Allele-specific genomic data elucidate the role of somatic gain and copy-number neutral loss of heterozygosity in cancer

Highlights

- Allele-specific analysis of TCGA collection identifies 18 million allelic imbalance events
- Wild-type and copy-number gain calls are reclassified as LOH
- LOH states associate with tumor suppressors reduced expression and prognosis
- Activation of TP53 downstream targets reflects its allele-specific genomic status

In brief

Ciani et al. delineated the purity and ploidy-adjusted allele-specific profiles across TCGA tumor types and identified 18 million allelic imbalance events. This led to the reclassification of wild-type and copy gain calls as loss of heterozygosity (LOH) and to an allele-specific genomic events catalog. The authors showed that the activation of p53 downstream targets is reflective of the allele-specific genomic status of TP53 and highlighted the pervasiveness of LOH and its association with prognosis and tumor suppressor genes expression.
Allele-specific genomic data elucidate the role of somatic gain and copy-number neutral loss of heterozygosity in cancer

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SUMMARY

Pan-cancer studies sketched the genomic landscape of the tumor types spectrum. We delineated the purity- and ploidy-adjusted allele-specific profiles of 4,950 patients across 27 tumor types from the Cancer Genome Atlas (TCGA). Leveraging allele-specific data, we reclassified as loss of heterozygosity (LOH) 9% and 7% of apparent copy-number wild-type and gain calls, respectively, and overall observed more than 18 million allelic imbalance somatic events at the gene level. Reclassification of copy-number events revealed associations between driver mutations and LOH, pointing out the timings between the occurrence of point mutations and copy-number events. Integrating allele-specific genomics and matched transcriptomics, we observed that allele-specific gene status is relevant in the regulation of TP53 and its targets. Further, we disclosed the role of copy-neutral LOH in the impairment of tumor suppressor genes and in disease progression. Our results highlight the role of LOH in cancer and contribute to the understanding of tumor progression.

INTRODUCTION

Pan-cancer genomic studies, pioneered by the Cancer Genome Atlas (TCGA), uncovered both tissue-specific and shared features of human tumors (Berger et al., 2018), enabled the characterization of the immune response to cancer (Thorsson et al., 2018), and detected at least one driver mutation in 91% of 2,658 analyzed whole-cancer genomes, mainly in coding regions (ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium, 2020).

In addition to mutations and changes in the tumor cell ploidy (aneuploidy) (Bieliski et al., 2018b; Pfister et al., 2018) (Zack et al., 2013), an important class of events in cancer cells is the loss of heterozygosity (LOH). LOH occurs via heterozygous deletion of one allele. These events can be simple deletions or be accompanied by duplications of the remaining allele, giving rise to copy-neutral LOH (CN-LOH) or even to copy gain-LOH events. LOH has been interrogated in search for actionable vulnerabilities, since the lack of one allele and the subsequent reduced genomic redundancy can be exploited to specifically target cancer cells, for instance using allele-specific gene editing technology to target the remaining allele of essential or haploinsufficient genes (Nichols et al., 2020). Despite the interest as putative targets, to our knowledge, no study has systematically shown the relevance of LOH events in cancer-related processes at pan-cancer level.

The accurate measurement of tumor cells ploidy and the use of methods that can discriminate between alleles (Prandi and Demichelis, 2019; Shen and Seshan, 2016; Taylor et al., 2018) is essential for the comprehensive characterization of somatic copy-number aberrations (SCNA) and the ultimate delineation of allele-specific informed events. This is particularly relevant for identifying genes in CN-LOH status otherwise classified as wild type; in fact, CN-LOH can in principle lead to the duplication of a mutated allele in an oncogene or in a tumor suppressor gene, and duplication or loss of a methylated allele thus impacting on gene expression (Hagenkord et al., 2010; Yeung et al., 2018). Allele-specific informed data have been considered in tumor-type-specific studies (Buchwald et al., 2020; Ged et al., 2020; Hoff et al., 2020; Wilkinson et al., 2020), in the setting of haploinsufficiency detection for tumor suppressor genes (TSGs) (Davoli et al., 2013), for DNA repair genes in breast tissues.
(Karayyaz-V以后irm et al., 2020), and also for the understanding of cancer aneuploidy (Taylor et al., 2018).

We hypothesized that a uniform harmonized characterization of tumor-allele-specific informed genomic landscape would deepen our understanding of the cancer genomes and of the role of previously unappreciated LOH events, such as CN-LOH and copy gain-LOH events, in cancer-related processes. This characterization can lead to the identification of molecular vulnerabilities (Nichols et al., 2020) and provide additional discovery tools for the assessment of biomarkers for patients’ enrollment into clinical trials. Therefore, here we present a framework for the analysis of allele-specific genomic features, a uniform harmonized characterization of the genomes of 4,950 patients from 27 TCGA datasets, and evidence that single-allele data provide an orthogonal component of information to the landscape of primary tumors whereby LOH is a common trait of impaired tumor-suppressive processes.

**RESULTS**

**A framework for allele-specific informed genomic features analysis**

To comprehensively characterize the genomic landscape of human tumors at the single-allele level and define the spectrum of LOH events (including CN-LOH, copy gain (i.e., the allele presents 3 or 4 copies) and amplification LOH (i.e., the allele presents 5 or more copies) events, here referred to as Gain-LOH and Amp-LOH, respectively), we designed a framework that integrates a set of widely used tools (STAR Methods; Figures S1 and S2) to seamlessly process matched tumor and normal samples profiled by next-generation sequencing technologies and extract allele-dependent genomic information from segmented data upon tumor ploidy and tumor purity correction. The pipeline implements the computation of allele-specific copy-number (asCN) data (Prandi and Demichelis, 2019) that broaden the spectrum of assessable copy-number states. As any DNA copy number higher than one can be explained by more than one-allele-based assessable copy-number states. As any DNA copy number

Building on the asCN data of each tumor sample, we here defined a measure of the tumor cell nuclear DNA content, similar to the concept of DNA index from DNA cytometry (Danielsen et al., 2016), which we termed allele-specific informed ploidy (asP) index (Figures 1A and S4), as it is computed as the weighted mean of the asCN of homologous chromosomes. By definition, perfectly diploid cells have asP equal to 2. The obtained asP values are overall concordant with ABSOLUTE ploidy measures (Carter et al., 2012) (Figure S5A). Overall, 1,305 tumors (26.4%) in the study cohort show high asP (defined as asP > 2.5) with marked variability among tumor types, ranging from 81.2% (n = 101) in testicular germ cell tumors (TGCT) to none (n = 16) in acute myeloid leukemia (LAML).

Given the widespread presence of allelic imbalance, we next looked at the representation of asCN states in the presence of
point mutation events. We observed depletion of wild-type and balanced states compared with unbalanced conditions for TSGs (Figure 2A), in line with the observation previously reported in advanced solid cancer patient samples (Bielski et al., 2018a).

We next explored at gene level the relationship between the incidence of SNVs and asCN of both OG and TSG by stratifying tumors in three asCN classes, namely LOH, heterozygous, and unbalanced. We observed multiple genes with uneven distributions of SNVs across classes (chi-square test, FDR < 0.05, only keeping classes with at least 20 SNV events, Table S8), with enrichment of TSGs (Fisher’s exact test, p-value = 0.008) in the LOH asCN class, with TP53, PTEN, SMAD4, CIC, VHL,
and RB1 as top-ranked (Figure 2B). Further, when querying their CN adjusted allelic fractions (AF) among asCN classes, we often observed AF close to 1 for SNVs in LOH states, thus indicating that deleterious SNVs within TSGs are driver events (Figure 2C).

On the other hand, OG data demonstrate a bimodal distribution (Figure 2C) evidencing that point mutations occur upon the loss of one allele, in line with the notion that bi-allelic mutations are not required for oncogenic activation.

We then hypothesized that LOH asCN states of TSG are enriched for deleterious SNVs as a means of increasing cell fitness (Figure 2C) evidencing that point mutations occur upon the loss of one allele, in line with the notion that bi-allelic mutations are not required for oncogenic activation.

