Genomic instability and TP53 genomic alterations associate with poor anti-proliferative response and intrinsic resistance to aromatase inhibitor treatment

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Running Head:
Genomic instability and resistance to aromatase inhibitor treatment

Conflicts of Interests: JR is employed by Oncimmune. JR holds stock in Oncimmune and FaHRAS. JR received honoraria from AstraZeneca, Bayer and Amgen. JR receives research funding from Oncimmune. JR holds patents from Oncimmune and is in the speakers’ bureau of AstraZeneca. MD is a paid adviser to Radius and receives financial benefits from the Institute of Cancer Research’s Rewards for Inventors Scheme; MD receives funding from Pfizer and Radius. The remaining authors declare no competing financial interests.
**Key objective:** Our study was focused on understanding the link between somatic copy number alterations (SCNAs) and intrinsic resistance to aromatase inhibition (AI) therapy and we observed tumors with high levels SCNAs had intrinsic resistance to therapy.

**Knowledge generated:** There is a well established link between high genomic instability (GI) and TP53 mutations and resistance to cancer treatment; however, we are the first to show that primary ER+ tumors with high GI have an intrinsic resistance to treatment that can be measured after a short two-week AI treatment. High GI tumors do not require time to evolve resistance to estrogen deprivation therapy as they already have de novo resistance to treatment.

**Relevance:** Estrogen deprivation therapy with AI treatment is highly effective in ER+ breast cancer (BC), but more than 20% of postmenopausal women with early-stage BC suffer a relapse. The POETIC phase III trial with 2-weeks of perioperative AI therapy offers the opportunity to identify mechanisms and biomarkers of intrinsic AI resistance, and in the POETIC study up to 20% of tumors showed resistance to AI treatment after just 2 weeks of treatment. The results show high genomic instability (GI) is associated with AI resistance and detection of copy number alterations and mutations in TP53 are predictive of high GI. Validation of these results in a larger study would provide a framework for better stratifying patients into high risk of AI-resistance that are likely to benefit from added or alternative treatment.
Abstract

Purpose: While aromatase inhibition (AI) is an effective treatment for estrogen receptor-positive postmenopausal breast cancer (BC) resistance is common and incompletely explained. Genomic instability (GI) as measured by somatic copy number alterations (SCNAs) is important in BC development and prognosis. SCNAs to specific genes may drive intrinsic resistance, or high GI may drive tumor heterogeneity allowing differential response across the tumors and surviving cells to rapidly evolve resistance to treatment.

We therefore evaluated the relationship between SCNAs and intrinsic resistance to treatment as measured by a poor anti-proliferative response.

Patients and Methods: SCNAs were determined by SNPArray in baseline and surgery core-cuts from 73 postmenopausal patients randomized to receive 2 weeks’ pre-operative AI or no AI in the POETIC trial. Fifty-six samples from the AI-group included 28 poor responders (PrRs, <60% reduction in Ki67) and 28 good responders (GdRs, >75% reduction in Ki67). Exome sequencing was available for 72 pairs of samples.

Results: GI correlated with Ki67 expression at both baseline (P=0.0003) and at surgery (P= 0.0002), and GI was higher in PrRs (P=0.048). The SCNA with the largest difference between GdRs and PrRs was loss of heterozygosity (LOH) observed at 17p (FDR=0.08), which includes TP53. Nine of 28 PrRs had loss of wildtype TP53 due to mutations and LOH compared to 3 of 28 GdRs. In PrRs, somatic alterations of TP53 were associated with higher GI, higher baseline Ki67 and greater resistance to AI treatment compared to wildtype TP53.

Conclusion: We observed that primary tumors with high GI have an intrinsic resistance to AI treatment and do not require further evolution to develop resistance to estrogen deprivation therapy.
Introduction

Estrogen deprivation is the major treatment strategy for hormone-dependent breast cancer (BC) and typically involves agents that inhibit aromatase, the enzyme catalyzing the conversion of androgens to estrogens. Despite near complete suppression of circulating estrogen levels by aromatase inhibitor (AI) treatment, acquired and de novo resistance to AI is common\(^1\). There are few pre-treatment biomarkers for AI resistance and mechanisms of resistance are incompletely understood\(^2\).

