Tumor copy number alteration burden is a pan-cancer prognostic factor associated with recurrence and death

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The level of copy number alteration (CNA), termed CNA burden, in the tumor genome is associated with recurrence of primary prostate cancer. Whether CNA burden is associated with prostate cancer survival or outcomes in other cancers is unknown. We analyzed the CNA landscape of conservatively treated prostate cancer in a biopsy and transurethral resection cohort, reflecting an increasingly common treatment approach. We find that CNA burden is prognostic for cancer-specific death, independent of standard clinical prognostic factors. More broadly, we find CNA burden is significantly associated with disease-free and overall survival in primary breast, endometrial, renal clear cell, thyroid, and colorectal cancer in TCGA cohorts. To assess clinical applicability, we validated these findings in an independent pan-cancer cohort of patients whose tumors were sequenced using a clinically-certified next generation sequencing assay (MSK-IMPACT), where prognostic value varied based on cancer type. This prognostic association was affected by incorporating tumor purity in some cohorts. Overall, CNA burden of primary and metastatic tumors is a prognostic factor, potentially modulated by sample purity and measurable by current clinical sequencing.

Significance Statement:

Tumor genomes often show alterations in copy number throughout their genomes, with the percentage of the genome altered termed copy number alteration (CNA) burden. As clinical genomic analysis of tumors and tumor biopsies becomes widespread, there is a growing need to understand the prognostic factors captured by genomic features including CNA. The present work finds that increased CNA burden in tumors is correlated with increased mortality. In prostate cancer, the most common malignancy in men, CNA burden is associated with cancer-specific death in a cohort treated conservatively, an increasingly common treatment approach. Broadening these findings to other cancer types, we find CNA burden is a prognostic factor in breast, endometrial, renal clear cell, thyroid, and colorectal cancer. Notably, CNA burden is prognostic for death in independent cancer cohorts using a clinically approved sequencing assay, demonstrating the potential for incorporating CNA burden assessment into clinical prognosis.

Introduction

Somatic copy number alterations (CNAs) are nearly ubiquitous in cancer (1, 2) and alter a greater portion of the cancer genome than any other type of somatic genetic alteration (2). Different cancer types vary in their balance of copy number alterations to somatic point mutations, with prostate cancer having relatively high rates of CNA compared to point mutation. Given the prevalence of CNAs in cancer, significant effort has been directed towards identifying specific CNAs associated with cancer clinical characteristics and prognosis as well as the potential driver genes they contain (3-5). There are well demonstrated associations between specific CNAs and CNA signatures to cancer state and characteristics (6-8). CNV patterns or clusters have been associated with high Gleason prostate cancer (Gleason 8+ compared to Gleason 6-7 (7)) and recurrent disease (compared to primary (6, 9, 10)). Nonetheless, most CNAs are large, (1, 11) and their associations with cancer outcome may not be well identified by gene-specific approaches. Increasing evidence indicates that large CNAs harbor multiple drivers (12, 13), emphasizing the need to study their biological and clinical significance beyond individual gene-focused standpoints.

The CNA burden of a tumor is the degree to which a tumor's genome is altered as a percentage of genome length and represents a fundamental measure of genome copy number alteration level. As such, tumor CNA burden, rather than individual CNAs, may be associated with cancer outcomes. While tumor mutational burden (TMB) predicts response to immunotherapy across multiple cancer types (14, 15), tumor CNA burden may be prognostic for outcomes such as recurrence and survival. Indeed, we and others have previously found CNA burden and genome-wide CNA patterns to be associated with biochemical recurrence and metastasis in primary prostate cancer, the most common cancer in men, across multiple cohorts (8, 16, 17). This prognostic significance of tumor CNA burden extends to low and intermediate risk prostate cancer (Gleason scores of 7 and less) (16) and has the potential to better stratify risk in patients who are considering conservative treatment approaches such as active surveillance to reduce overtreatment (18, 19).

In addition to questions about the prognostic potential and overall landscape of CNA in conservatively treated prostate cancer, it is unknown whether CNA burden is prognostic for prostate cancer survival, rather than only recurrence and metastasis. Nor is it known whether the prognostic significance of tumor CNA burden extends to other cancer types. Here we set out to address these questions, as well as whether tumor CNA burden can be prognostic in a clinical practice setting, including (i) in cancers treated conservatively rather than through immediate surgery or radiation, (ii) in biopsy or resection samples, and (iii) using a clinical targeted sequencing that allows rapid and cost-effective measurement of tumor CNA burden.

To address these questions, we first examine the genomic CNA landscape of conservatively treated prostate cancer in more than a hundred diagnostic biopsy and resection specimens from a conservatively treated cohort; this cohort consisted of patients with localized prostate who were not treated with surgery or radiation within six months of diagnosis. We demonstrate that tumor CNA burden is associated with cancer-specific death, independent of standard clinical predictors. To explore the prognostic significance of tumor CNA burden more broadly in other cancer types, we find that tumor CNA burden is also associated with disease-free and overall survival in TCGA cohorts of primary breast, endometrial, renal clear cell, thyroid, and colorectal cancer in addition to prostate cancer, with the degree of association varying in some cancer types. We then establish the clinical feasibility of measuring tumor CNA burden using the FDA-cleared MSK-IMPACT clinical next generation sequencing (NGS) assay and confirm that tumor CNA burden is associated with overall and disease-specific survival in both primary and metastatic tumors across cancer types. In all, we demonstrate that tumor CNA burden is a prognostic factor associated with cancer recurrence and death in multiple cancer types, including in conservatively treated prostate cancer which would benefit from increased risk stratification.

Results

The genomic copy number landscape of conservatively treated prostate cancer

To explore the genomic copy number landscape of conservatively treated prostate cancer, we set out to analyze copy number alteration (CNA) in cancer obtained non-surgically through biopsy and transurethral prostate resection (TURP) using a widely studied, conservatively treated primary prostate cancer cohort (20). This retrospective Transatlantic Prostate Group 1 (TAPG1) cohort (n=1675) consists of men below age 76 with clinically localized prostate cancer and prostate-specific antigen (PSA) below 100 ng/ml who did not receive surgery or radiation within 6 months of diagnosis (20). This population-based cohort was drawn from six cancer registries in Great Britain, and the majority of the cohort was followed without treatment, while a subset received hormonal therapy. The original diagnostic samples, either biopsy or TURP, were obtained and centrally reviewed to obtain consistent pathological evaluation to the current standards. Drawing from this cohort, we carried out genome-wide CNA analysis by array-based comparative genomic hybridization (aCGH) of 107 biopsies or TURP samples from the TAPG1 cohort, as tissue availability is limited for much of the full cohort. The subset of cases used for CNA analysis, which make up our conservative treatment CNA cohort, have similar clinical characteristics to the full TAPG1 cohort, including median diagnosis age, baseline PSA, hormonal treatment, and clinical stage, with the exception of higher Gleason score distribution, likely due to selection for cases with sufficient DNA for analysis (Suppl. Table 1). As expected for a cohort not subject to PSA screening, the patients are older and have higher grade at diagnosis than is typical for contemporary US cohorts. Among the cohort, 47 patients developed metastasis and 43 died of prostate cancer. The median follow-up time for survivors was 10.3 years from diagnosis.

The copy number alteration landscape of the conservative treatment cohort revealed canonical copy number alterations of prostate cancer, including gain of chromosome 8q and losses on chromosomes 6p, 8p, 13q and 16p, though with lower frequency than seen in prostate cancer cohorts analyzed by our group (MSKCC cohort) (8) and TCGA (9) (Figure 1a). The percentage of the cancer genome showing copy number changes, termed tumor CNA burden (TCB), is similar between the conservative treatment

CNA cohort and other cohorts (Figure 1b), with a mean tumor CNA burden of 5.7% (median 1.5%, IQR 0.05%-8.5%) compared to 5.2% (median 3.0%, IQR 0.04%-6.9%) for the 2010 MSKCC primary prostate cancer cohort (8) and 4.0% (median 0.7%, IQR 0.08%-5.1%) for the 2014 MSKCC primary prostate cancer cohort (16). The tumor CNA burden of the conservative treatment CNA cohort is, however, somewhat lower than the 8.7% average tumor CNA burden of the TCGA prostate cohort (9) (mean 8.7%, median 6.2%, IQR 1.7%-11.9%).

Tumor CNA burden is prognostic for prostate cancer-specific death

Since tumor CNA burden is associated with prostate cancer recurrence and metastasis in prostatectomy cohorts (8, 16), we sought to determine whether tumor CNA burden was prognostic for cancer-specific death in biopsies of conservatively treated prostate cancer. In our conservative treatment CNA cohort, we find that tumor CNA burden as a continuous variable is significantly associated with prostate cancer-specific death (per 5% tumor CNA burden, HR 1.49; 95% CI 1.30, 1.70; $p < 0.0001$; Table 1). Greater tumor CNA burden correlates with an increase in death from disease compared to a lower tumor CNA burden (Figure 2a). The risk of death due to prostate cancer within 5 years of diagnosis increases with tumor CNA burden over the majority of the tumor CNA burden distribution (Figure 2b). For example, the 5-year risk of death due to prostate cancer would be 13% for patients with a 2% tumor CNA burden and 28% for patients with a 10% tumor CNA burden (Figure 2b). Tumor CNA burden may therefore serve as a prognostic factor for cancer-specific death in patients who undergo increasingly common conservative treatment approaches.