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via full impairment of tumor-suppressive processes. We therefore tested whether in-depth allele-specific informed analysis of the recurrently mutated TSG TP53 (Hollstein et al., 1991; Olivier et al., 2010; Pettitjean et al., 2007) could lead to new insights on cancer-related biological processes with respect to conventional CN analysis (Figure 2D) where CN-LOH are frequently misclassified as wild-type copy number. For instance, we detected a significantly lower proportion of deleterious SNVs when focusing on asCN-based wild-type segments as opposed to apparent wild-type segments (based on log2 ratio only analysis) (proportion test, p value = 1.6e-54; 7% versus 24%). Conversely, we observed an enrichment of SNVs in Gain-LOH asCN segments with respect to log2 ratio = 1 segments (proportion test, p value = 2.8e-11; 55.7% versus 16.9%). Taking all asCN statuses into account (WT, CN-LOH, Hemi-del, Gain-LOH, and Gain), TP53 SNV status is not independent of the allele-specific status (chi-square test, p value < 2.2e-16). Altogether, TP53 LOH tumors are enriched for TP53 deleterious SNVs (37.2%, 51.4%, and 55.7% in Hemi-delns, CN-LOH, and Gain-LOH, respectively). The distribution of AF of loss-of-function (LOF) SNVs of TP53 has higher values in CN-LOH and Gain-LOH events with respect to WT, supporting the concept that SNVs and LOH are driver events and that gene amplifications (leading to CN-LOH and Gain-LOH) occur at a later time (Figure 2E). Despite the variability of TP53 mutation frequency, this association is conserved across tumor types (Figures 2F and 2G). When TP53 is mutated, some tumors still retain a wild-type copy (Figure 2G, right). This can modulate the effects of TP53 mutations through a ‘‘dominant-positive mechanism,’’ where a wild-type copy can change the stoichiometry and function of p53 tetramers (Gogna et al., 2015), in the impact of SCNA on the amount of proteins (Stingele et al., 2012), and in proteasome regulation (Walerych et al., 2018).

We next investigated the transcript levels of the p53 target genes CDKN1A (coding for p21) and MDM2 with respect to TP53-allele-specific status (Figure 2H). Independently of TP53 expression level (Figure S1O), significantly lower levels of expression of CDKN1A and MDM2 (Mann-Whitney test, p value = 0.012 and p value = 0.0002, respectively) were observed in tumors harboring exclusively mutated copies of TP53 compared with tumors that retained at least one wild-type copy. These pan-cancer observations suggest a TP53-dependent transcriptional regulation mechanism for MDM2 that is coherent with previous publications (Midgley and Lane, 1997; Terzian et al., 2008; Vijayakumaran et al., 2015), whereby the lack of wild-type TP53 impairs the transcriptional activation of MDM2, which is responsible for the ubiquitination and translocation of p53 to the proteasome. RB1 expression is also reduced in absence of wild-type TP53 (Mann-Whitney test, p value = 0.007 considering only LOF mutations, p value = 6.8e-05 considering all deleterious SNVs). In line with previous work showing that TP53 mutational status is linked to ploidy and that aneuploid mammalian cells activate p53 (Hinchcliffe et al., 2016) (Li et al., 2010; Soto et al., 2017; Thompson and Compton, 2010), we observed that TP53 SNVs are enriched in high asP samples (chi-square test p value = 4.7e-21, Table S7). As TP53 is involved in cell-cycle regulation and, based on recent data, in aneuploidy-mediated activation of proteotoxic stress response in cells (Santaguida et al., 2015), in the impact of SCNA on the amount of proteins (Stingele et al., 2012), and in proteasome regulation (Walerych et al., 2018), we tested the activation of a set of selected proliferative (STAR Methods; Table S18) (Sheltzer, 2013) and proteasome-related pathways (Levin et al., 2018; Wang et al., 2017) in high asP samples compared with diploid samples (Figure S1O). The results suggest the activation of opposite transcriptional programs upon the presence of high asP in different tumor types (Table S16) possibly related to different genetic and tissue-specific transcriptional backgrounds and their interactions with TP53 status, affecting proliferation and global protein homeostasis. Altogether, the observation in this pan-cancer setting of the allele-specific effect of TP53 on downstream targets expression and processes corroborates the hypothesis that the tumor genomic make-up contributes to shaping the proliferative response to aneuploidy by regulating both transcription and protein degradation.

CN-LOH events are frequent and associate with prognosis

While LOH events due to monoallelic deletions are commonly reported, CN-LOH events most often remain hidden in large genomic studies, either incorrectly classified as wild-type segments or not explored for their functional relevance. Here, we estimated an overall pan-cancer median CN-LOH burden per sample of 2% (IQR [0%, 9%]), with significant increase in high asP samples (p value < 0.001, Wilcoxon rank sum test with continuity correction) (Figure S11A) with median values of 14% (IQR [8%, 22%]) compared with 0.4% (IQR [0%, 4%]) and 0.2% (IQR [0%, 4%]) in diploid and low asP tumors, respectively. Remarkably, 30% of the high asP samples have at least 20% of the genome with CN-LOH signal (Figure S11B), while the percentage drops below 3% for diploid and low asP samples. When considering tumor-specific data (Figure S11C), few exceptions emerged as TGCT and colon adenocarcinoma (COAD) (Table S9). Albeit less frequent, LOH events also include Gain-LOH and Amp-LOH, observed in 36.5% of the study samples and enriched in high asP samples (Figure S11D; Table S10). Since LOH events can involve entire chromosomal arms (Figure S11E), a great number of genes can be affected by Hemi-del and CN-LOH in each sample (Figure S12A). We observed that Hemi-delns are underrepresented in high asP samples (p value < 0.001, Wilcoxon rank sum test with continuity correction), which are enriched for CN-LOH events instead (Figure S12A, inset). In a recent work, Nichols et al. (Nichols et al., 2020) focused on LOH events on essential genes to identify putative cancer vulnerabilities: we estimated frequencies of LOH in essential genes obtaining results concordant with previously published data (Figure S12B). With respect to what was reported by Nichols et al., we here add the characterization of Gain-LOH and Amp-LOH events in essential genes, potentially expanding the panel of putative cancer vulnerabilities to genes with copy-number gains.

When studying the extension of LOH genomic events at the level of chromosomal arms (arm-wide versus partial) across tumor types, we observed that TSGs such as TP53, BCL2, and CDKN2A are included in chromosomal arms which show extremely widespread LOH regions (Figures 3A and S11E). We estimated that almost 40% and 15% of samples have at least two TSG with CN-LOH and Gain-LOH, respectively (Figure 3B). Patterns of Hemi-delns are not random, preferentially encompassing TSGs and antiproliferative genes (Solimini et al., 2012)
and cumulative haploinsufficiency may contribute to tumor evolution and maintenance (Davoli et al., 2013). Given these premises, we tested whether the asCN status of TSGs (Table S20) has prognostic value (considering all TSGs with at least 10 events per asCN status at pan-cancer level). We performed pan-cancer multivariate cox hazard analysis in diploid samples using PFI as readout, with asCN gene status combined with the presence of SNVs as predictor and tumor type (study) as covariate. A total of 20 TSGs resulted as significant predictors of risk when comparing Hemi-del versus WT (N = 11) or CN-LOH versus WT (N = 12), in the absence of deleterious SNVs (Table S11, results for all samples, independently of ploidy, are shown in Table S12). Of note, 12 genes showed statistical significance when considering CN-LOH status versus WT (in absence of deleterious SNVs), thus supporting the relevance of explicitly assessing the asCN status of TSG. Among the top significant genes of the multivariate cox hazard models, the analysis unveiled the associations between disease progression and events in CDKN2A and in the multiple endocrine neoplasia type I gene, MEN1, already reported as a haploinsufficient tumor suppressor (Lejonklou et al., 2012) (Figures 3C–3E). Despite the limitations stemming from the correlative nature of the analysis, we here observed clear evidence for TSGs CN-LOH association with tumor progression.

**LOH associates with TSG expression**

It has been observed that deletions of TSGs are early events (Deng et al., 1996) and, therefore, the most plausible scenario for the origin of CN-LOH involving TSGs is likely the loss of one allele followed by the duplication of the remaining allele. This is supported by our observation of the AF of SNVs in TSGs which present CN-LOH or Gain-LOH status (Figure 2C). On this premise, we next tested whether CN-LOH events, despite restoring two copies of the involved genes, demonstrated reduced gene expression with respect to the WT counterpart as opposed to rescuing the basal levels. Specifically, we hypothesize that genes that lose one allele and then regain additional copies (CN-LOH, Gain-LOH, or Amp-LOH) cannot rescue their basal
expression level if that is at a disadvantage to the cancer cell, such as in the case of TSGs.

To test this hypothesis, we built a linear model to predict the expression level of a gene based on two variables derived by asCN information: the total number of copies (CN tot) and the presence of LOH (Figure 4A). In the specific scenario of a TSG, we can test whether CN-LOH events associate with reduced expression level with respect to the WT level and further if Gain-LOH and Amp-LOH also show a reduction in expression, independently of the total number of copies.