Mutations and somatic copy number alterations (SCNAs) can play important roles in activating oncogenes or inactivating tumor suppressors, and BC is characterised by multiple recurrent SCNAs and few recurrent mutations\(^3\). We have previously shown that TP53 mutations occur at a higher rate in tumors with poor response to AI treatment suggesting these patients received less benefit from AI\(^4\) but SCNAs to specific genes may also play an important role in AI resistance\(^5\). It is known that non-specific genomic alterations like high genomic instability (GI) is associated with poor prognosis and probably due at least partly to tumor heterogeneity allowing some cells to survive and evolve resistance to treatment\(^6\). There is evidence in other solid tumors for an association of high GI and intrinsic resistance to chemotherapy\(^7\). However, there are few studies of GI and response to endocrine treatment. The aim of this work was therefore to determine if genome-wide measures of SCNAs (i.e. genomic instability - GI) and/or focal SCNAs are associated with intrinsic resistance to AI treatment.

Response to AI treatment can be measured by change in the proliferation marker Ki67, following 2-4 weeks of presurgical therapy, and AI resistance in primary tumors can be characterized and defined by limited or no Ki67 response to AI treatment\(^8-10\). This change
in Ki67 has been found to predict benefit from endocrine therapy better than clinical response\textsuperscript{10}. We therefore extended our earlier study on the relationship between mutations and resistance to AIs in the pre-surgical Perioperative Endocrine Therapy—Individualising Care (POETIC) trial. We used SNPArray technology to identify SCNAs and included paired baseline and surgery samples to assess the degree of intra-tumoral heterogeneity and selection during AI treatment.

**Methods**

**Patients and tissues**

The POETIC trial (CRUK/07/015) is a pre-surgical randomized study with 4,486 post-menopausal patients receiving non-steroidal AI (anastrozole 1 mg/day or letrozole 2.5 mg/day) or no treatment (2:1) two-weeks before surgery\textsuperscript{11}. Core-cut biopsies (14-G) were collected from c.15\% of patients into RNA\textit{later} (Qiagen). Whole blood was collected for germline DNA analysis. The trial was approved by the NRES Committee London-South East. Patients gave informed consent for DNA analysis.

**Biomarker analyses**

Ki67\% staining was centrally analyzed on formalin-fixed samples as previously described\textsuperscript{8}. HER2 status was measured locally. Ki67 and HER2 results are shown in Supplementary Table 1.

**Sample Selection**

DNA was extracted from 192 baseline/surgery samples from the subset of POETIC ER+ tumors stored in RNA\textit{later} and matching blood controls from 73 patients with baseline Ki67 scores greater than 5\%. Poor responders (n=28) were defined as having a Ki67 decrease
of <60% between baseline and surgery, and good responders (n=28) with >75% Ki67
decrease (Supplemental Figure 1). Patients with intermediate Ki67 decrease between 60-
75% were not considered. Exome sequencing was available for 72 tumors from a previous
study⁴. Samples from 17 patients who received no-AI were also analysed to ensure that
changes in SCNAs ascribed to AI treatment were not artefactual. Aliquots were taken from
10 tumor DNA samples and assessed as technical replicates (Supplemental Figure 1).

**DNA extractions**

8-μm sections were taken from RNAlater-stored core-cuts embedded in OCT (Cryo-M-
Bed, Bright Instruments, UK) and stained with Nuclear Fast Red (0.1% (w/v)). Needle
microdissection was used to achieve >60% pure tumor cells when necessary. DNA was
extracted from the sections using the DNeasy Tissue and Blood kit (Qiagen), and from
peripheral blood using the EZ1 system (LifeTechnologies).