We next asked whether tumor CNA burden was associated with outcome after adjusting for established prognostic variables, including Gleason sum score and the UCSF Cancer of the Prostate Risk Assessment (CAPRA) score (21, 22) which combines PSA, Gleason score, percentage positive biopsy cores, clinical stage, and age (Figure 2c). Tumor CNA burden is significantly associated with cancer-specific death even after adjusting for biopsy Gleason score (per 5% tumor CNA burden, HR 1.44; 95% CI 1.24, 1.67; $p < 0.0001$) or CAPRA score (per 5% tumor CNA burden, HR 1.44; 95% CI 1.24, 1.68; p

<0.0001) (Table 1, Figure 2c). The addition of tumor CNA burden into the model with the CAPRA score increased Harrell's concordance index from 0.756 to 0.805 for cancer-specific survival in our cohort of men with conservatively treated prostate cancer.

Tumor CNA burden is prognostic for cancer-free and overall survival in multiple cancer types

Large, clinically annotated cancer genomic efforts such as TCGA now provide an opportunity to examine whether CNA burden is prognostic for primary cancer outcomes across many cancer types. In the TCGA primary prostate cancer cohort (9), tumor CNA burden is significantly associated with biochemical recurrence individually ($p < 0.0001$; per 5% tumor CNA burden, HR = 1.27; 95% CI, 1.13, 1.42) and after adjustment for Gleason score and mutation burden ($p = 0.015$; per 5% tumor CNA burden, HR = 1.18; 95% CI, 1.03, 1.35), validating our findings from other prostate cancer cohorts (Figure 2c, Table 2, Suppl. Fig. 1). There were insufficient deaths in this cohort to analyze survival. CNA burden was still significantly associated with biochemical recurrence after adjusting for tumor sample purity determined by ABSOLUTE ($p < 0.003$; per 5% CNA burden, HR = 1.22; 95% CI, 1.07, 1.40; Table 2). Since tumor CNA burden could potentially reflect simply the prognostic significance of aneuploidy as determined by cytometric DNA index in various cancers (23, 24), we examined the tumor CNA burden in a multivariable model together with ploidy. Ploidy, generated by CLONET and previously published for this cohort, estimates the average DNA index of the tumor cells (25, 26). Tumor CNA burden was associated with recurrence independent of tumor ploidy ($p = 0.002$; per 5% tumor CNA burden, HR = 1.32; 95% CI 1.11, 1.56; Table 2). Moreover, for a multivariable model that includes tumor CNA burden, Gleason grade, and mutation burden, the Harrell's C-index is 0.691. In contrast, the C-index for a model including ploidy instead of tumor CNA burden is only 0.606, indicating that a model with clinical factors and ploidy does not perform as well as a model with the same clinical factors and tumor CNA burden.

The prognostic significance of tumor CNA burden in prostate cancer led us to ask whether tumor CNA burden is prognostic in other cancer types. Towards this end, we examined published TCGA

cohorts for multiple cancer types with available disease-free survival and overall survival data, including breast (27), endometrial (28), renal clear cell (29), thyroid (30), and colorectal (31) cancers. We found that tumor CNA burden is associated with recurrence (disease-free survival) in these cancer types (Figure 2c, Table 2, Suppl. Fig. 2). This association between tumor CNA burden and lower disease-free survival was independent of disease stage in all cancer types except colorectal cancer, where the association was independent of tumor stage but not disease stage (Table 2). In addition to lower disease-free survival, higher tumor CNA burden was also significantly associated with lower overall survival in breast, endometrial, thyroid, and colorectal cancer (Table 2). This association with overall survival was independent of disease stage in breast and endometrial cancer and independent of tumor stage in colorectal cancer (Table 2). There were insufficient cases of thyroid cancer with stage data for this analysis. In summary, tumor CNA burden is prognostic for recurrence and/or overall survival in multiple cancer types beyond prostate cancer, including breast, endometrial, colorectal, renal clear cell, and thyroid cancer.

Tumor CNA burden determined by clinical targeted sequencing of primary and metastatic tumors is prognostic for survival

We next wanted to determine whether CNA burden's prognostic associations could be observed using panel-based targeted sequencing assays that are increasingly entering clinical use, in contrast to CGH array-based determination of tumor CNA burden. The Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay is a clinical laboratory improvement amendments (CLIA)-certified sequencing-based assay (32) of several hundred cancer genes and 1042 common single nucleotide polymorphisms (SNPs) that has been used to profile 504 prostate tumors (33) and more than ten thousand tumors across other cancer types (34). The IMPACT assay identifies both somatic point mutations and copy number alterations in the genes included in the panel. Overall copy number burden is calculated across the whole genome (Figure 1a) using segmentation derived from a combination of the profiled SNPs to provide low resolution copy number data and the

genes sequenced in the panel (32-34). To address the possibility that CNA burden from the IMPACT panel might differ from that derived from more comprehensive sequencing, we directly compared CNA burden calculations from 1005 tumors that were profiled using both IMPACT and whole exome sequencing. CNA burden determined by the two methods were highly correlated (p-value < 0.0001, rho = 0.88, n = 1005), indicating that CNA burden is not significantly affected by the reduced resolution in moving from whole exome to targeted panel sequencing (Suppl. Fig. 3).

We find that tumor CNA burden assayed by targeted clinical sequencing is significantly associated with overall survival in primary prostate tumors (per 5% tumor CNA burden, HR = 1.17; 95% CI, 1.04, 1.3; p = 0.007; Table 3, Figure 2c, Suppl. Fig. 4) in the IMPACT prostate cohort (33). As clinical sequencing assays such as MSK-IMPACT are principally used in the metastatic patient population, the IMPACT cohorts also provide an opportunity to investigate the prognostic significance of tumor CNA burden in late stage disease. We find that tumor CNA burden of metastatic prostate tumors assayed by clinical sequencing is also significantly associated with survival (per 5% tumor CNA burden, HR = 1.07; 95% CI, 1.01, 1.14; p = 0.020; Table 3, Figure 2c, Suppl. Fig. 4).

Since clinical sequencing assays also provide point mutation information for several hundred cancer genes, we asked if tumor CNA burden is prognostic after adjusting for known prostate cancer driver alterations. In separate multivariable regression models adjusting for *TP53*, *RB1*, or *PTEN* loss and/or mutation, tumor CNA burden is still associated with overall survival independent of these alterations in primary prostate tumors (Table 3). In metastatic tumors, these specific gene mutations do not reach prognostic significance when combined with tumor CNA burden (Table 3). Notably, tumor CNA burden remains significant in metastatic tumors after adjusting for overall tumor mutation burden (per 5% tumor CNA burden, HR = 1.08; 95% CI = 1.02, 1.15; p = 0.011; Table 3).

As targeted clinical sequencing is applied to a wide range of cancer types, we expanded our survival analysis to a pan-cancer cohort, consisting of 6610 primary tumors and 4864 metastatic tumors across 53 cancer types assayed by MSK-IMPACT sequencing panel (Methods and Suppl. Table 2). We find that tumor CNA burden is prognostic for overall survival pan-cancer in primary tumors (p < 0.0001;

per 5% tumor CNA burden, HR = 1.04; 95% CI, 1.02, 1.05) and in metastatic tumors (p = 0.005; per 5% tumor CNA burden, HR = 1.02; 95% CI, 1.01, 1.03) in a univariate analysis of these pan-cancer cohorts (Table 3, Figure 2c). Tumor CNA burden is also prognostic for cancer-specific death in the metastatic tumor cohort (p = 0.026; per 5% tumor CNA burden, HR = 1.05; 95% CI, 1.01, 1.10). Adjustment for sample tumor purity determined by FACETS (35) found that CNA burden was still significantly associated with overall survival in primary tumors in the pan-cancer analysis approached significance for metastatic tumors (p = 0.06; Suppl. Table 3), though purity-adjusted CNA burden was no longer significantly associated with overall survival in the prostate tumor subsets (Suppl. Table 3). Tumor mutation burden (TMB), in contrast to tumor CNA burden, was not associated with overall survival or cancer-specific survival (p = 0.4 and p > 0.9, respectively; Table 3).

Since the pan-cancer prognostic significance of tumor CNA burden is likely to be influenced by the distribution of cancer types within the IMPACT cohorts, we stratified the primary and metastatic pan-cancer IMPACT cohorts by their ten most prevalent cancer types, which make up nearly three-quarters of the cohort (Suppl. Table 2). A multivariable Cox model was used for each cancer type to adjust for mutation burden and extract the effect size, which was then entered into a meta-analysis. After stratifying by cancer type, the CNA burden of primary tumors measured by the MSK-IMPACT assay is still significantly associated with death (overall fixed effects HR = 1.04; 95% CI 1.02, 1.05; test of effects size p <0.0001; Table 3; Figure 2c). Similarly, metastatic tumor CNA burden was associated with death (overall fixed effects HR = 1.02; 95% CI 1.01, 1.04; test of effects size p = 0.005; Table 3; Figure 2c).

