in mammals. *Nat. Genet.* 38(1):47–53

Okabe T, Okamoto I, Tamura K, Terashima M, Yoshida T, et al. 2007. Differential constitutive activation of the epidermal growth factor receptor in non–small cell lung cancer cells bearing *EGFR* gene mutation and amplification. *Cancer Res.* 67(5):2046–53

Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. 2013. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* 503(7477):548–51

Patricelli MP, Janes MR, Li L-S, Hansen R, Peters U, et al. 2016. Selective inhibition of oncogenic KRAS output with small molecules targeting the inactive state. *Cancer Discov.* 6(3):316–29

Pires JC, Conant GC. 2016. Robust yet fragile: expression noise, protein misfolding, and gene dosage in the evolution of genomes. *Annu. Rev. Genet.* 50:113–31

Raznahan A, Parikshak NN, Chandran V, Blumenthal JD, Clasen LS, et al. 2018. Sex-chromosome dosage effects on gene expression in humans. *PNAS* 115(28):7398–403

Rodon J, Dienstmann R, Serra V, Tabernero J. 2013. Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat. Rev. Clin. Oncol.* 10(3):143–53

Saito Y, Koya J, Araki M, Kogure Y, Shingaki S, et al. 2020. Landscape and function of multiple mutations within individual oncogenes. *Nature* 582(7810):95–99

Shen R, Seshan VE. 2016. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res*. 44(16):e131

Smyth LM, Piha-Paul SA, Won HH, Schram AM, Saura C, et al. 2020. Efficacy and determinants of response to HER kinase inhibition in *HER2*-mutant metastatic breast cancer. *Cancer Discov*. 10(2):198–213

Speicher MR, Carter NP. 2005. The new cytogenetics: blurring the boundaries with molecular biology. *Nat. Rev. Genet.* 6(10):782–92

Stephen AG, Esposito D, Bagni RK, McCormick F. 2014. Dragging Ras back in the ring. *Cancer Cell* 25(3):272–81

Stephens PJ, Greenman CD, Fu B, Yang F, Bignell GR, et al. 2011. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell* 144(1):27–40

Stingele S, Stoehr G, Peplowska K, Cox J, Mann M, Storchova Z. 2012. Global analysis of genome, transcriptome and proteome reveals the response to aneuploidy in human cells. *Mol. Syst. Biol.* 8:608

Tikoo A, Roh V, Montgomery KG, Ivetac I, Waring P, et al. 2012. Physiological levels of *Pik3ca*^{H1047R} mutation in the mouse mammary gland results in ductal hyperplasia and formation of ERα-positive tumors. *PLOS ONE* 7(5):e36924

To MD, Rosario RD, Westcott PMK, Banta KL, Balmain A. 2013. Interactions between wild-type and mutant *Ras* genes in lung and skin carcinogenesis. *Oncogene* 32(34):4028–33

Toy W, Shen Y, Won H, Green B, Sakr RA, et al. 2013. *ESR1* ligand-binding domain mutations in hormone-resistant breast cancer. *Nat. Genet.* 45(12):1439–45

Umbreit NT, Zhang C-Z, Lynch LD, Blaine LJ, Cheng AM, et al. 2020. Mechanisms generating cancer genome complexity from a single cell division error. *Science* 368(6488):eaba0712

Van Keymeulen A, Lee MY, Ousset M, Brohée S, Rorive S, et al. 2015. Reactivation of multipotency by oncogenic PIK3CA induces breast tumour heterogeneity. *Nature* 525(7567):119–23

Van Loo P, Nordgard SH, Lingjærde OC, Russnes HG, Rye IH, et al. 2010. Allele-specific copy number analysis of tumors. *PNAS* 107(39):16910–15

Varmus HE. 1984. The molecular genetics of cellular oncogenes. *Annu. Rev. Genet.* 18:553–612

Vasan N, Razavi P, Johnson JL, Shao H, Shah H, et al. 2019. Double *PIK3CA* mutations in cis increase oncogenicity and sensitivity to PI3Kα inhibitors. *Science* 366(6466):714–23

Vivanco I, Sawyers CL. 2002. The phosphatidylinositol 3-kinase–AKT pathway in human cancer. *Nat. Rev. Cancer* 2(7):489–501

Wang YK, Bashashati A, Anglesio MS, Cochrane DR, Grewal DS, et al. 2017. Genomic consequences of aberrant DNA repair mechanisms stratify ovarian cancer histotypes. *Nat. Genet.* 49(6):856–65

Westcott PMK, Halliwill KD, To MD, Rashid M, Rust AG, et al. 2015. The mutational landscapes of genetic and chemical models of *Kras*-driven lung cancer. *Nature* 517(7535):489–92

Yao Z, Torres NM, Tao A, Gao Y, Luo L, et al. 2015. BRAF mutants evade ERK-dependent feedback by different mechanisms that determine their sensitivity to pharmacologic inhibition. *Cancer Cell* 28(3):370–83

Yao Z, Yaeger R, Rodrik-Outmezguine VS, Tao A, Torres NM, et al. 2017. Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS. *Nature* 548(7666):234–38

Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, et al. 2011. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* 478(7367):64–69

Yuan TL, Cantley LC. 2008. PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 27(41):5497–510

Yuan W, Stawiski E, Janakiraman V, Chan E, Durinck S, et al. 2013. Conditional activation of *Pik3ca*^{H1047R} in a knock-in mouse model promotes mammary tumorigenesis and emergence of mutations. *Oncogene* 32(3):318–26

Yueh AE, Payne SN, Leystra AA, Van De Hey DR, Foley TM, et al. 2016. Colon cancer tumorigenesis initiated by the H1047R mutant PI3K. *PLOS ONE* 11(2):e0148730

Zack TI, Schumacher SE, Carter SL, Cherniack AD, Saksena G, et al. 2013. Pan-cancer patterns of somatic copy number alteration. *Nat. Genet.* 45(10):1134–40

Zhang Z, Wang Y, Vikis HG, Johnson L, Liu G, et al. 2001. Wildtype *Kras2* can inhibit lung carcinogenesis in mice. *Nat. Genet.* 29(1):25–33

Zhou Q, Derti A, Ruddy D, Rakiec D, Kao I, et al. 2015. A chemical genetics approach for the functional assessment of novel cancer genes. *Cancer Res.* 75(10):1949–58