**SNParray Analysis**

Illumina Human OmniExpress Exome BeadChip v.3 was used to generate genotype and
intensity data for blood and tumor samples, and ASCAT¹² for the estimate of ploidy,
fraction of tumor cells and copy number alterations (CNA) in the tumor samples. Two
samples did not pass ONCOSNP QC¹³ and visual inspection of the SNP-array data. Ploidy
and purity using default parameters and a range of higher segmentation penalties were
estimated with ASCAT and OncoSNP. The segmentation penalty in ASCAT was increased
(22-samples) or the estimate of ploidy and purity from OncoSNP was used in ASCAT (4-
samples) to generate SCNA calls that best described the data. For five samples, germline
genotype predictions generated by ASCAT were due to contamination or QC failure of
blood controls. Bedtool multiintersect¹⁴ was used to identify 47807 non-overlapping
segments from all samples. Data has been deposited in the European Genome-phenome Archive (EGAS00001001940).

**Measures of Genomic Instability**

Chromosomal gains and losses were determined relative to estimates of tumor ploidy by ASCAT (sum of major and minor allele calls minus tumor ploidy rounded to nearest integer). Loss of heterozygosity (LOH) was assigned when the estimated copy number was zero for the minor allele (LOH). Genomic Instability (GI) was defined as the percentage of the genome with SCNAs calculated by summing the total basepairs (bp) of segments with gains, loss or LOH relative to paired normal blood control samples for each tumor sample and dividing by the size of the genome (3x10^9bp).

**Intrinsic Subtypes**

PAM50 intrinsic subtypes were determined for 36 tumors. Details are available in Supplemental Table 1.

**Statistical methods**

Mann-Whitney, F, chi-squared, Pearson’s correlation (Pearson’s), Fisher’s exact tests (Fisher’s), and multiple correction by Benjamini-Hochberg method (FDR) were also carried out using R with the wilcox.test, var.test chisq.test, cor.test, fisher.test, p.adjust functions, respectively. Fisher’s exact tests were one-sided and remaining reported p-values were two-sided tests unless otherwise specified. Boxplot plots were generated with the boxplot function in R to show median, interquartile and range of values excluding outliers.
Results

SCNA characteristics in the overall population

SCNAs were identified in 28 patients with tumors classified as PrRs, 28 classified as GdRs and 17 tumors from the no-treatment control group (Figure 1A). The median percent of the genome with SCNAs was 46% for all tumors with a single representative tumor sample chosen from matched baseline, surgery or technical replicate samples to calculate the median percentage of SCNAs. The median percent of the genome with gains relative to tumor ploidy, losses relative to tumor ploidy, and LOH was 15%, 16%, and 15%, respectively (Figure 1B, Supplemental Table 2). Highly recurrent SCNAs (gains at 1q, 16p, 20q and 8q, and losses/LOH at 11q 16q, 17p and 8p) occurred in >50% of all representative samples (Supplemental Figure 2A-D). The majority of sites with losses overlapped with LOH (Supplemental Figure 2E), as expected\(^\text{17,18}\).

Intratumoral heterogeneity of SCNAs

Overlap of SCNAs between paired core-cuts

Discordance between baseline and surgery time points was significantly greater than differences between technical replicate samples taken from the same DNA extraction (Supplemental Figure 2F). Discordance in SCNAs was observed in >10% of the genome in only one pair of technical replicate samples; notably, these samples had the highest GI with >90% of the genome with SCNAs (P088 samples, Supplemental Figure 3A).

Overall SCNA calls in baseline and surgery AI pairs were very similar (Supplemental Figure 3) with the median overlap for SCNAs at 87% and 88% for 33 baseline/surgery AI pairs and 11 no-AI pairs respectively. There was no significant difference between the frequency of discordant SNCA calls between baseline and surgery AI pairs after correction
for multiple testing and only 4% of 47807 non-overlapping regions have >10% more events in baseline or surgery samples (>4 additional SCNA events in the baseline or surgery samples in the 33 pairs) (Supplemental Figure 4). Much larger sample sizes are required to determine if these regions are significantly different between baseline and surgery.

**Concordance of SCNAs between paired core-cuts**

For pairs of baseline and surgery samples, the median percentage of the genome with discordant SCNA calls was 5% (Figure 1C), and discordance between samples was associated with the percentage of the genome with SCNAs (Supplemental Figure 5). There was only one paired set of core-cuts in which discordant SCNAs were greater than the SCNAs shared between the pair of samples, suggesting two independently evolved tumors (Supplemental Figure 6).