A closer look at the pan-cancer analysis reveals statistically significant heterogeneity in the relationship between tumor CNA burden and survival across tumor types (p = 0.003 and p = 0.024 in primary and metastatic tumor cohorts respectively, Suppl. Fig 4). In primary tumors, heterogeneity appears to be driven by colorectal and pancreatic cancers, where an inverse association between tumor CNA burden and death is seen (Suppl. Fig. 5a). After excluding colorectal and pancreatic cancers, heterogeneity is no longer statistically significant (overall fixed effects HR = 1.05; 95% CI 1.03, 1.07; test of effects size p <0.0001; test for heterogeneity p = 0.3; Suppl. Fig. 5a). In metastatic tumors, two

outlying cancer types drive this heterogeneity: pancreatic cancer, which shows the same inverse association of tumor CNA burden with death as in primary pancreatic tumors, and prostate, which shows the opposite effect (Suppl. Fig. 5b). Exclusion of either cancer type eliminates the significant heterogeneity in effects size, such that higher tumor CNA burden is associated with increased death in the remaining homogenous set of cancer types (overall fixed effects HR = 1.03; 95% CI 1.01, 1.04; test of effects size p = 0.002; test for heterogeneity p = 0.8, Suppl. Fig. 5b). These results indicate that tumor CNA burden can have differing levels of prognostic effect depending on the cancer type, while a core set of cancer types show a statistically similar association between overall survival and tumor CNA burden assayed by targeted sequencing. More generally, we find that tumor CNA burden determined by a clinically-certified sequencing panel is associated with overall and disease-specific mortality in a large multi-cancer population, including in patients with metastatic cancer where clinical sequencing is increasingly applied.

Discussion

Many specific genes altered by CNA have been associated with cancer outcomes (3-5), however the relationship between outcome and the overall level of CNA harbored by a tumor is less well studied. Here we expanded on our previous work showing that tumor CNA burden is associated with recurrence in surgically treated primary prostate cancer (8, 16) by showing a significant association with death from prostate cancer, including in conservatively treated patients where the tumor CNA burden measurement was made from biopsies. Importantly, this association remains significant even after adjusting for Gleason score or for CAPRA score, demonstrating that CNA burden is independent of previously identified associations with these measures of cancer pathology or disease state. Thus, tumor CNA burden assessment from prostate biopsies could have a role in deciding between surgery and surveillance

for men at the low end of intermediate risk. Conversely, it may also have role in men at high risk where multimodal treatment may be needed.

An unanticipated outcome of our analysis beyond prostate cancer is the prognostic role of tumor CNA burden across a range of tumor types. The pan-cancer tumor CNA burden association is significant but also heterogeneous depending on cancer type. Recent work has similarly found that the presence of any CNA, regardless of gene identity, is associated with overall and event-free survival in pediatric AML (36) and that the percentage of subclonal CNAs but not subclonal somatic point mutations is associated with overall survival in non-small cell lung cancer (37). Moreover, survival time was associated with a CNA signature derived from supervised analyses in prostate cancer and extended to breast and lung cancer (38). Prognostic individual CNAs or sets of CNAs, as opposed to the broader measure of genome-wide CNA level examined here may be specific to individual cancer types, whereas we have demonstrated the prognostic potential of a generalized measure of overall copy number dysregulation. Further work will be needed to address the trade-offs between generalizability of CNA burden and discriminatory power. In addition, it will be important to investigate whether the prognostic associations of CNA burden from the pan-cancer analysis are independent of known cancer- or subtype-specific prognostic factors, such as ER receptor status in breast cancer, ultra- and hypermutated (POLE and MSI+) status in endometrial cancer and MSI-positive/CIN-negative status in colorectal cancer (23).

We find it notable that tumor CNA burden assessment using a targeted sequencing can serve as a surrogate for tumor CNA burden calculated using more comprehensive genomic assays such as array CGH. With the proliferation of different clinical sequencing panels for mutation detection, it will be of interest to see how much resolution, depth, and coverage can be reduced and still retain the prognostic association of CNA burden; future work in this area will also need to incorporate the predictive clinical utility of the point mutation data to address the multimodal uses of clinical sequencing assays. Another important variable is tumor purity. The prognostic significance of CNA burden can be affected by sample tumor purity, with purity being independently associated with outcome. The effect of purity on the

association between CNA burden and outcome appears complex and may be influenced by the analysis platform, cancer type, and outcome type. For example, pan-cancer CNA burden from clinical sequencing panel remained prognostic for survival after purity adjustment in primary tumors and was just below significance for metastatic tumors, though the CNA burden of the prostate tumor subset assayed by IMPACT sequencing panel did not. However, the CNA burden of prostate tumors assayed by SNP array showed continued association with recurrence after adjustment for purity. Tumor purity alone was also independently associated with survival, revealing a complex interaction between these tumor features that will need further exploration. As targeted sequencing moves from tumor samples to liquid biopsy in the form of cell-free DNA (cfDNA) (2, 39, 40), it will be important to determine whether tumor CNA burden determined by analysis of cfDNA has similar prognostic utility as that determined by direct analysis of tumor DNA. There is already some evidence this may be possible, as the summed CNA level of the most highly copy number altered genes assayed from whole genome sequencing of cfDNA in twenty metastatic prostate cancer patients correlated with overall survival (39). As sequencing costs continue to drop and computational power improves, it would be interesting to investigate low pass whole genome sequencing as an alternative approach for determining tumor CNA burden that provides complete genome coverage.

Another interesting feature of the association of tumor CNA burden with outcome demonstrated here is that it has prognostic significance independent of tumor mutation burden (TMB). This is consistent with recent work in glioblastoma, breast, lung, and ovarian cancer showing that CNA-derived signatures have more prognostic power than somatic point mutation-based signatures, as measured by concordance index (41). Thus, tumor CNA burden could complement clinical analyses of actionable driver mutations using a single panel-based sequencing assay.

The prognostic significance of tumor CNA burden raises intriguing questions regarding the underlying biology. Tumor CNA burden may be a simple measure that correlates with the extent of oncogenic driver alterations. Yet, we show that tumor CNA burden retains its prognostic significance after adjustment for a number of known oncogenic alterations in primary prostate cancer, including *PTEN*

loss associated with increased tumor CNA burden (7, 42). In metastatic tumors, combining tumor CNA burden with *TP53* or *RB1* loss in multivariable analyses renders both slightly below conventional significance thresholds, raising the possibility of biological interplay between these genes (particularly *TP53*) and subsequent copy number alteration that develops during tumor evolution. Further, the prognostic associations of tumor CNA burden are independent of tumor ploidy, which suggests that tumor CNA burden may not simply reflect aneuploidy, defined as abnormal DNA content (24). It is also possible that tumor CNA burden captures prognostic information about currently unidentified driver alterations and/or the rate of ongoing CNA within a tumor that may generate additional driver alterations, including those reflecting intratumoral heterogeneity, thereby affecting outcome. Ongoing work by others has begun to develop genomic methods for identifying mechanisms of somatic CNA (43) and identify prognostic CNA signatures and the mechanisms underlying the component CNA (44). Ultimately, the biology underlying the significant association of tumor CNA burden with multiple cancer outcomes will be a fruitful area for future investigation.

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Author Contributions

H.H., B.S.C., P.T.S., A.J.V., J.C., and C.L.S. designed and planned the TAPG copy number cohort. R.M. and K.K.Y. reviewed and dissected the TAPG copy number cohort tumor samples. D.M.B. carried out the pathology review of the TAPG1 cohort. H.H. and R.M. designed and oversaw the aCGH copy number profiling of the TAPG copy number cohort. B.S.T. carried out the aCGH copy number data analysis. H.H., C.L.S., A.L.T., and A.J.V. designed the statistical analyses and A.L.T. carried out these statistical analyses. H.H. wrote the initial draft of the manuscript and all authors contributed to the final version.

Methods

aCGH copy number analysis of conservative-treatment TAPG cohort. Of the TAPG1 cohort (20), FFPE prostate tumor tissue from 180 patients was macrodissected from slides. DNA was isolated (Agilent FFPE DNA isolation for aCGH protocol) and quantified by picogreen-based quantification. 107 cases yielded greater than 500 ug DNA and were analyzed by Agilent 180K human CGH arrays (Agilent, 4X180K G4449A arrays, per manufacturer's instructions). Copy number data from patients in the TAPG copy number cohort were quantified, normalized, segmented, and analyzed with RAE, as previously described (8, 16). The conservative treatment TAPG copy number cohort array data was deposited in NCBI GEO under accession number GSE103665.