This is opposed to a model that uses classical CN information instead of asCN and considers the level of expression dependent solely on the total number of copies (Figure S13A). We tested the model (STAR Methods) on all genes at study (cancer type) specific level and observed that, globally, the total number of copies positively impacts on expression while LOH has a negative impact (Figure 4B, left panel): this effect is even more pronounced in specific studies such as BRCA (Figure 4B, right panel) and it is stronger for TSGs with respect to OGs and all other genes (Figure 4C). The most relevant terms are related to RNA metabolic processes as well as to molecular localization and transport. Other enriched molecular functions, such as “vacuolar transport,” “autophagy,” and “oligosaccharide-lipid intermediate biosynthetic process” are relevant for tumorigenesis and cancer progression (Mulcahy Levy and Thorburn, 2020).

In total, we identified 18 TSGs under putative selective pressure for reduced expression upon CN-LOH in at least two studies (Figure S13C). When comparing the magnitude of the expression reduction with respect to WT, we observed that Hemi-del and CN-LOH have a similar impact, with a median ratio (see STAR Methods) of 0.54 and 0.66, respectively. Globally, we observed that CN-LOH, Gain-LOH, and Amp-LOH can all be associated with reduced expression compared with the

Figure 4. Loss of heterozygosity events and their impact on gene expression
(A) Synthetic example showing the expression levels, stratified by asCN, of a gene for which LOH has negative effect on expression.
(B) Density of association coefficients for parameters $a$ (CN tot) and $b$ (LOH) of the linear model. Negative values indicate a negative impact on gene expression. Sets of genes included are in Table S20.
(C) Proportion of genes showing a significant decrease of gene expression upon LOH ($p$-values are computed with the proportion test).
(D) Distribution of ratios of aberrant asCN to WT expression levels. Dashed line indicates half expression with respect to WT. Ticks on the x axis indicate single events ($N = 37$).
wild-type state for specific TSGs in a tumor-specific manner (Figures S13D–S13G).

**DISCUSSION**

The quantitative assessment of tumor ploidy and asCN alterations that we here proposed broadens the characterization of primary tumor genomes. For instance, low ploidy tumors are naturally distinguished from high ploidy by asP as in the case of ACC, an observation that would have been missed by other genomic indices (Beroukhim et al., 2010; Burrell et al., 2013; Carter et al., 2012; Mouliere et al., 2018; Taylor et al., 2018). The combined utilization of multiple genomic stability indices and ploidy assessment could help distinguish between markedly diverse cancer genomes states, from chaotic disruptions to whole-genome duplications, in turn pointing to the study of specific molecular targets.

Allele-specific informed analysis, applied via our framework, allows for precise detection of a variety of LOH states such as CN-LOH, Amp-LOH, and Gain-LOH. This is particularly relevant for CN-LOH events, which are otherwise incorrectly classified as WT, potentially leading to incorrect biological interpretations. In fact, we observed that CN-LOH results in the enrichment of loss and gain-of-function mutations in TSGs. Although we did not explicitly study in detail the time dependency between imbalance and point mutations, it recently emerged that whereas allelic imbalance is not driven by the occurrence of a mutant allele, a mutant allele dosage increase favors the fitness of a malignant clone (“exaptation” phenomenon) (Bielski et al., 2018a). In the context of copy-neutral and Gain-LOH events of cancer genes, the analysis of CN adjusted AF of deleterious SNVs suggests that point mutations rarely occur after the relevant amplification event (i.e., concomitant or prior events) as opposed to what is observed in the context of heterozygous states. Unbalanced status of point mutations could be an additional feature, beyond the position and the type of substitution, by which OG might achieve the ideal level of signaling (“sweet spot”), as recently suggested for KRAS (Li et al., 2018).

This study also quantified the widespread presence of allelic imbalance events, more pronounced in high asP tumors. These events could be a result of the processing of DNA double-strand breaks occurring via breakage-induced replication (Elango et al., 2017), particularly for the cases where arm-wide events are prevalent, and, which may also contribute to the high asP phenotype. Our results suggest that these events are more widespread than expected, possibly related to alterations in DNA-damage checkpoint and DNA repair capacity or underlying the acquisition of an alternative lengthening of telomeres (ALT) phenotype (Heaphy et al., 2011). It is possible that p53 status, which impacts on DNA replication and repair mechanisms (Klusmann et al., 2016) (Janic et al., 2018), can also impact on the occurrence of CN-LOH events.

In-depth characterization of TP53 genomics through an unsupervised integrated pan-cancer analysis highlighted the relationship between mutations and aneuploidy state, providing a link between TP53 status, aneuploidy, cell proliferation, and proteasome activation. Further, TP53 LOH associates with the presence of TP53 SNVs, exacerbating the deregulation of tumor-suppressive pathways. On one hand, the complete loss of wild-type copies impairs the ability of TP53 to regulate its targets, on the other hand, the presence of mutated copies allows for new interactions and oncogenic processes such as the establishment of a mutant-p53 proteasome axis (Walerych et al., 2016).

Integration of allele-specific genomics and matched transcriptomic data pointed to processes of RNA metabolisms, known to be quantitatively fine-tuned in the maintenance of normal cells, as perturbed upon LOH. We further confirmed a more prominent association of LOH and TSGs expression, as opposed to the whole transcriptome (Davoli et al., 2013), and showed that this is heavily contributed by CN-LOH events often undisclosed in genome-wide studies. In-depth analysis of LOH can shed the light on previously unexplored selective pressure mechanisms involving specific genes or pathways. Although we made the effort to study asCN genomic features and their functional impact by considering the specific contribution of diverse tumor types, our results are intrinsically limited by the modest frequencies of specific event subclasses in the study cohorts. Further, we omitted to consider additional type of events that could provide compensatory mechanisms in tumor evolution and disease progression (Persi et al., 2021).

Broadly, we showed that the detailed characterization of cancer genomic alterations benefits the study of oncogenic and tumor progression events while empowering cancer mechanistic investigations. To acknowledge the importance of reproducibility and easy access to asCN-based future studies, we implemented the study pipeline also using the common workflow language (CWL) and provide the full set of allele-specific informed genomic data for the studied cohort. Altogether, we envision that this orthogonal genomic feature will eventually allow the refined charting of tumor evolution paths, the detection of synthetic lethality combinations, and the accurate assessment of genomic biomarkers for patients’ enrollment in clinical trials.

**STAR★METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **METHOD DETAILS**
  - TCGA studies inclusion criteria
  - The allele-specific informed pipeline
  - Resources organization
  - Performance
  - Pipeline output tables
  - CWL implementation of the SPICE pipeline
  - Genotype based analyses, SPIA and EthSeq
  - Purity and ploidy correction of log2 ratios
  - Allele-specific ploidy (asP) and other indices
  - Allele-specific copy number and SNV analyses
  - Dimensionality reduction and clustering
SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cels.2021.10.001.

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AUTHOR CONTRIBUTIONS

Y.C., T.F., D.P., and F.D. conceived the study. Y.C., T.F., D.P., A.L., F.L., and P.G. designed and/or performed data analysis. G.M.F., M.B., O.E., L.L.F., and A.I. contributed to data discussion and interpretation. All authors participated in editing or reviewing of the manuscript, and all authors approved the submitted manuscript. F.D. supervised the work.

DECLARATION OF INTERESTS

The authors declare no competing interests. Co-authors D.P. and A.L. are currently at Fondazione Bruno Kessler, Trento, Italy and Biotechnology Research and Innovation Center, University of Copenhagen, Denmark.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Deposited data      |        |            |
| Allele-specific genomic data, SNVs, indels, association of expression with copy number and LOH | This paper | 10.5281/zenodo.5266542 |
| TCGA WES data       | Genomic Data Commons | dbGAP phs000178.v11.p8 |
| Recount2 data       | recount2 | TCGA |
| Microsatellite instability annotations | Bonneville R. et al., 2017 | https://doi.org/10.1200/PO.17.00073 (Data supplement 4) |
| Absolute ploidy and WGD calls | Pan-cancer atlas publications | TCGA_mastercalls.abs_tables_JSedit.fixed.txt |
| Aneuploidy score    | Taylor et al., 2018 | https://doi.org/10.1016/j.ccell.2018.03.007 (Table S2) |
| TCGA clinical information | Liu J. et al., 2018 | https://doi.org/10.1016/j.ccell.2018.02.052 (Table S1) |
| Functional annotations of mutations | Chakravarty D. et al., 2017 | Cancer genes and mutation functional annotations |
| Tumor suppressors and Cancer genes | Futreal P.A. et al., 2004 | https://doi.org/10.1038/nrc1299 (Table S1) |
| Tumor suppressors and Cancer genes | Zhao M. et al., 2016 | https://bioinfo.uth.edu/TSGene/ |
| Absolute ploidy and WGD calls | Pan-cancer atlas publications | TCGA_mastercalls.abs_tables_JSedit.fixed.txt |
| Aneuploidy score    | Taylor et al., 2018 | https://doi.org/10.1016/j.ccell.2018.03.007 (Table S2) |
| TCGA clinical information | Liu J. et al., 2018 | https://doi.org/10.1016/j.ccell.2018.02.052 (Table S1) |
| Functional annotations of mutations | Chakravarty D. et al., 2017 | Cancer genes and mutation functional annotations |
| Tumor suppressors and Cancer genes | Futreal P.A. et al., 2004 | https://doi.org/10.1038/nrc1299 (Table S1) |
| Tumor suppressors and Cancer genes | Zhao M. et al., 2016 | https://bioinfo.uth.edu/TSGene/ |