**Discordance in PrR and GdR paired samples**

There was a trend for PrRs to have more discordant SCNAs between paired samples than GdRs (average 10% in PrRs and 6% in GdRs) but this difference was not significant. However, the variance in the percent of the genome with discordant SCNAs was significantly greater in PrRs than GdRs ($P < 10^{-6}$, $F$ test) (Figure 1D). These data indicate that the tumors with the highest topographic heterogeneity in SCNAs were more frequent among the PrRs.

**Intrinsic subtypes**

PAM50 intrinsic subtype calls$^{15}$ were performed on 36 baseline tumors. There is an enrichment of poor prognosis intrinsic subtypes (PrR non-luminal/luminal-B) in PrR
samples (64%) compared to GrR (20%); however, >30% of measured PrR samples are luminal-A subtypes suggesting intrinsic subtyping is not fully capturing the higher risk of recurrence in these samples (Supplemental Table 1).

**Inter-tumoral heterogeneity in SCNAs**

*Comparison between PrRs and GdRs in percent of genome altered*

Given the overall concordance between baseline and surgery core-cuts in SCNAs and the results of previous observations of minimal impact of AI treatment on mutation counts, we merged all the SCNA events from multiple samples from the same tumor to represent the SCNAs events in that tumor (merged 35-baseline and surgery; 9-baseline, surgery and technical replicates; 1-baseline technical replicates). The GI was higher in the 28 PrR combined samples than the 28 GdR combined samples ($P=0.048$, Mann-Whitney) and GI was significantly correlated with baseline ($r=0.41, P=0.0003$, Pearson’s) and surgery ($r=0.48, P=0.0002$, Pearson’s) Ki67 (Figure 2).

*Comparison of SCNAs between PrRs or GdRs*

The percentage of a chromosomal arm with gains, losses and LOH was calculated, and PrRs showed a significantly higher percentage of gains in chromosome 6p, losses in 5q, and LOH in 10q, 17p, and 19p (FDR < 0.1, one-sided Mann-Whitney) (Figure 3A-C, Supplemental Figure 7). The largest difference in percentage values (mean and median values) for arms between GdRs and PrRs was observed in LOH at 17p (Figure 3D-G), followed by LOH in 8p and gains in 8q. There were no chromosomal arms with significantly greater gains, losses or LOH in GdRs.
Analysis of smaller regions, based on the 47807 non-overlapping segments, revealed the most significant differences in gains were observed at 10p12.31 and 10p13 ($P=0.0004$, Fisher’s), losses at 5q11.2 ($P=0.0002$), and LOH at 17p13.3 ($P=0.0005$). These regions had approximately 40% more events in PrRs (13 to 10 more SCNA events in the 28 PrR samples than GrRs) but were not significant after multiple correction (Supplemental Figure 8A).

**TP53 alterations**

*Occurrence of TP53 mutations and LOH in cohort*

Our previous work from exome sequencing showed PrRs and TP53 mutations associated with a higher mutational load and that the mutational load was correlated with Ki67 levels at a surgery after 2-weeks of AI treatment\(^4\). We did not observe a significant correlation between the percent of the genome with SCNAs and mutational load, but we did observe greater GI in tumors with TP53 mutations (Figure 4E).

As expected for a tumor suppressor, LOH at the TP53 locus in 17p was associated with TP53 mutations across all tumors (driving loss of the functioning copy of the tumor suppressor gene) ($P=0.004$, Fisher’s). Of the 17 patients with TP53 mutations in baseline or surgery samples, 15 had LOH at the TP53 locus (9-PrRs, 5-GdRs, 3-Controls). All nine PrR samples and three out of five GrR samples with TP53 mutations also had LOH at the TP53 locus. There was a significant enrichment of TP53 genomic alterations in PrRs ($P=0.03$, Fisher's) and significant difference in the distribution of TP53 genetic alterations between PrRs and GdRs ($P=0.02$, Chi-squared) (Figure 4A).