Tumor CNA burden (tumor CNA burden) was analyzed as percent CNA burden, defined as the length of the genome altered by copy number alteration multiplied by 100. For regression analyses, tumor CNA burden was scaled as per 5 percent so that the estimates of our hazard ratios were more interpretable. All statistical analyses were performed using Stata 13 (StataCorp, College Station, TX).

TAPG copy number cohort statistical analyses

For Cox regression analyses, the primary aim was to determine whether tumor CNA burden is associated with cancer specific survival (CSS). First, we assessed whether there was an association between tumor CNA burden and CSS by utilizing a univariate Cox model, censoring patients who did not die at the date of their last follow-up and patients who died of other causes at their death date. Secondly, in order to assess whether there is information from tumor CNA burden over and above biopsy Gleason score, we utilized a multivariable Cox model, adjusting for biopsy Gleason sum categorized as $\leq$6, 7, and $\geq$8. Finally, to assess whether there is an association between tumor CNA burden and CSS after accounting for the preoperative predictors of CSS, we utilized a multivariable Cox model, adjusting for the UCSF-CAPRA score, a preoperative risk score calculated by incorporating the patient's age at diagnosis, PSA at diagnosis, primary and secondary Gleason score at biopsy and clinical tumor stage. As percent of positive biopsy cores was not available for the cohort, a modified CAPRA score was utilized not incorporating this information. Among our cohort of 107 patients, 47 patients were missing clinical tumor stage;

multiple imputation was used to impute the missing values. Statistical analyses were performed utilizing the measured and imputed values combined across 10 imputations using Rubin's method. Furthermore, to evaluate the discriminative accuracy of the model including tumor CNA burden, we calculated bootstrap optimism-corrected Harrell's C-index. It should be noted that the discrimination of the CAPRA score is lower in the TAPG1 conservative treatment CNA cohort than seen in some other prostate cancer cohorts, and this may impact the degree to which tumor CNA burden increases the concordance index. All data used for these analyses are available in Supplementary Table 4.

For illustrative purposes, we utilized competing risk methods to estimate the probability of death from prostate cancer in the setting of death from other causes. Cumulative incidence was shown for patients who died from prostate cancer, or died from other causes, stratified on tumor CNA burden in relation to the median tumor CNA burden among the cohort, using the *stcompet* command in Stata.

Statistical analyses of IMPACT cohorts

For analysis of the prostate cancer MSK-IMPACT cohort (33), the published cases were analyzed by Cox regression for association between overall survival and tumor CNA burden (Supplementary Tables 5 and 6). The IMPACT cases were separated into groups consisting of primary tumors or metastatic tumors, including loco-regional, non-resistant to treatment, and treatment resistant, though primary tumor samples include cases sampled after metastatic spread. Among our primary and metastatic IMPACT prostate cancer cohorts, we excluded men with unknown overall survival status and unknown time until overall survival status, leaving us with a final cohort of 261 and 216 men, respectively. Among these two groups of patients, we assessed the association between tumor CNA burden and overall survival using a univariate Cox model. Multivariable Cox models were then used to determine whether the association between tumor CNA burden and overall survival remained after accounting for purity determined by FACETS (35), the overall point mutation burden, or specific somatic gene alterations (shallow or deep copy number loss or mutation) occurring in prostate cancer (*BRCA1*, *BRCA2*, *ATM*, *TP53*, *RB1*, and *PTEN*), using separate models for each alteration. As the overall point mutation burden was not available for all patients, 34 patients with primary prostate cancer and 11 patients with metastatic prostate cancer were excluded from this portion of the analysis in their respective cohorts.

For analysis of our pan-cancer IMPACT cohort (MSK-IMPACT cohort (34) and additionally accrued IMPACT samples), outcome data at time of analysis, mutation burden, and fraction genome altered data used were derived and available in updated form the cBio Portal (http://www.cbioportal.org/study?id=msk_impact_2017, samples and annotation used at time of analysis available as Supplementary Tables 7 and 8). A cohort of 7305 primary tumor cases across 53 different cancer types and a cohort 5907 metastatic tumor cases, across 47 different cancer types, were identified. Within the primary and metastatic disease cohorts, we excluded patients with unknown tumor CNA burden, overall survival status, unreported follow-up time, death or censoring immediately after treatment, unknown cancer type, and unknown mutation burden. The final cohort used here therefore included 6610 and 4864 patients, respectively. Within both of these cohorts, univariate Cox models were used to determine whether CNA or mutation burden is associated with overall survival. Reported follow-up time was used. As it is probable that the association between tumor CNA burden and survival likely varies based on the particular cancer type, we focused on patients with the ten most prevalent cancer types in both of the respective cohorts (Supplementary Table 2, 5198 and 3886 patients with primary and metastatic disease respectively) and proceeded with a meta-analysis in order to stratify by cancer type. In particular, we utilized a multivariable Cox model, adjusting for mutation burden for each cancer type and extracted the effect size. The effect size for each cancer type was then entered into a meta-analysis using the *metan* command in Stata. Both fixed effects and random effects estimates were calculated. Fixed effects weights were calculated using inverse-variance weighting, *metan* weights were calculated using the DerSimonian and Laird method.

Statistical analyses of TCGA cohorts

For analyses of TCGA cohorts, the following published cohorts were filtered for only primary, non-neoadjuvantly treated cases and analyzed: TCGA prostate adenocarcinoma (2015)(9), breast carcinoma(27), uterine endometriod cancer(45), renal clear cell carcinoma(29), papillary thyroid carcinoma(30), and colorectal adenocarcinoma(31). The number of cases and exclusions based on unavailable data are detailed in Supplementary Table 9. Cox regression was used to test the association of tumor CNA burden as a continuous variable with (i) cancer free status and (ii) overall survival in univariate models and in multivariable models with disease stage. For the TCGA colorectal cancer cohort, tumor stage was also used. For the TCGA prostate adenocarcinoma cohort, multivariable Cox regression models that included Gleason score, mutation count, ploidy, and/or ABSOLUTE purity (25) originally reported with this cohort were also used. Analyses including purity exclude 37 patients without absolute tumor purity measured, resulting in analysis with 243 men, 29 of whom had BCR, and a median followup time for survivors of 20.1 (7.0, 37.9) months.

Data access. The conservative treatment TAPG copy number cohort array data was deposited in NCBI GEO under accession number GSE103665 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE103665, reviewer access token czwruyesnzqbbyn).

Tables and Figures

Table 1. Tumor CNA burden is associated with prostate cancer-specific death in conservative treatment cohort independent of Gleason sum score and CAPRA score. Cox Regression model assessing the association between CNA burden (per 5%) and cancer specific survival. N=107*

Model	HR	95% CI	P-value
Univariate, tumor CNA burden	1.49	1.30, 1.70	<0.0001
Multivariable – adjusting for Gleason sum (≤6, 7, ≥8)	1.44	1.24, 1.67	<0.0001
Multivariable – adjusting for UCSF-CAPRA score utilizing multiple imputation	1.44	1.24, 1.68	<0.0001
Multivariable – adjusting for UCSF-CAPRA score without utilizing multiple imputation * N = 60 (excludes 47 patients with unknown stage)	1.57	1.29, 1.92	<0.0001

Table 2. Tumor CNA burden is associated with recurrence and overall survival independent of disease stage in multiple cancer types

Cohort	*Model*	*Disease Free Time*				*Overall Survival*			
		cases	**HR**	**95% CI**	**P**	**cases**	**HR**	**95% CI**	**P**
Prostate cancer TCGA	Tumor CNA burden, per 5% tumor CNA burden, univariate	280	1.27	1.13, 1.42	<0.0001				
	Tumor CNA burden, per 5% tumor CNA burden, adjusted for Gleason grade and mutation burden	279	1.18	1.03, 1.35	0.015				
	Tumor CNA burden, per 5% tumor CNA burden, adjusted for purity (ABSOLUTE)*	243	1.22	1.07, 1.40	0.003	Insufficient events			
	Tumor CNA burden, per 5% tumor CNA burden, adjusted for ploidy	243	1.32	1.11, 1.56	0.002				
Breast cancer TCGA	Tumor CNA burden, per 5% tumor CNA burden, univariate	709	1.07	1.01, 1.14	0.028	794	1.08	1.03, 1.13	0.0005
	Tumor CNA burden, per 5% tumor CNA burden, Multivariable, adjusted for disease stage	695	1.07	1.00, 1.14	0.049	777	1.08	1.03, 1.13	0.002
Endometrial Cancer TCGA	Tumor CNA burden, per 5% tumor CNA burden, univariate	496	1.10	1.06, 1.14	<0.0001	536	1.13	1.08, 1.17	<0.0001
	Tumor CNA burden, per 5% tumor CNA burden, multivariable, adjusted for disease stage	496	1.08	1.04, 1.13	<0.0001	536	1.10	1.05, 1.15	<0.0001
Renal clear cell cancer TCGA	Tumor CNA burden, per 5% tumor CNA burden, univariate	425	1.05	1.01, 1.09	0.028	525	1.02	0.98, 1.06	NS (0.4)
	Tumor CNA burden, per 5% tumor CNA burden, multivariable, adjusted for disease stage	423	1.05	1.00, 1.11	0.035	522	1.01	0.97, 1.06	NS (0.7)
Thyroid cancer TCGA	Tumor CNA burden, per 5% tumor CNA burden, univariate	483	1.17	1.01, 1.35	0.033	497	1.30	1.04, 1.63	0.021
	Tumor CNA burden, per 5% tumor CNA burden, multivariable, adjusted for disease stage	481	1.18	1.00, 1.39	0.048	Insufficient events			
Colorectal cancer TCGA	Tumor CNA burden, per 5% tumor CNA burden, univariate	512	1.05	1.00, 1.11	0.037	587	1.06	1.01, 1.12	0.012
	Tumor CNA burden, per 5% tumor CNA burden, multivariable, adjusted for disease stage	496	1.03	0.98, 1.09	NS (0.3)	567	1.03	0.97, 1.09	NS (0.3)
	Tumor CNA burden, per 5% tumor CNA burden, multivariable, adjusted for tumor stage	511	1.06	1.01, 1.12	0.028	585	1.07	1.02, 1.13	0.009