Software and algorithms

| Pipeline used to analyze the samples in this work (bash version) | This paper | https://doi.org/10.5281/zenodo.5266412 |
| Pipeline version based on CWL with containerized tools | This paper | https://doi.org/10.5281/zenodo.5266410 |
| PaCBAM | http://bcglab.cibio.unitt.in/PaCBAM | https://doi.org/10.1186/s12864-019-6386-6 |
| Picard | http://broadinstitute.github.io/picard/ | N/A |
| SPIA | https://cran.r-project.org/package=SPIAssay | https://doi.org/10.1093/nar/gkn089 |
| EthSEQ | https://cran.r-project.org/package=EthSeq | https://doi.org/10.1093/bioinformatics/btx165 |
| CNVkit | https://github.com/etal/cnvkit | https://doi.org/10.1371/journal.pcbi.1004873 |
| SLMSuite | https://sourceforge.net/projects/slmSuite/ | https://doi.org/10.1186/s12859-017-1734-5 |
| FACETS | https://github.com/mskcc/facets | https://doi.org/10.1093/nar/gkw520 |
| Mutect2 | https://gatk.broadinstitute.org/hc/en-us/articles/360037593851-Mutect2 | https://doi.org/10.1101/861054 |
| Variant Effect Predictor | https://www.ensembl.org/info/docs/tools/vep/index.html | https://doi.org/10.1186/s13059-016-0974-4 |
| CLONETv2 | https://cran.r-project.org/package=CLONETv2 | https://doi.org/10.1002/cpbi.81 |
| TPES | https://cran.r-project.org/package=TPES | https://doi.org/10.1093/bioinformatics/btz406 |
| GNU Parallel | https://www.gnu.org/software/parallel | http://doi.org/10.5281/zenodo.1146014 |
| Common Workflow Language | https://github.com/common-workflow-language/cwltool | https://doi.org/10.6084/m9.figshare.3115156.v2 |
| REVIGO | http://revigo.irb.hr/ | https://doi.org/10.1371/journal.pone.0021800 |
| clusterProfiler | https://doi.org/10.18129/B9.bioc.clusterProfiler | https://doi.org/10.1016/j.xinn.2021.100141 |
| UMAP | https://cran.r-project.org/package=uwot | https://arxiv.org/abs/1802.03426 |
| DBSCAN | https://cran.r-project.org/package=dbscan | https://dl.acm.org/doi/10.5555/3001460.3001507 |

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Yari Ciani (yari.ciani@unitn.it).
**Materials availability**
This study did not generate new materials.

**Data and code availability**
- This paper analyzes existing, publicly available data. Processed data have been deposited at 10.5281/zenodo.5266542 and are publicly available as of the date of publication. Accession numbers of analyzed data and DOI of processed data are listed in the key resources table.
- Original source code for data processing is publicly available; DOIs are listed in the key resources table. The code implemented to generate the figures is available from the lead contact upon request.
- Any additional information required to reproduce this work is available from the lead contact.

**METHOD DETAILS**

**TCGA studies inclusion criteria**
Via the GDC Data Transfer Tool Client provided by the Genomic Data Commons (GDC) (Grossman et al., 2016), all Whole Exome Sequencing (WES) BAM files of the TCGA collection available on January 2018 were downloaded (N BAM=22,196). Figure S2D reports inclusion criteria and relevant numbers of the current study. Briefly, samples for which either kit annotation was unavailable or multiple kits were specified were excluded from downstream processing (N samples excluded=2,885). All the possible pairs of normal-tumor samples were identified per patient (N pairs=10,581). Tumor-normal pairs were excluded if gender information was not available (gender required for log2 ratio inference) (N pairs excluded=75) or MuTect2 SNV calls from GDC were not available (N pairs excluded=1353). The SPIA genetic distance based tool (Demichelis et al., 2008) applied to verify the correct annotation of paired samples nominated 14 alleged pairs with incompatible genotypes (11 of which from the same tumor type study (DLBC)), which we excluded from subsequent analyses (N patients excluded=14). When more than one pair was available for the same patient, we included the one with the highest tumor purity. Only patients with a primary tumor available were retained (i.e. metastases or recurrent tumors were excluded). Last, studies with less than 60 patients, prior to ploidy and purity correction, were excluded. Based on the above requirements, a total number of 8,183 patients (i.e. normal-tumor pairs) across 27 tumor types was selected (see Table S1; Figure S2D). Further quality filters for allele-specific genomic analysis then nominated 4,950 pairs as adequate for downstream study investigations. (reliable copy number based clonality by CLONETv2: 4,950; reliable SNV based clonality by TPES: 4,299, of which 1,246, not included in the CLONETv2 set).

**The allele-specific informed pipeline**
The pipeline was designed to generate a comprehensive analysis of matched normal and tumor next-generation sequencing aligned data, including whole-exome, whole-genome, and targeted panels. The peculiarity of the pipeline is the heavy use of individual-specific germline information, from quality check steps to the assessment of somatic lesion clonality and allele-specific events. The pipeline named SPICE is composed of several modules each designed to handle a different part of the analysis (Figure S1A). Each module is self-contained and, by maintaining the input/output interface, can be replaced with custom modules. The pipeline is structured so that each normal/tumor pair is analyzed independently. All tools used in the pipeline modules have been previously published either by our group or by others and custom scripts were created for their integration. The bash version of the pipeline, including integration scripts, is available at https://github.com/demichelislab/SPICE-pipeline. A CWL version of the pipeline is available at https://github.com/demichelislab/SPICE-CWL-pipeline. A list of the pipeline modules with relevant references is available below and at https://github.com/demichelislab/SPICE-CWL-pipeline.

**Resources organization**

**Preparation:**
The pipeline configuration file includes the parameters needed to perform all analyses, such as the reference genome build, the dbSNP version, the identifier of the sequencing kit, the gender of the individual, and the BAM files paths. During this phase, the folder structure used by each tool during the analysis is created. After consistency checks related to the configuration setup, the pipeline verifies if the indices of the BAM files are present otherwise BAM indexing is run. The last step of the preparation phase is the computation of the SNP pileups (Valentini et al., 2019) confined to regions covered by the sequencing kit, as utilized multiple times throughout the pipeline.

**Quality Control (QC):**
As part of this module the following steps are run; collection of statistics of the sequencing data (picard HsMetrics, URL http://broadinstitute.github.io/picard/); inference of individual’s ethnicity (EthSEQ (Romanel et al., 2017)); normal-tumor match check by genetic distance based on a set of relevant SNPs (SPIA (Demichelis et al., 2008)). Number of SNPs used for each sample spans from 223 to 497, median 460.

**Segmentation:**
After the QC phase is completed the pipeline proceeds with the “Copy number segmentation phase,” where the copy number profile of the tumor is computed by CNVKit (Talevich et al., 2016) CBS segmentation that returns a log2 ratio of tumor against control for each
Segment; given the low computational cost (Figures S2A–S2C), two additional segmentation methods are run for ancillary analyses, i.e. CNVKit with SLM based segmentation (Orlandini et al., 2017) and FACETS (Shen and Seshan, 2016). By default, the CNVKit CBS-based segmentation is used; the user can select other segmentation outputs via a parameter (i.e. configuration file).

**Variant calling:**
In this phase, SNVs and indels are called using MuTect2 (https://doi.org/10.1101/861054) and annotated with Variant Effect Predictor (VEP) (McLaren et al., 2016). The information about the coverage of the SNV sites is integrated with the annotation produced by VEP.

**CLONETv2:**
As last phase, the pipeline runs tools to assess copy number data, allele-specific copy number data, and SNV features upon tumor ploidy and purity correction. First, the copy number based tool CLONETv2 (Prandi and Demichelis, 2019) is applied. CLONET corrects the effects that tumor ploidy and admixture have on the copy number of the tumor and determines the level of SCNA clonality. Second, an SNV based tool, TPES (Locallo et al., 2019), is applied to ensure tumor purity assessment of tumors with quiet genomes. Last, the pipeline combines the information about clonality that is generated by CLONETv2 with the SNVs to estimate the clonality of each SNV.