**AI resistance and TP53 status**
Within the PrR group, samples with no LOH and $TP53^{WT}$ had the best anti-proliferative response to AI compared with samples with $TP53^{WT}$+LOH and $TP53^{MUT}$+LOH as measured by the change in Ki67 ($P=0.01$ and $P=0.05$ respectively, Mann-Whitney) (Figure 4B). The difference in the change in Ki67 between $TP53^{WT}$+LOH and $TP53^{MUT}$+LOH was not significant but there were significant differences between $TP53^{WT}$+LOH and $TP53^{MUT}$+LOH for baseline Ki67 scores ($P=0.02$), for surgery Ki67 scores ($P=0.04$) and for the percentage of the genome with SCNAs ($P=0.0004$), (Figure 4B-E).

**Impact of HER2 status**

There were seven HER2 positive samples in the PrR group and none in the GdR group. HER2 positive samples had a significantly higher percentage of the genome with gains in copy number compared to HER2-negative PrR samples ($P=0.03$, Mann-Whitney) but did not have significantly higher percentage of SCNAs in general, losses or LOH (Supplemental Figure 8B-E). The results with HER2-negative cases were similar to those with all samples with the most significant differences between PrRs and GdRs being loss at 5q and LOH at 17p for HER2-negative samples (Supplemental Figures 8F-G, 9-10). There was also a significant enrichment of TP53 genomic alterations in PrRs ($P=0.02$, Fisher’s) and significant difference in the distribution of $TP53$ genetic alterations between PrRs and GdRs in HER2-negative samples ($P=0.03$, Chi-squared) (Supplemental Figure 10C).

**Discussion**

Our primary goal was to identify global and focal SCNAs that were associated with the anti-proliferative response of ER+ BC to short-term estrogen deprivation using AIs. Our selection of samples from >3000 patients in the AI-group from the POETIC study aimed to
exploit this large study to understand good/poor response to AI treatment in a general ER+ BC population but not to represent the trial population per se. The sampling of tumors before and after 2-weeks of AI treatment allowed the impact of tissue heterogeneity to be assessed, and prior exome sequencing gave the opportunity to integrate the SCNA and mutation data to better understand intrinsic resistance. While the number studied seems modest, the ability to assess response in individual tumors allows much greater confidence with molecular associations that larger studies with time to recurrence. HER2 positivity was enriched in the PrPs, as previously noted, but the genomic changes were similar in HER2-negative cases and the overall population.

The lack of recurrent alterations specific to only baseline or surgery in AI-treated samples indicates a limited impact and selection for SCNAs after 2-weeks of AI treatment in line with other studies. Notably, mean tumor volume did not change significantly in the nearly 3,000 POETIC AI-treated patients within the 2-week treatment window (data not shown) indicating little opportunity for selection of resistant cells in that time. Reduced heterogeneity might be observed from longer treatment. These data therefore indicate that a small biopsy before or after short-term AI treatment is likely to be representative of the whole tumor for most BCs; however for tumors with high GI and greater heterogeneity, multiple biopsies may be necessary to capture all genomic alterations.

There is a large body of evidence to associate GI poor outcomes in solid tumors, and incorporation of GI scores can greatly improve molecular prognostic models for BC. It is not known if high GI and greater tumor heterogeneity allows the few surviving tumors to evolve resistance to AI treatment or if there is intrinsic resistance to AI in these tumors. Our data here support the latter with tumors with high GI showing de novo resistance to AI
therapy as measured by a poor Ki67 response after two weeks of treatment, a validated intermediate marker of benefit from endocrine therapy\textsuperscript{10}. This also suggests that GI not only has prognostic value but also predicts which postmenopausal ER+ primary tumors are likely to be resistant to AI therapy.

LOH in 17p was significantly SCNA associated with poor Ki67 change, and LOH was significantly greater in PrR tumors compared to GdRs in HER2-negative tumors and the overall population. This region encodes for several cancer driver genes including \textit{TP53}, a key regulator of cellular processes controlling proliferation and genomic stability. LOH and mutations in \textit{TP53} has been shown to result in worse outcomes\textsuperscript{23} and we have now shown that it is also associated with poor anti-proliferation response to AI and intrinsic resistance to treatment. Clearly there are other factors besides \textit{TP53} that can modulate GI and AI resistance, and GI is significantly inversely correlated with the average expression of the ER-regulated genes \textit{TFF1}, \textit{GREB1}, \textit{PGR} and \textit{PDZJK1} in ER+ tumors from METABRIC\textsuperscript{24} ($r=-0.24,P<10^{-16}$, Pearson’s) suggesting other factors besides ER are driving proliferation and resistance to AI in tumors with high GI. Even in tumors with high ER expression and good prognosis, \textit{TP53} genomic alterations can results in worse outcomes (Supplemental Figure 11).