* Result differed with similar adjustment in IMPACT prostate cancer cohort utilizing FACETS, see Suppl. Table

Table 3. Tumor CNA burden determined by clinically approved sequencing panel is associated with overall survival in primary and metastatic tumors

Model	*Overall Survival*					
	Primary tumors			*Metastatic tumors*		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Prostate Cancer[a, b]						
Univariate, tumor CNA burden, per 5%	1.17	1.04, 1.31	0.007	1.07	1.01, 1.14	0.020
Multivariable						
Tumor CNA burden, per 5%	1.11	0.98, 1.26	0.10	1.08	1.02, 1.15	0.011
Mutation burden (per mutation)	1.22	1.12, 1.33	<0.0001	1.05	1.02, 1.08	0.001
Multivariable						
Tumor CNA burden, per 5%	1.17	1.04, 1.31	0.007	1.06	1.00, 1.13	NS (0.069)
TP53 CN loss or mutation	4.12	2.02, 8.41	<0.0001	1.24	0.76, 2.02	NS (0.4)
Multivariable						
Tumor CNA burden, per 5%	1.15	1.02, 1.30	0.026	1.06	0.99, 1.13	NS (0.091)
RB1 CN loss or mutation	3.24	0.70, 14.98	NS (0.13)	1.68	0.94, 2.99	NS (0.080)
Multivariable						
Tumor CNA burden, per 5%	1.17	1.04, 1.32	0.008	1.07	1.01, 1.14	0.023
PTEN CN loss or mutation	2.38	1.03, 5.51	0.042	1.15	0.70, 1.89	NS (0.6)
Pan- Cancer						
Univariate, tumor CNA burden, per 5%[c,d]	1.04	1.02, 1.05	<0.0001	1.02	1.01, 1.03	0.005
Univariate, mutation burden (per 5 units)[c,d]	0.98	0.97, 1.00	NS (0.072)	0.99	0.97, 1.01	NS (0.4)
Meta-analysis, tumor CNA burden (per 5%)[e]	1.04	1.02, 1.05	<0.0001[f]	1.02	1.01, 1.04	0.005[g]
Meta-analysis, tumor CNA burden (per 5%), excluding outlier cancer types[h]	1.05	1.03, 1.07	<0.0001[i]	1.03	1.01, 1.04	0.002[j]

[a] Prostate primary tumors: patient n=261 for all models except multivariable model with mutation burden, where n = 227; event n=33; median follow-up time for survivors 40 (IQR 25,81) months

[b] Prostate metastatic tumors: patient n=216 for all models except multivariable model with mutation burden, where n = 205; event n=80; median follow-up time for survivors 59.5 (IQR 32, 129) months

[c] Pan-cancer primary tumors, univariate models: patient n=6610, event n= 1535, median follow-up time for survivors 24 (IQR 11, 61) months

[d] Pan-cancer metastatic tumors, univariate models: patient n=4864, event n=1467, median follow-up time for survivors 51 (IQR 23, 109) months

[e] Pan-cancer meta-analysis, among ten most prevalent cancer types: primary tumor patient n = 4863, metastatic tumor patient n = 3676. Estimates are based on overall fixed effects.

[f] p-value corresponds with test of effects size. Chi-square test for heterogeneity p-value = 0.003

[g] p-value corresponds with test of effects size. Chi-square test for heterogeneity p-value = 0.024

[h] Exclusion of cancer types to reduce heterogeneity: primary tumor patient n = 3887, metastatic tumor patient n = 3098. Estimates are based on overall fixed effects.

[i] Excluding pancreatic and colorectal cancer, test of effects size p-value. Chi-square test for heterogeneity p-value = 0.3

[j] Excluding pancreatic and prostate cancer, test of effects size p-value. Chi-square test for heterogeneity p-value = 0.8

Figure 1. Tumor copy number landscape of conservatively treated primary prostate cancer, compared to other primary prostate cancer cohorts. (**a**) Heat map of copy number alterations in conservative treatment CNA cohort, as well as TCGA, MSKCC, and IMPACT primary prostate cancer cohorts. (**b**) Frequency distribution of CNA burden, as log of percentage of genome copy number altered, for the conservative treatment prostate cancer cohort and three other primary prostate cancer cohorts.