An option to compute the MuTect2 panel of normal is available and that is reported as phase “Other” in the figure.

**Performance**
The pipeline collects the runtime of each step. The execution time on a single core machine is moderate (Figure S2A) and allows to scale to large parallel machines. All tools were run on all the study samples, with the exception of MuTect2; in-house MuTect2 calls for 2,000 randomly selected patients were compared to those generated by the Genomic Data Commons (GDC), resulting in high concordance. The more time-consuming steps are those which process the entire BAMs namely PaCBAM (Valentini et al., 2019) (SNP pileup, SNV pileup), Picard HSMetrics, and CNVkit.

The median execution times of the entire pipeline for a tumor/normal pair with and without MuTect2 computations on a single core are ~21 hours and 5.5 hours, respectively. We analyzed the entire set of selected patients in ~20 days of computing time on 3 HPC machines with 40 cores (for a total of 120 cores) and 256 Gb of RAM each.

The pipeline analyzes each sample on a single core thus allowing the easy implementation of external parallelization strategies. The pipeline uses GNU parallel (DOI http://doi.org/10.5281/zenodo.1146014) as parallelization mechanism. CPU and memory usage of a test run on 158 normal/tumor pairs were assessed (Figures S2B and S2C). The test was conducted on a machine with 40 physical cores and 256 Gb of RAM. By default, each sample is configured so to use a single core in order to have a predictable behavior both in terms of CPU and memory usage. This single-threaded nature enables us to maximize the load on the machines as visible in Figure S2C. The pipeline peak memory usage was of ~130 GB. If we consider the memory usage per-core, the pipeline used less than 3.5GB throughout the whole execution. The entire per-core memory usage is shown as a gray line in the bottom part of Figure S2C.

**Pipeline output tables**
Table S21 lists the steps that are included in the bash version of the pipeline and the output files produced by each step. Figure S1 shows the dependencies between all the steps that are part of the pipeline using a flowchart.

**CWL implementation of the SPICE pipeline**
The SPICE pipeline is also available using the Common Workflow Language (Amstutz et al., 2016), a standard specification for the description of computational workflows that enables easily portable and scalable pipelines. Using one of the many available CWL implementations, it is possible to run SPICE on a variety of architectures (from single machines to clusters or cloud services) to easily scale up as needed. In order to enable ease of use and reproducible analyses, the tools that are used in the pipeline are ready on Docker Hub as containers. To run the pipeline, it is sufficient to create a single configuration file per tumor/normal pair, where the user provides the required options (e.g. BAM files, reference genome) (https://github.com/demichelislab/SPICE-CWL-pipeline).

**Genotype based analyses, SPIA and EthSeq**
A genotype-based tool (SPIA) and an ethnicity caller (EthSeq) were applied to all study samples (Demichelis et al., 2008) (Romanel et al., 2017). Briefly, SPIA measures the similarity between two samples using a set of high-MAF selected SNPs (N SNP median = 460; range: 223:497), whereby matched normal and tumor samples are expected to have high similarity. Figure S3A reports the results of the analysis on all possible pairings of the study samples. The vast majority of the samples were correctly paired with few exceptions (red dots), where samples annotated as related demonstrate high genotype distance (N=13) and samples annotated as unrelated demonstrate distances compatible with a match (N=15). Most of the unexpected results involve samples from the TCGA-DLBC (Diffuse Large B-cell Lymphoma) project (complete list of samples in Table S15). Those samples were excluded from the study (Figure S2). The total numbers of samples included in the test is 18,309 (i.e. 167,600,582, pairs tested) with number of pairs expected to match equal to 12,383 (504 SNPs used; Probability mismatch match=0.1; Probability mismatch non-match=0.6; Standard deviations match=2; Standard deviations mismatch=4; Similar maximum threshold=0.13; Different minimum threshold=0.50.

As somatic copy number aberrations might affect the genotype of variants within the genomic stretch, we plotted the genotype distance of matching pairs against the genomic burden; Figure S2B shows the relationship between genomic burden and the
Purity and ploidy correction of log2 ratios

Purity and ploidy estimation and log2 ratios correction have been performed using CLONETv2 (Prandi and Demichelis, 2019). Briefly, for each segment spanning a set of SNPs that are heterozygous in the individual under study (informative SNPs), a Beta value (i.e. an estimation of the fraction of reads equally representing the two parental alleles) is computed by comparing the observed distribution of the SNPs allelic fractions against a set of expected distributions that assume diploid genomes for non-transformed cells in the tumor sample. The tumor sample purity and ploidy are then inferred from the log2 ratios and the Beta values with error minimization approaches and segmented data are adjusted for tumor ploidy and purity. Finally, allele-specific copy number (asCN) are assigned to each segment. Complex and ambiguous samples have been manually inspected in the log2 ratio/Beta space and adjusted accordingly.

Allele-specific ploidy (asP) and other indices

Segmentation algorithms assign to each identified genomic segment s few values, including the log2 ratio (tumor over normal), \( rs \in \mathbb{R} \), and the segment coordinates, therefore the length \( ws \in \mathbb{N} \). The total copy number of \( s \) is defined as \( cn(s) = 2 \times 2^{\alpha} \); further, the asCN of the genomic segment \( s \) is represented as a pair of real values \((cn_A(s), cn_B(s))\), where \( cn(s) = (cn_A(s) + cn_B(s)) \), with \( cn_A(s) \geq cn_B(s) \) by definition. We here implemented a measure that is proportional to the average amount of DNA per cell. Given a genome \( G \) defined by a set of segments \( s \in G \), the allele-specific ploidy (asP) is defined as the weighted mean of the allele-specific copy number of the segments \( s \) in \( G \), that is

\[
\text{asP}(G) = \frac{\sum_{s \in G} (cn_A(s) + cn_B(s)) \times ws}{\sum_{s \in G} ws}
\]

Discretized asP identifies three classes: low asP when \( \text{asP}(G) < 1.5 \), for instance when at least half of the genome \( G \) retains only one allele; high asP when \( \text{asP}(G) \geq 2.5 \), for instance when half of the genome presents at least three copies; diploid otherwise. By definition, \( \text{asP}(G) \) range is \([0, \infty)\). A diploid cell without any CN aberration by definition has \( \text{asP} = 2 \).

Hereafter, we report the definition of six indexes related to the genomic status of tumor cells. In addition to allele-specific ploidy introduced in this study, the other indexes were introduced and/or used in recent landscape studies exploiting next-generation sequencing or high-density array data from human tumor samples. A set of examples to highlight the behavior of each measure in different genomic contexts is listed in Figure S4C. A direct comparison between asP and ABSOLUTE ploidy is shown in Figure S5. Despite the overall concordance between the measures, there is a fundamental difference in the calculation: whereby ABSOLUTE relies on modeling of karyotypes and, for ambiguous samples, it relies on the most common study cohort karyotypes, asP is instead calculated independently for each sample and the cohort composition does not contribute to the calculation.

1. **Median absolute deviation (MAD):** MAD has been calculated as in (Mouliere et al., 2018). The median absolute deviation (MAD) statistics quantifies the spread of a distribution. In genomics, MAD is conventionally the median absolute deviation from copy number neutrality, computed as \( \text{MAD}(G) = \text{median}(|rs - 0|) \). MAD has been used extensively to normalize and improve the quality of genotype calling in array data (Mouliere et al., 2018). MAD ranges in the interval \([0, \infty)\).

2. **Genomic burden (GB):** The genomic burden (GB) has been calculated as in (Beroukhim et al., 2010). It is a measure of the quietness of the genome; it is defined as the percentage of a genome \( G \) that is not wild-type (i.e., number of alleles different from two). GB is equal to 0 when no SCNA is detected and equal to 1 when no wild type genomic segment is present. Triploid and tetraploid cells have genomic burden equal to 1. The Genomic burden range is \([0,1]\).

3. **Whole genome doubling (WGD):** Whole genome doubling (WGD) is computed with ABSOLUTE (Carter et al., 2012) from Affymetrix SNP 6.0 array data of tumor samples (Carter et al., 2012). Briefly, ABSOLUTE estimates sample purity and ploidy from segmented copy number data and pre-computed models of cancer karyotypes. WGD assumes values \( 0, 1, \) and \( 2 \), corresponding to no duplication event, one duplication event, and more than one duplication event, respectively.