Recent work by other groups has associated mutations in DNA repair pathways\textsuperscript{25} or mismatch repair pathways\textsuperscript{19} and co-amplification of \textit{FGFR1} and \textit{CCND1}\textsuperscript{5} with resistance to AI treatment, but we have not observed enrichment of these genomic alterations in our PrRs. This may be due to small samples sizes in each study and additional differences in how AI resistance is classified: we classified response/resistance based on changes of Ki67 between baseline and AI-treated tumors since this dynamic assessment relates to
benefit from treatment. Others have used the level of residual Ki67 in AI-treated tumors as the endpoint to define resistance which reflects residual risk of recurrence on AI. Notably, a patient with a large reduction in proliferation after treatment has clearly benefited from and responded to AI treatment regardless of her residual risk based on Ki67 measurements at surgery.

We conclude that the poor prognosis of ER+ postmenopausal tumors associated with high GI, TP53 LOH and TP53 mutations is at least in part due to intrinsic resistance of these tumors to AI therapy. The short two-week AI treatment can reveal poor anti-proliferative response in these primary tumors indicating that they continue to proliferate in an estrogen deprived environment and do not require further evolution to enable the tumor to resist treatment. It is not clear if high GI or TP53 genomic alterations directly play a role in AI resistance or if these are biomarkers for other drivers of resistance. Further analysis of the >3000 AI-treated patients from POETIC may reveal additional links between GI, TP53 and AI resistance and lead to better treatment for those patients with high GI and intrinsic resistance to AI treatment.

References


Figure Legends:

Figure 1.

A. Arrow plot showing the change in Ki67 between baseline and surgery for GdRs (green), PrRs (red) and untreated Controls (blue). B. Boxplot showing percent of the genome with SCNAs, gains relative tumor ploidy, losses relative to tumor ploidy, LOH and HD for 127 tumor samples (PrRs in red, GdRs in green and controls in blue). Barplot (C) and boxplot (D) showing the average percentage of genome discordant between pairs of core-cuts (baseline and surgery) for all SCNAs (GdRs - green, PrRs - red and untreated Controls - blue).

Figure 2.

Boxplot showing the difference in GI (the percentage of genome with SCNAs) between GdRs (green) and PrRs (red) tumors (A). Comparisons of Ki67 baseline IHC scores with GI (the percent of the genome with SCNAs). B. for GdRs (green circles), PrRs (red squares) and untreated Controls (light blue squares). Comparisons of Ki67 surgery IHC scores after AI treatment with GI (the percent of the genome with with SCNAs). C. PrRs (red squares) and GdRs (green circles). Grey lines represent regression line.

Figure 3.
Percentage of samples with gains relative to tumor ploidy for PrRs (dark red) and GdRs (pink) (A), with losses (light blue – GdR, dark blue – PrR) (B) and with LOH (light green GdR, dark green – PrR) (C) at 47807 segments generated from POETIC SCNA analysis.

Percentage of samples with LOH (light green GdRs, dark green – PrRs) for chromosome 17 (D) including table for LOH events at TP53 (E) and difference in the % of samples with LOH between PrRs and GdRs (F). Boxplots showing the percent of 17p with LOH for GdRs (green) and PrRs (red) (G) and barplots showing the percent of LOH at 17p for each tumor (PrRs –red, GdRs – green) (H).

Figure 4.