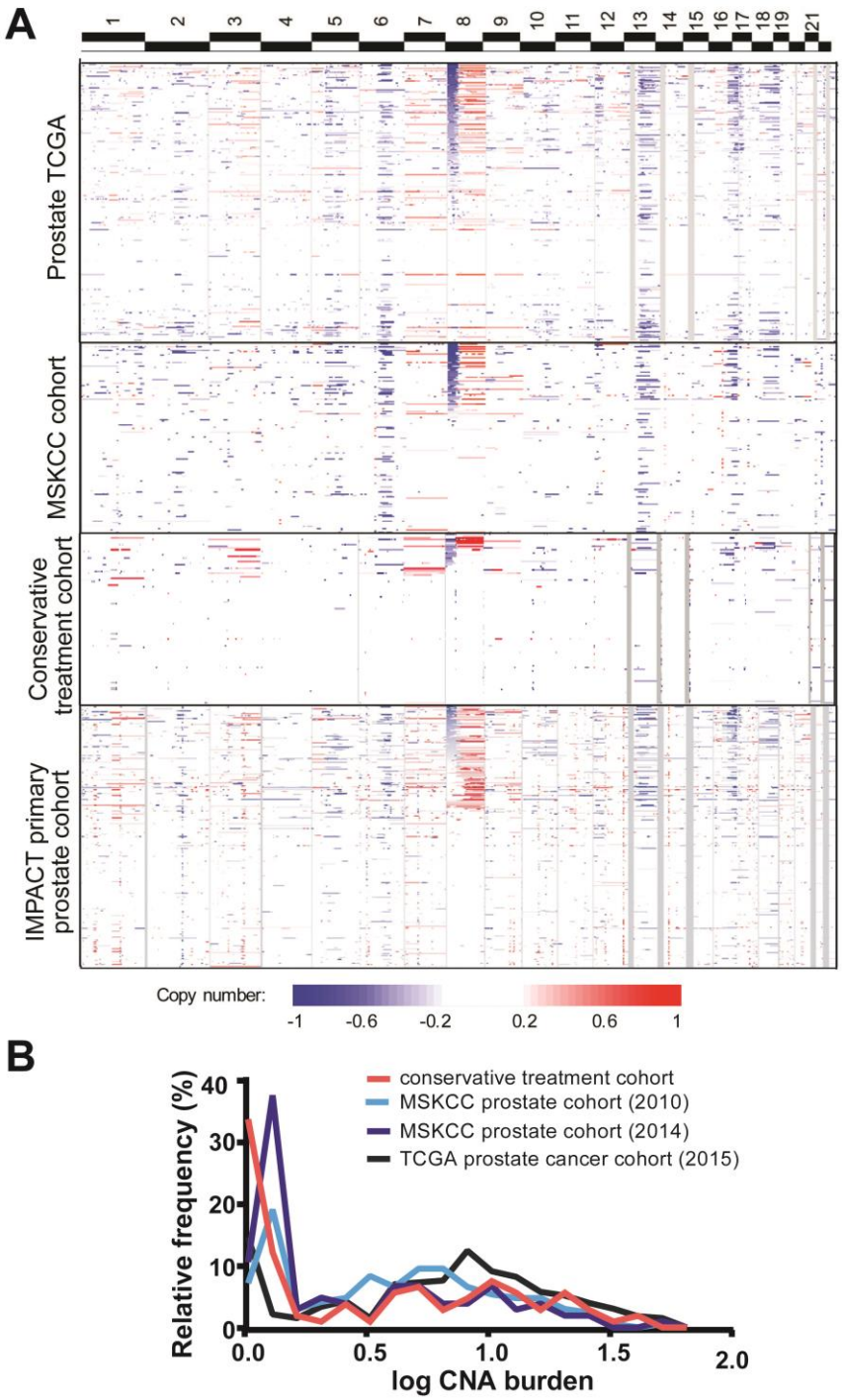
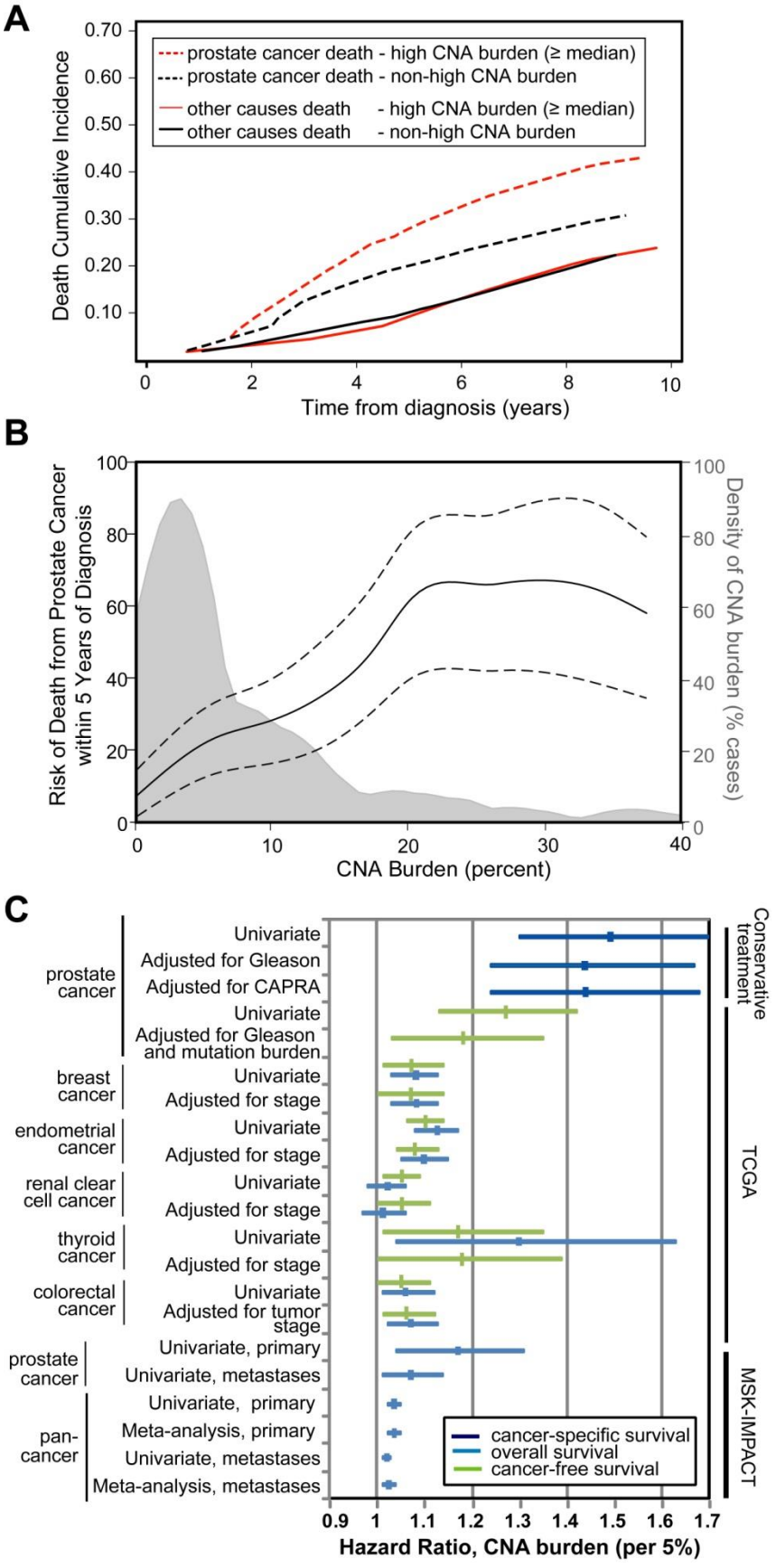


Figure 2. Tumor copy number alteration burden is associated with death from prostate cancer in conservatively treated patients. **(a)** Cumulative Incidence of death from disease (dashed lines) and death from other causes (solid lines) based in cases with high CNA burden (red lines, CNA Burden greater than or equal to the median CNA burden of this cohort, 1.48) or non-high CNA burden (black lines, CNA Burden < median). **(b)** Risk for death from prostate cancer within 5 years of diagnosis. Univariate risk for 5-year prostate cancer-specific death, calculated by locally weighted Kaplan–Meier estimates (solid black line) with 95% confidence interval (dashed black lines) overlaid on the distribution of CNA burden (gray). **(c)** Association of tumor CNA burden with available cancer outcomes in the conservative treatment primary prostate cancer TAPG1 cohort, TCGA primary cancer cohorts, and the MSK-IMPACT clinical sequencing prostate and pan-cancer cohorts of primary and metastatic tumors. Forest plot of hazard ratio (per 5% CNA burden) with 95% confidence interval shown for cancer-specific mortality (dark blue), overall mortality (light blue), and cancer recurrence (green).


A
Death Cumulative Incidence
0.70
0.60
0.50
0.40
0.30
0.20
0.10
prostate cancer death - high CNA burden (≥ median)
prostate cancer death - non-high CNA burden
other causes death - high CNA burden (≥ median)
other causes death - non-high CNA burden
0
2
4
6
8
10
Time from diagnosis (years)
B
Risk of Death from Prostate Cancer within 5 Years of Diagnosis
Density of CNA burden (% cases)
100
80
60
40
20
0
0
10
20
30
40
CNA Burden (percent)
C
prostate cancer
Univariate
Adjusted for Gleason
Adjusted for CAPRA
Univariate
Adjusted for Gleason and mutation burden
breast cancer
Univariate
Adjusted for stage
endometrial cancer
Univariate
Adjusted for stage
renal clear cell cancer
Univariate
Adjusted for stage
thyroid cancer
Univariate
Adjusted for stage
colorectal cancer
Univariate
Adjusted for tumor stage
prostate cancer
Univariate, primary
Univariate, metastases
pan-cancer
Univariate, primary
Meta-analysis, primary
Univariate, metastases
Meta-analysis, metastases
cancer-specific survival
overall survival
cancer-free survival
Conservative treatment
TCGA
MSK-IMPACT
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
Hazard Ratio, CNA burden (per 5%)

Supplementary Tables and Figures

Supplementary Table 1. Cohort characteristics

Characteristic	Conservative Treatment CNA cohort (n=107)[a]	TAPG1 cohort (n=2333)[b]
Age (years)	70 (67, 74)	70 (66, 73)
Median follow-up time (years) among survivors	10.3 (5.0, 14.1)	14.7 (13.8, 15.8)
Patients Underwent Hormonal Treatment	30 (28%)	670 (29%)
Baseline PSA (ng/ml)	11.0 (4.0, 28.9)	13.0 (4.8,30.4)
CAPRA score (n=60)	5 (2, 7)	
Mortality		
Death from prostate cancer	43	638
Death from other causes	43	1012
Alive	21	680
Gleason Score at Biopsy		
≤6	39 (36%)	749 (45%)
7	26 (24%)	514 (31%)
≥8	42 (39%)	420 (25%)
Clinical Stage		
T1	20 (33%)	506 (36%)
T2	25 (42%)	612 (44%)
T3	15 (25%)	269 (19%)

Values are displayed as median (IQR) and frequency (percentage).
[a] n = 60 with available clinical stage
[b] n = 2170 with available baseline PSA; n = 2330 with available survival status and censoring date; n =1683 with available Gleason and histology; n=1387 with evaluated clinical stage

Supplementary Table 2. Distribution of cancer types in IMPACT cohorts

	Primary tumor cohort		Metastatic cohort	
	Frequency	Percent	Frequency	Percent
Non-Small Cell Lung	1089	16.5	719	14.8
Breast	757	11.5	937	19.3
Colorectal	628	9.5	544	11.2
Glioma	627	9.5	15	0.3
Prostate	394	6.0	335	6.9
Pancreatic	348	5.3	243	5.0
Bladder	329	5.0	105	2.2
Hepatobiliary	262	4.0	102	2.1
Esophagogastric	229	3.5	97	2.0
Renal Cell Carcinoma	220	3.3	144	3.0
Soft Tissue Sarcoma	200	3.0	152	3.1
Non-Hodgkin Lymphoma	175	2.6	12	0.2
Endometrial	166	2.5	127	2.6
Germ Cell Tumor	120	1.8	87	1.8
Melanoma	102	1.5	324	6.7