4. **Aneuploidy score (Taylor et al., 2018):** Of a tumor sample is defined as the number of chromosome arms with “large” somatic copy number alterations (SCNA). For each chromosome arm, the size of the SCNA is computed by first applying Gaussian mixture model to cluster SCNAS with similar length and genomic location. Then, three classes were identified based on the percentage of chromosome arm covered by the nominated SCNA cluster: more than 80% (value +1), less than the 20% (value 0), and intermediate length (no call). The Aneuploidy score is the sum of the arm level values returned by the described procedure. As not all chromosomal arms are typically sequenced, the Aneuploidy score range is \([0,39]\).
5. **Weighted genomic instability index (GII):** The genomic instability index GII (Chin et al., 2007) was originally defined for Affymetrix SNP 6.0 array assay as the percentage of SNPs within aberrant copy number segments. **Weighted GII** (wGII) (Burrell et al., 2013) improves over GII to account for different chromosome sizes: GII computed for each chromosome and wGII is the mean over the 22 chromosomes.

We define weighted ploidy as:

$$p_{lw}(G) = \frac{\sum_{s \in G} cn(s) \times ws}{\sum_{s \in G} ws}$$

Following (Bakhoum et al., 2018), we adapted wGII to whole exome sequencing data by:

- computing the weighted ploidy \(p_{lw}(A)\) for each chromosome arm \(A\)
- extracting the set of segments \(T \subseteq A\) such that \(t \in T\) iff \(|cn(t)| - p_{lw}(A)| > 0.5\)
- calculating the GII of chromosome arm \(A\) as the fraction of \(A\) that differs from \(p_{lw}(A)\) as

$$wGII_A = \frac{\sum_{s_\in A} wt}{\sum_{s_\in A} wa}$$

The wGII of a genome \(G\) is the mean \(GII_A\) for each chromosome arm \(A\) in genome \(G\). The wGII range is \([0,1]\).

6. **Microsatellite instability (MSI):** Microsatellites are short repeated DNA sequences. Microsatellite instability (MSI) implies a change in the length of the inherited microsatellites and it is usually associated to defects in the mismatch repair mechanism. The extent of MSI has been recently characterized in 39 TCGA cancer types (Bonneville et al., 2017). The MSI detection tool MANTIS (Kauko et al., 2017) distinguishes MSI positive (named MSI-high or MSI-H) from microsatellite stable tumor samples (MSI-stable or MSS).

In summary, \(asP\) is proportional to the total amount of DNA (e.g. equals to 2, 3, and 4 for diploid, triploid and tetraploid genomes, respectively), can measure the DNA quantity resulting from catastrophic events as chromothripsis, and reflects the difference between monoallelic gain or monoallelic loss of the same genomic fraction, as opposed to other genomic indexes (see Figure S4C).

### Allele-specific copy number and SNV analyses

Somatic copy number levels are conventionally grouped into five classes (Cerami et al., 2012; Gao et al., 2013), deep or homozygous deletions, shallow or hemizygous deletions, wild type, gain (3 and 4 copies), and amplification (5+ copies), based on log2 ratio values from tumor over matched normal signals. However, this abstraction masks relevant allele-specific copy number features as diverse combinations of allele counts result in the same group; for instance, for all autosomal chromosomes, both wild type copy number (one copy per allele; \(cnA=1, cnB=1\)) and copy-neutral loss of heterozygosity (one allele lost and one allele duplicated; \(cnA=2, cnB=0\)) result in the conventional wild type class. To solve these ambiguities and to study the landscape of allele-specific copy number and SNVs the “Transactivation Class” annotation of the IARC TP53 database (R20, (Bouaoun et al., 2016)) has been used. The current study uses TCGA WES data and does not utilize the matched SNP arrays. On one hand, this choice allows for the study expansion to other WES data cohorts and exploits the higher coverage with respect to WGS data. Allele-specific copy number and SNVs statuses of genes are annotated using the gene model reported in Table S17.

### Dimensionality reduction and clustering

Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction algorithm (McInnes and Healy, 2018) and fast Principal Component Analysis (fPCA) (Zheng et al., 2017) were applied on copy number allele-specific data to look for similarities within and across tumor types (Figure S9; Table S4). Genomic segments that lacked allele-specific cnA and cnB status (due to coverage and/or informative SNPs restrictions) were first assigned a proxy value via interpolation. Briefly, given a genomic segment \(g\) with undefined allele-specific copy number, we identified the nearest \(3’\) and \(5’\), \(g^a\) and \(g^b\), with defined allele-specific copy number and assigned to \(g\) the mean of the allele-specific copy number of \(g^a\) and \(g^b\), weighted by the length of \(g^a\) and \(g^b\). To remove the \(asP\) effect from UMAP analysis and to characterize the allele-specific profile of each tumor sample (Figures S9A and S9B), we first applied fPCA to allele-specific copy number data and then we applied UMAP to all but the first fPCA component allele-specific data. As input for
UMAP analysis we used continuous allele A and B (allele A and B corresponding to the allele present with more and less copies, respectively) copy values at gene level. Continuous values from bulk DNA analysis allow for subclonal events signal (together with uncertainty around the estimates). Finally, we identified clusters of tumor samples with similar allele-specific copy number profiles utilizing the Density-Based Spatial Clustering of Applications with Noise (DBSCAN) algorithm (Ester et al., 1996). DBSCAN groups together samples that are close (reachability distance and reachability minimum number of points, set to 0.24 and 15) in the UMAP space, while isolated samples result as outliers. Aberration enrichment analysis in DBSCAN clusters is performed with two tailed Fisher’s exact test.

To measure the stability of clusters, after dimensionality reduction (UMAP), we randomly subdivided diploid samples into ten groups. We then ran the clustering algorithm (DBSCAN) while excluding each group alternatively for a total of ten runs. As a measure of stability, we used the Adjusted Rand Index (ARI) (https://doi.org/10.2307/2284239), a rescaled version of the Rand Index. Figure S8 shows the Adjusted Rand Index computed on each subset of samples. To calculate the measure, we excluded, from the original clustering, the points that were removed in each fold. As clearly visible from the figure, the values are very high (median: 0.9895, SD: 8.9e-3), suggesting that our original clustering is indeed stable. Moreover, if we compare the original clustering to a version where the labels are randomly shuffled, we obtain very low ARI values (median: 0.02175, SD: 1.6e-3; Figure S8B).

**Association of LOH with gene expression**

The matched expression data was downloaded from recount2 project (Collado-Torres et al., 2017). Genomic and transcriptomic data were matched using the case_id provided by the NCI Genomic Data Commons (GDC) (Grossman et al., 2016) (Table S1). Recount2 expression data were normalized with function scale_counts of R package recount using default parameters. To estimate the impact of LOH on gene expression we built a linear model for each gene in each TCGA study \( \text{Exp} \sim \alpha \text{CN} + \beta \text{LOH} \), using the copy number (CN, defined as the sum of copies of alleles A and B) and the presence of LOH as variables. We retained genes for which the model returned \( \beta < 0 \) and statistical significance for the LOH variable (fdr <0.05). Coefficient of association for CN and LOH are calculated by dividing \( \beta \) by the standard error.

Only genes with mean expression \( \geq 20 \) and at least 10 events of LOH in each specific cancer type were tested. To calculate enrichment for LOH impact on expression in classes of genes (TSG, OG, ESSENTIAL and OTHER) we considered genes with \( \beta < 0 \) and significance for LOH variable (fdr < 0.05) in at least two tumor types and performed independence test (Chi-squared test).

In order to take into account expression due to non-cancer cells (1-purity), the ratio of aberrant asCN (Hemi del, CN-LOH, Gain-LOH and Amp-LOH) with respect to WT asCN, was calculated for each gene as follows:

\[
\frac{\text{median(\text{aberrant asCN})} - \text{median(Homo del)}}{\text{median(WT)}}
\]

Functional annotation analysis was performed using ClusterProfiler (Yu et al., 2012) (Table S13). To reduce the redundancy and improve visualization of GO biological terms, we clustered the significant ones (fdr<0.1) in at least two tumor types in the semantic space, based on the Resnick distance (arXiv:cmp-lg/9511007), using ReviGO (Supek et al., 2011). Only terms with dispensability < 0.2 are labelled with text in Figure S13B.

Synthetic data used in Figures 4A and S13A are generated as follows: for each asCN class we generated a vector of n=100 normally distributed random numbers. The mean of each distribution is defined based on the expected level for that asCN, with mean of WT = 1. For instance, for Hemi-del we expect half the expression in respect to WT, so mean of Hemi-del = 0.5. These data have been used exclusively to generate Figures 4A and S13A and have no impact on the calculation of the linear models or any other result.

**Gene signatures analysis**

The selection of the gene signatures was hypothesis-driven; gene signatures were obtained from the literature (Table S18). Each signature was tested in each study comparing high asP and diploid samples using Mann-Whitney test (p<0.05, one-sided based on biological hypothesis). Hierarchical clustering was performed using Pearson’s correlation as distance applying hierarchical clustering algorithm with complete linkage. In this context, focal copy number events were defined spanning genomic sizes shorter than 0.2 are labelled with text in Figure S13B.  

Synthetic data used in Figures 4A and S13A are generated as follows: for each asCN class we generated a vector of n=100 normally distributed random numbers. The mean of each distribution is defined based on the expected level for that asCN, with mean of WT = 1. For instance, for Hemi-del we expect half the expression in respect to WT, so mean of Hemi-del = 0.5. These data have been used exclusively to generate Figures 4A and S13A and have no impact on the calculation of the linear models or any other result.

**Tumor suppressor genes and Oncogenes lists**

Lists of TSGs and OG were obtained from Futreal and Zhao publications (Futreal et al., 2004; Zhao et al., 2016). For TSGs, only genes present in both lists were kept (Table S20).

**TP53 status analysis**

asCN calls of TP53 were obtained through the SPICE pipeline. Proportions of asCN and SNV states were calculated and plotted using the mosaic function from the vcd package (Meyer D, Zeileis A, Hornik K (2020). vcd: Visualizing Categorical Data. R package version 1.4-7) and graphically adapted for the figure.

**Survival Analysis**

Univariate and multivariate analysis were performed using the survival (http://CRAN.R-project.org/package=survival) and survminer (https://CRAN.R-project.org/package=survminer) packages for R. Proportional hazard regression models were calculated using type of tumor (study, reference=BRCA) and genes genomic status (reference as wt_0, 0=SNV absent, 1=SNV present) as predictor
variables and progression free interval (PFI) as response. For TSG and OG, for each gene, genomic status values were considered only if in the number of events is at least 10 in the cohort.

Significant variables (fdr<0.05) in the univariate analysis were used in the multivariate analysis. Forest plot, survival curves and Kaplan-Meier estimator were calculated and plotted using the `survminer` package.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Statistical tests applied throughout the study are specified in results, figure legends, and in the methods accordingly.
Supplemental information

Allele-specific genomic data elucidate the role of somatic gain and copy-number neutral loss of heterozygosity in cancer

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This manuscript contains the following supplemental materials:

**Supplemental Figures**
- Supplementary Fig. 1 | Flowchart of the pipeline. Related to Figure 1.
- Supplementary Fig. 2 | Performance analysis of the pipeline. Related to Figure 1.
- Supplementary Fig. 3 | Genetic distance and inference of ethnicity. Related to Figure 1.
- Supplementary Fig. 4 | Distribution of genomic instability measures. Related to Figure 1.
- Supplementary Fig. 5 | Comparison between asP and ABSOLUTE ploidy. Related to Figure 1.
- Supplementary Fig. 6 | Association with prognosis of genomic instability measures. Related to Figure 1.
- Supplementary Fig. 7 | Examples of asCN classification in two samples. Related to Figure 1.
- Supplementary Fig. 8 | Clusters stability. Related to Figure 1.
- Supplementary Fig. 9 | Allele-specific copy number data. Related to Figure 1.
- Supplementary Fig. 10 | Effect of concomitant SNV and loss of wt copy of TP53 on target genes. Related to Figure 2.
- Supplementary Fig. 11 | Loss of Heterozygosity burden across TCGA studies. Related to Figure 3.
- Supplementary Fig. 12 | LOH events across samples and tumor types. Related to Figure 3.
- Supplementary Fig. 13 | Loss of Heterozygosity. Related to Figure 4.
Supplementary Fig. 1 | Flowchart of the pipeline. Related to Figure 1. **A**, Overview of the tools included in the SPICE pipeline and dependencies between the tools. Color code denotes main analysis modules. Technical details at https://github.com/demichelislab/SPICE-pipeline-CWL. **B**, table showing references and links related to the tools used in the modules of the pipeline.
Supplementary Fig. 2 | Performance analysis of the pipeline. Related to Figure 1. A, Violin plot of the execution times of the SPICE pipeline colored by analysis module. B, Usage of the cores of the machine (40 cores) stratified by analysis step, including MuTect processing of reference model using panel of normal (N~200) (pink). C, Aggregate usage of memory of the machine stratified by analysis step (top). The gray line (magnified in the bottom part of the panel) reports the average usage of memory per core. D, The panel shows the selection steps performed. The top figure reports the total number of downloaded samples and number of samples excluded because of the WES kit (red). The bottom section of the figure refers to the number of sample pairs: left (green), number of pairs in the cohort at each step; central (red), number of excluded pairs; right, exclusion criteria.
Supplementary Fig. 3 | Genetic distance and inference of ethnicity. Related to Figure 1. **A**, Distribution of the SPIA genotype distance (STAR Methods) for all possible pairing of study samples stratified by reported annotation. Red dots are pairs whose distance deviates from the expected distance range, suggesting wrong matching between sample and patient in the annotation. In the right boxplot a subsample of 1 million points among of all non-matching (total: 167,588,199) is shown (n samples: 9,153). **B**, Effect of the copy number of the sample on the genotype distance between correctly paired tumor and normal samples (n: 4,950). **C**, Ethnicity of the whole cohort (EthSeQ, see STAR Methods) and stratified by tumor type (n: 8,183). **D**, Distribution of genotype distances of matching tumor-normal pairs stratified by ethnicity (n: 12,383). **E**, Median distances of non-matching pairs stratified by combinations of ethnicities (n: 167,588,199).
C

|                  | Moulere, et al., 2018 | Beroukhim, et al., 2010 | Carter, et al., 2012 | Taylor, et al., 2018 | Burrell, et al., 2013 | This study** |
|------------------|----------------------|-------------------------|---------------------|----------------------|-----------------------|--------------|
| MAD [0,∞]        | GB [0,1]             | WGD [0,1.2]             | AS [0..0.39]        | wGI [0,1]           | asP [0,∞]            |              |

**Genomic status of tumor cells**

|                  | Diploid | Triploid | Tetraploid | 1 copy loss of 20% of each chr | 1 copy gain of 20% of chr1 | 1 copy gain of 20% of each chr | 20 copies of chr1 | Gain-rich chromothripsis (10% of genome involved) |
|------------------|---------|----------|------------|--------------------------------|-----------------------------|-------------------|----------------|-----------------------------------------------|
|                  | 0       | 0        | 0.58       | 0                              | 0                           | 3                 | 0              | 0                              |
| Triploid         | 1       | 1        | 1          | 1                              | 1                           | 39                | 0              | 0                              |
| Tetraploid       | 1       | 1        | 1          | 1                              | 1                           | 39                | 0              | 0                              |
| 1 copy loss of 20% of each chr | 0*      | 0.2      | 0          | 0                              | 0                           | 0.2               | 1.8             |
| 1 copy gain of 20% of chr1 | 0       | ~0.017   | 0          | 0                              | 0                           | ~0.005            | ~2.02           |
| 1 copy gain of 20% of each chr | 0*      | 0.2      | 0          | 0                              | 0                           | 0.2               | 2.2             |
| 20 copies of chr1 | 0       | ~0.087   | 1          | 2                              | 0                           | 0                 | ~3.56           |
| Gain-rich chromothripsis (10% of genome involved) | 0       | 0.1      | 0          | 0                              | 0                           | ~0.1              | ~2.1            |

*MAD >0 only if gained portion is telomeric.
**Measures proportional to total DNA quantity, including Carter, et al., Carter, et al., Nat Biotechnol, 2012.
Supplementary Fig. 4 | Distribution of genomic instability measures. Related to Figure 1. A Sankey diagram linking the distribution of the raw log2 ratios with the purity and ploidy adjusted log2(tumor/normal) values for the study cohort. Percentages within the parallel sets plot provide relative fraction of genomic segments corresponding to discretized CN states. Peaks in the right-side distribution are annotated with corresponding CN state (far right column). Tumor ploidy and purity correction using de-facto standard thresholds (STAR Methods) led to the reclassification of 32% of the genomic segments, with significant increment of gains (from 18% to 28%, p-value<0.001, proportion test) and amplifications (from 3% to 9%, p-value<0.001, proportion test) (Table S2). Further, the number of homozygous deletions almost doubled upon data adjustment (from 4,241 to 9,031, p-value<0.001, proportion test), while a modest but significant reduction in the hemizygous deletions (from 14% to 13%, p-value<0.001, proportion test) was observed; vice-versa, 718 genomic segments previously marked as homozygous deletions (corresponding to 17% of the events) were re-classified as hemizygous deletion and 5,286 hemizygous deletions (corresponding to 7%) changed to homozygous deletions. B, Comparison of allele-specific ploidy (asP) with genomic MAD, AS and WGD. Each dot represents a sample color coded by ploidy status. C, Genomic indexes values for toy examples of a set of tumor genomic statuses. Each index has own peculiarities; for instance, GB, WGD, and AS don’t distinguish triploid from tetraploid and wGII, by definition, is insensitive to whole genome events. D, Density distribution of allele-specific ploidy. Vertical dashed lines indicate values of 1.5 and 2.5, delimiting low asP and high asP status, respectively. E, Distribution of different genomic instability measures in our cohort. From left to right: whole genome duplication (n=4,780); aneuploidy score (n=4,780); weighted Genomic Instability Index (n=4,950); microsatellite instability score (n=4,945) stratified in microsatellite stable (MSS) and MSI high (MSI-H) samples. Vertical dashed lines separate low from high scores.
Supplementary Fig. 5 | Comparison between asP and ABSOLUTE ploidy. Related to Figure 1.