Thyroid	98	1.5	124	2.5
Ovarian	97	1.5	154	3.2
Mesothelioma	93	1.4	13	0.3
Head and Neck	81	1.2	100	2.1
Gastrointestinal Stromal Tumor	72	1.1	55	1.1
Bone	60	0.9	41	0.8
Small Cell Lung	42	0.6	57	1.2
Appendiceal	41	0.6	45	0.9
Skin, Non-Melanoma	39	0.6	37	0.8
CNS	39	0.6	1	<0.1
Salivary Gland	37	0.6	78	1.6
Embryonal Tumor	32	0.5	20	0.4
Uterine Sarcoma	29	0.4	58	1.2
Small Bowel	27	0.4	15	0.3
Cervical	24	0.4	30	0.6
Ampullary Carcinoma	22	0.3	10	0.2
Gastrointestinal Neuroendocrine Tumor	20	0.3	29	0.6
Anal	14	0.2	20	0.4
Adrenocortical Carcinoma	12	0.2	10	0.2
Nerve Sheath Tumor	12	0.2	2	<0.1
Thymic Tumor	10	0.2	1	<0.1
Sex Cord Stromal Tumor	6	0.1	8	0.2
Miscellaneous Brain Tumor	6	0.1	0	0.0
Hodgkin Lymphoma	6	0.1	0	0.0
Retinoblastoma	6	0.1	0	0.0
Miscellaneous Neuroepithelial Tumor	5	0.1	2	<0.1
Histiocytosis	5	0.1	1	<0.1
Vaginal	5	0.1	1	<0.1
Wilms Tumor	4	0.1	2	<0.1
Penile	4	0.1	2	<0.1
Sellar Tumor	4	0.1	0	0.0
Breast Sarcoma	3	<0.1	2	<0.1
Leukemia	3	<0.1	1	<0.1
Gestational Trophoblastic Disease	3	<0.1	0	0.0
Pheochromocytoma	1	<0.1	2	<0.1
Multiple Myeloma	1	<0.1	0	0.0
Pineal Tumor	1	<0.1	0	0.0
Total	6610	100	4864	100

Supplementary Table 3. Association between overall survival and CNA burden after adjustment for purity in IMPACT prostate and pan-cancer cohorts. Purity was determined by FACETS (35).

Model	*Overall Survival*					
	Primary tumors [a, c]			*Metastatic tumors* [b, d]		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Prostate Cancer: Tumor CNA burden, per 5%, adjusted for purity [a, b]	1.04	0.98, 1.11	NS (0.2)	1.00	0.96, 1.05	NS (0.9)
Pan- Cancer: Tumor CNA burden, per 5%, adjusted for purity [c,d]	1.02	1.01, 1.03	0.002	1.01	1.00, 1.02	NS (0.061)

[a] Prostate primary tumors: patient n=193; event n=28; median follow-up time for survivors 37 (IQR 25,83) months
[b] Prostate metastatic tumors: patient n=201; event n=77; median follow-up time for survivors 62.5 (IQR 33, 131) months
[c,] Pan-cancer primary tumors, n=4052
[d] Pan-cancer metastatic tumors n=3175

Supplementary Table 4. TAPG1 conservative treatment primary prostate CNA cohort annotation. Available as associated file.

Supplementary Table 5. MSK-IMPACT primary prostate tumor cohort annotation. Available as associated file.

Supplementary Table 6. MSK-IMPACT metastatic prostate tumor cohort annotation. Available as associated file.

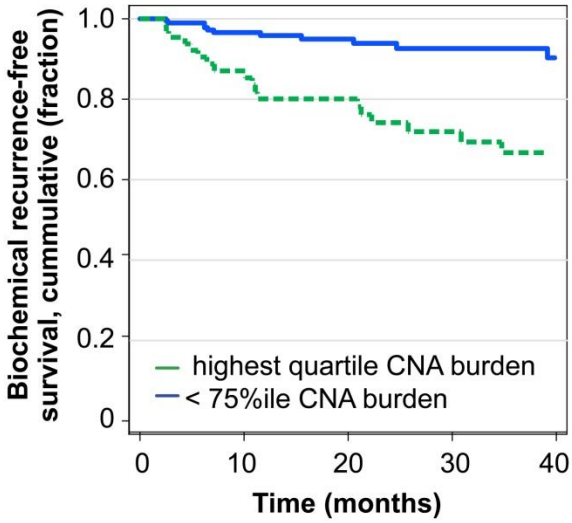
Supplementary Table 7. MSK-IMPACT primary pan-cancer cohort annotation. Available as associated file.

Supplementary Table 8. MSK-IMPACT metastatic pan-cancer cohort annotation. Available as associated file.

Supplementary Table 9. TCGA Cohort statistics: patient exclusion, events, and follow-up

	Initial Cohort Size	**Unknown CNA Burden**	**Unknown recur-ence status or time**	**Unknown OS status or time to death**	**Immedi-ately censored after treatment**	**Final Cohort Size**	**RF event n**	**Median followup time for RF survivors and IQR (months)**	**OS event n**	**Median followup time for OS survivors and IQR (months)**
Prostate	330	0	50	N/A	0	280	32	18 (7, 36)	--	--
Breast	817	0	N/A	0	23	794	53	20 (12, 38)	100	20 (12, 39)
Endometrial	547	8	N/A	2	1	536	105	30 (17, 58)	90	31 (18, 58)
Kidney	537	9	N/A	1	2	525	124	45 (21, 64)	175	48 (23, 71)
Thyroid	507	8	N/A	1	1	497	46	31 (17, 48)	16	31 (18, 50)
Colorectal	629	13	N/A	7	22	587	117	22 (13, 34)	124	24 (14, 36)

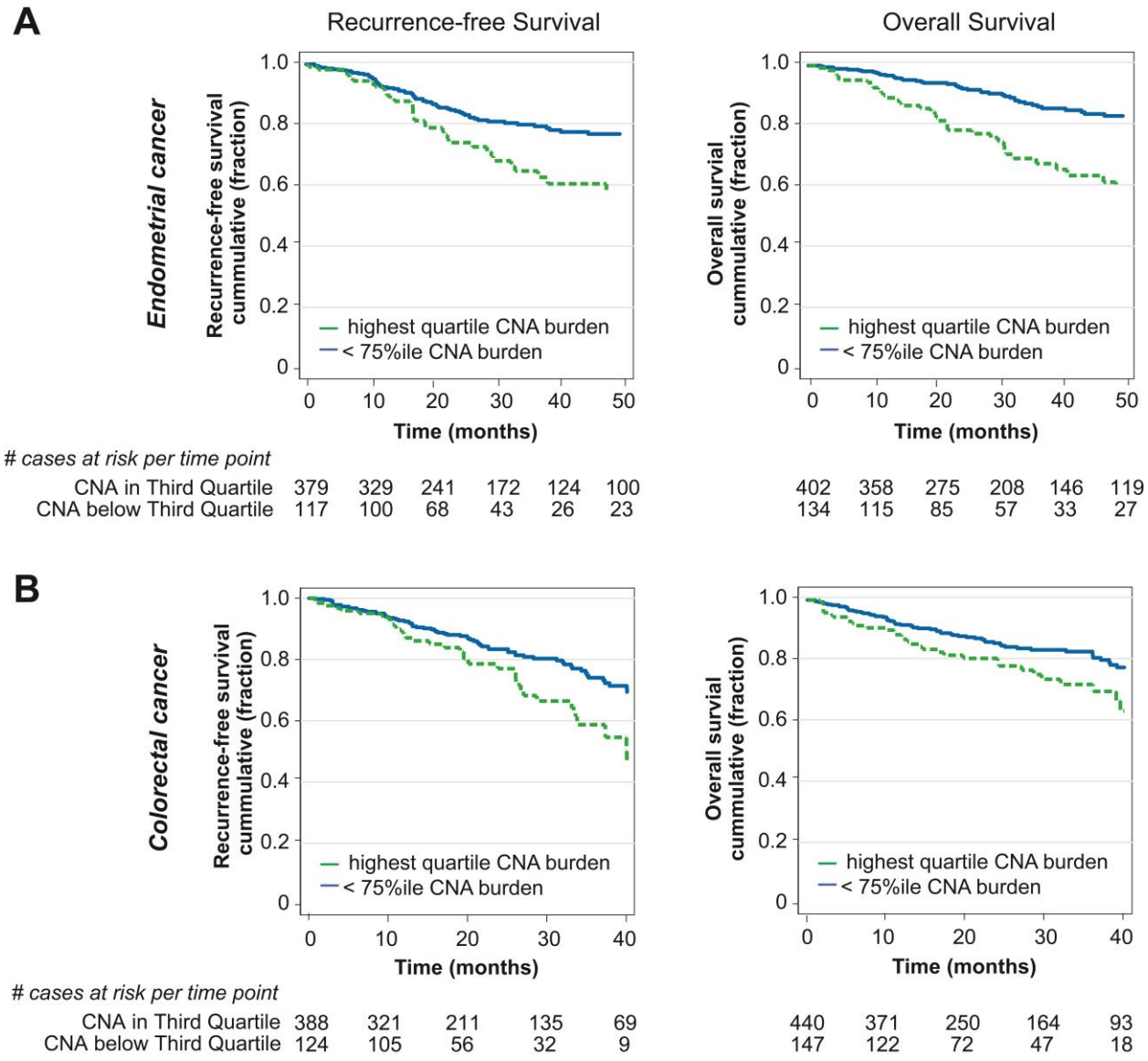
Supplementary Figure 1. Kaplan-Meier plot of biochemical recurrence in TCGA primary prostate cohort. The highest quartile tumor CNA burden (above 75 percentile CNA burden, green) is compared to lower three quartiles (blue) with risk table showing the number of patients present at each time point.