A, Scatter plot of ploidy calls as reported by ABSOLUTE (y-axis) and by CLONETv2 (x-axis) (TCGA tumor data). There’s good concordance between the methods, differences arise mainly in samples that one of the two methods estimate as high ploidy. B and C, details of two high purity tumor cases where ABSOLUTE calls ploidy >2, whereas CLONET v2 calls ploidy <2 and D shows a case where CLONET v2 calls high asP whereas ABSOLUTE returns a ploidy of ~2. scatterplot showing adjusted log2 ratio and Beta values of segments: each point represents a segment (labelled segments are shown in panels F, G) Beta values represent the fraction of reads in a segment equally representing the two parental alleles. asP values are calculated exclusively from asCN values while ABSOLUTE ploidy measures are derived from karyotypes also informed using the most common cancer karyotypes in a study cohort.
Supplementary Fig. 6 | Association with prognosis of genomic instability measures. Related to Figure 1. A. Kaplan-Meier curves of overall survival or progression free (for ACC) probabilities for different genomic measures. P-value of log-rank test statistics are reported. (GB: genomic burden, WGD: whole genome doubling, AS: aneuploidy score, wGII: weighted genome instability index).
Supplementary Fig. 7 | Examples of asCN classification in two samples. Related to Figure 1. 
A, E Sankey diagram showing classification of segments based on discretized raw log2 (left), purity and ploidy adjusted log2 (middle), and allele-specific copy number (asCN, right). Detailed visualization of segments for the corresponding patients are shown in panels B, C, D, F, G and H. B, F scatterplot showing adjusted log2 and Beta space of segments: each point represents a segment, labelled segments are shown in panels C, D and G, H. Beta values are estimations of the fraction of reads equally representing the two parental alleles. C, G allelic fraction of informative SNPs on chr5 and chr17 in the normal sample (top panels) and tumor samples (bottom panels). D, H, distribution of allelic fractions in chr5 and chr17 in normal sample (top panels), and tumor samples (bottom panels) stratified by asCN (defined based on tumor sample).
Supplementary Fig. 8 | Clusters stability. Related to Figure 1. A. Visualization of the clusters found by DBSCAN in each fold; the points excluded from each fold are not shown. B. On the left: boxplot of the Adjusted Rand Index (ARI) computed on each of the folds; on the right: boxplot of the ARI computed on 10 random shuffles of the cluster labels.
Supplementary Fig. 9 | Allele-specific copy number data. Related to Figure 1. A, scatter plot of the first component of the PCA of the asCN matrix of all the samples against the asP. This demonstrate that the first component of the PCA is highly related to PCA (p-value of r-squared test). B, UMAP run on the complete dataset annotated by asP. It is clear that the three groups of points are induced by the difference in terms of asP C, UMAP non-linear dimensionality reduction of the gene level allele-specific CN data run on the PCA of the data where the first component (related to asP) was removed. Samples are color coded by tumor type. Boundaries of clusters identified by DBSCAN are shown. All genes are used and interpolation using flanking segments (weights based on log2 values similarities) is applied as necessary. D, Pie chart matrix of main tumor aberrations (rows) in panel D DBSCAN clusters (columns). Each pie reports the number of aberrant samples and the proportions per tumor type. The triangle indicates that two or more tumor types are enriched for the aberration in the cluster. Rows are annotated with the tumor type distribution of each aberration. Columns are annotated with the tumor type distribution in each DBSCAN cluster. Only data for clusters with at least one enriched aberration are shown.
Supplementary Fig. 10 | Effect of concomitant SNV and loss of wt copy of TP53 on target genes. Related to Figure 2. A, Dendrogram and corresponding heatmap of significance (-log(P-value)) of differential expression of proliferative signatures (left) and proteasome signatures (right) when comparing high asP against diploid samples in tumor type. Data is clustered in two groups: cluster #1, characterized by the activation of proliferative signatures in high asP samples; and cluster #2 by repression of proliferation in high asP samples. Five tumor types excluded due to the low number or absence of high asP samples. Activation of the proteasome signature is observed almost exclusively in high asP samples of tumor types included in cluster 1, while inhibition of the proteasome (Levin et al., 2018; Wang et al., 2017) was mainly associated to cluster 2. B, Fraction of samples in the two clusters, stratified by asCN and concomitant presence of TP53 SNV and wild-type copy number. We observe significant enrichment for TP53 SNVs in cluster 1 (Chi-squared test, fdr=3.95e-05). Cluster 1 is also enriched for TP53 LOH events with respect to cluster 2 (top panel, 59% and 47%, respectively, Chi-squared test, p=1.33e-12), and number of samples with SNVs and concomitant loss of wild-type TP53 (mut/mut) (bottom panel, 92% and 86%, respectively, Chi-squared test, p<0.01). We also detected enrichment of copy gain focal events in cluster 1 high asP samples (Table S19). C, Expression levels of the proteasome signature and E2F7 stratified by cluster, TP53 status, and ploidy. D, Expression level of TP53 in samples with TP53 SNV, stratified by the presence of a wt TP53 copy (E, F and G). Expression levels of E2F1, KI67 and CDK6 in samples with TP53 SNV stratified by cluster, wt allele presence, and asP. These analyses include all deleterious mutations.
Supplementary Fig. 11 | Loss of Heterozygosity burden across TCGA studies. Related to Figure 3.

A, Boxplot of copy number neutral loss (CN-LOH) burden against ploidy status. Significant levels of Wilcoxon rank-sum test are reported (*** indicate p-val < 0.001, ns = non-significant). B, Percentage of TCGA samples (y-axis) with CN-LOH ≥ x, for each value x of the CN-LOH burden (x-axis), stratified by ploidy status. C, For each TCGA study, the plot reports the distribution of CN-LOH burden on a sample basis. Samples from the same TCGA study are stratified by ploidy status. Statistics are reported in Table S9. D, Boxplot of the sum of copy gain loss of heterozygosity (Gain-LOH) and copy amplification loss of
heterozygosity (Amp-LOH) burden against ploidy status. Significant levels of Wilcoxon rank-sum test are reported (*** indicate p-val < 0.001, ns = non-significant). E, Dot plots and dendrogram reporting the fractions of samples showing LOH burden in each interval.
Supplementary Fig. 12 | LOH events across samples and tumor types. Related to Figure 3. A. Visualization of the number of genes that have undergone loss of heterozygosity (either hemizygous deletion or copy neutral LOH) within the TCGA cohort ordered by the total number of aberrations in each
sample. Under the barplot are reported the annotations from the TCGA project and the ploidy status for each sample. The inset compares the number genes with a hemizygous deletion (on the x axis) to the number of genes with CN-LOH (on the y axis). The plot shows that samples have either a high number of hemizygous deletion or a high number of CN-LOH. As evident from the colors of the points this phenomenon is related to the asP of the sample. B. Number of aberrations in essential genes (tot: 1478) stratified by aberration class and TCGA study. The black line in each group of points represents the median of the group.
Supplementary Fig. 13 | Loss of heterozygosity events and their impact on gene expression. Related to Figure 4. A, synthetic data showing the expression levels, stratified by asCN, of a gene for which LOH is not associated on expression and CN is positively associated to expression. B, semantic clustering of GO terms associated to genes with LOH status negatively associated to expression. The term “ncRNA metabolic process” is enriched in 6 studies, suggesting a shared mechanism linked to regulation through ncRNAs that is fine-tuned by the number of alleles. C, dotmap showing TSG genes with decrease of gene expression upon LOH in each TCGA study. Empty dots indicate “no significance” while the absence of a dot indicates that the test has not been performed because of the low number of events (<10). D, E, F, G, examples showing the level of expression of TSG genes stratified by asCN. Width of boxplots is proportional to the number of events. All p-values of Mann-Whitney tests.