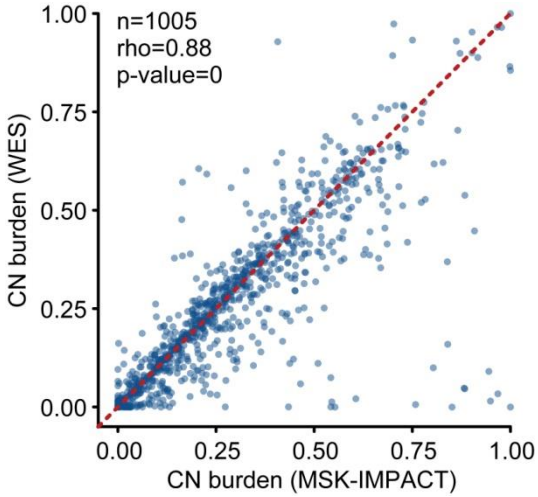
 # cases at risk per time point

CNA in Third Quartile	209	135	91	55	38
CNA below Third Quartile	71	50	41	28	19

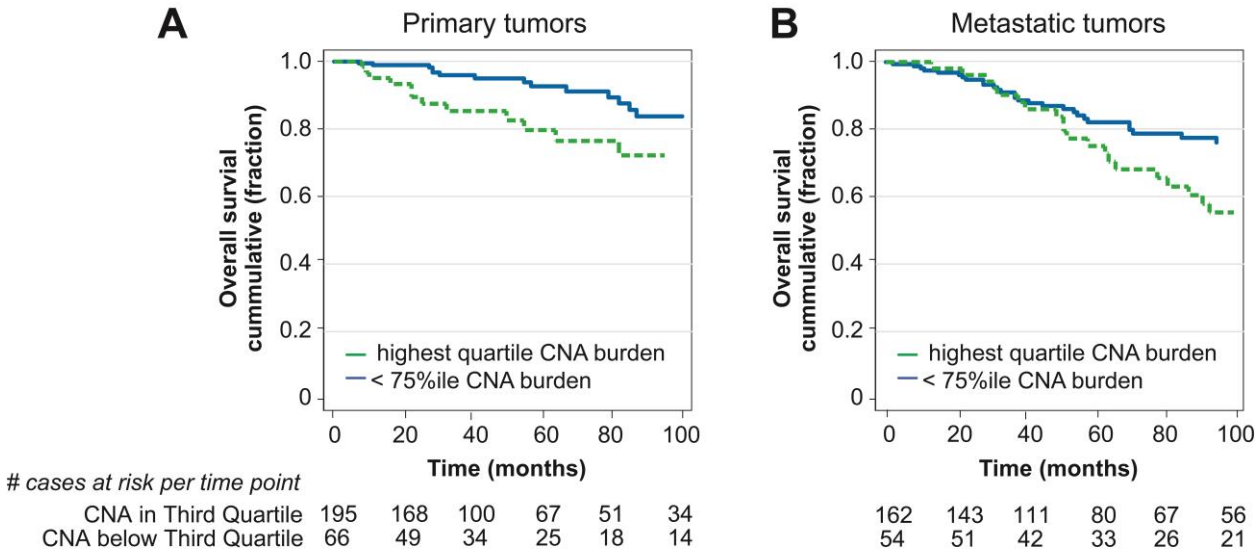
Supplementary Figure 2. Tumor CNA burden in multiple cancers is associated with disease free survival and overall survival. Kaplan-Meier plot of disease free survival (left) and overall survival (right) of TCGA cohorts of (**a**) endometrial cancer and (**b**) colorectal cancer. The highest quartile CNA (above 75 percentile CNA burden, green) is compared to lower three quartiles (blue).



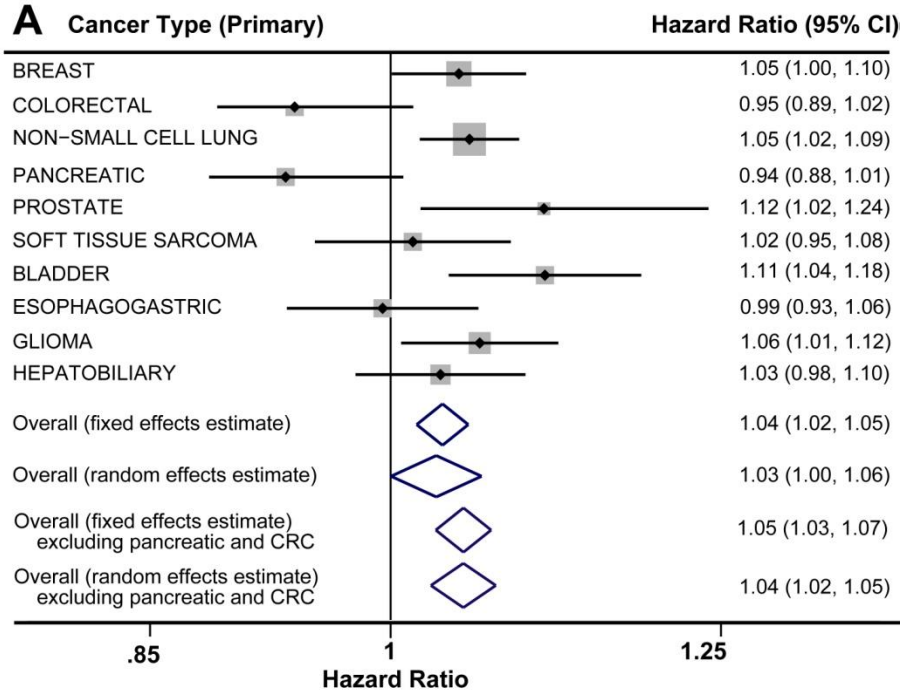
Supplementary Figure 3. Correlation between CNA burden from IMPACT targeted sequencing assay and whole exome sequencing (WES) of same samples, pan-cancer. The relationship between CNA burden determined by IMPACT targeted sequencing and WES in a subset of pan-cancer IMPACT cohort samples analyzed by both approaches (n = 1005) is shown (rho=0.88, p-value=0).

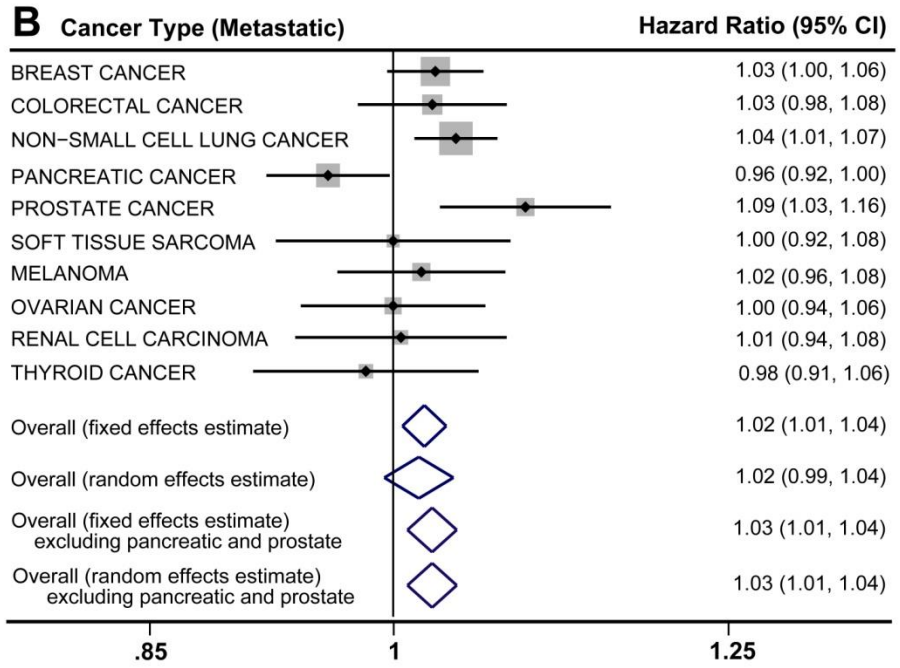


Supplementary Figure 4. Tumor CNA burden in primary prostate cancer is prognostic for overall survival when assayed by clinically approved sequencing panel. Kaplan-Meier plot of overall survival of IMPACT primary prostate cancer cohort by CNA burden quartile in (a) primary and (b) metastatic tumors. The highest quartile CNA (above 75 percentile CNA burden, green) is compared to lower three quartiles (blue).



Supplementary Figure 5. Forest Plot of Hazard Ratios (individual and pooled) for meta-analysis assessing the association between tumor CNA burden and overall survival in (**a**) primary cancer and (**b**) patients with metastatic cancer in the pan-cancer IMPACT cohort





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