A. Barplot showing percentage of GdR (green) and PrR (red) samples with TP53\textsuperscript{WT} and no LOH at the TP53 locus, TP53\textsuperscript{WT} and LOH at the TP53 locus, TP53\textsuperscript{MUT} and no LOH at the TP53 locus and TP53\textsuperscript{MUT} and LOH at the TP53 locus. Note: One GrR does not have exome sequencing data. Boxplot showing the % change in Ki67 (B), Ki67 baseline scores (C), Ki67 surgery score (D) and GI (the percentage of the genome with SCNAs) (E) for PrRs with TP53\textsuperscript{WT} and no LOH at the TP53 locus, TP53\textsuperscript{WT} and LOH at the TP53 locus, and TP53\textsuperscript{MUT} and LOH at the TP53 locus. There are no PrR samples with TP53\textsuperscript{MUT} and no LOH at the TP53 locus.

Supplemental Tables/Figures

Supplemental Table 1. Clinical, Ki67 and genomic data for 127 tumors in study.

Supplemental Table 2. ASCAT estimates and segments for 127 Tumors in study.

Supplemental Figure 1. Consort Diagram.
Supplemental Figure 2. Percent of representative tumors from each of the 73 patients with gains, losses or LOH and overlaps between segments. Percentage of discordance for technical and biological replicates.

Supplemental Figure 3. ASCAT estimates of SCNA gains, losses and LOH for 127 tumors.

Supplemental Figure 4. Percentage of gains, losses and LOH for 33 AI treated pairs.

Supplemental Figure 5. Percentage of genome with SCNAs and discordance.

Supplemental Figure 6. Independent tumors from same patient.

Supplemental Figure 7. Chromosomal arm analysis of gains, losses and LOH.

Supplemental Figure 8. Differences in percentage of samples with gains, losses and LOH with and without HER2 positive samples.

Supplemental Figure 9. Differences in percentage of samples with SCNAs when including or excluding HER2 positive tumors.

Supplemental Figure 10. Differences in percentage of samples with LOH at 17p when including or excluding HER2 positive tumors.

Supplemental Figure 11. Breast cancer-specific survival (BCSS) survival plots for METABRIC LumA stratified by TP53 mutation.
Figure 1. A. Arrow plot showing the change in Ki67 between baseline and surgery for GdRs (green), PrRs (red) and untreated Controls (blue). B. Boxplot showing percent of the genome with SCNAs, gains relative tumor ploidy, losses relative to tumor ploidy, LOH and HD for 127 tumor samples (PrRs in red, GdRs in green and controls in blue). Barplot (C) and boxplot (D) showing the average percentage of genome discordant between pairs of core-cuts (baseline and surgery) for all SCNAs (GdRs - green, PrRs - red and untreated Controls - blue).
Figure 2. Boxplot showing the difference in GI (the percentage of genome with SCNAs) between GdRs (green) and PrRs (red) tumors (A). Comparisons of Ki67 baseline IHC scores with GI (the percent of the genome with SCNAs) (B) for GdRs (green circles), PrRs (red squares) and untreated Controls (light blue squares). Comparisons of Ki67 surgery IHC scores after Al treatment with GI (the percent of the genome with with SCNAs) (C) PrRs (red squares) and GdRs (green circles). Grey lines represent regression line.
Figure 3. Percentage of samples with gains relative to tumor ploidy for PrRs (dark red) and GdRs (pink) (A), with losses (light blue – GdR, dark blue – PrR) (B) and with LOH (light green GdR, dark green – PrR) (C) at 47807 segments generated from POETIC SCNA analysis. Percentage of samples with LOH (light green GdRs, dark green – PrRs) for chromosome 17 (D) including table for LOH events at TP53 (E) and difference in the % of samples with LOH between PrRs and GdRs (F). Boxplots showing the percent of 17p with LOH for GdRs (green) and PrRs (red) (G) and barplots showing the percent of LOH at 17p for each tumor (PrRs –red, GdRs – green) (H).
Figure 4. A. Barplot showing percentage of GdR (green) and PrR (red) samples with TP53WT and no LOH at the TP53 locus, TP53WT and LOH at the TP53 locus, TP53MUT and no LOH at the TP53 locus and TP53MUT and LOH at the TP53 locus. Note: One GrR does not have exome sequencing data. Boxplot showing the % change in Ki67 (B), Ki67 baseline scores (C), Ki67 surgery score (D) and GI (the percentage of the genome with SCNAs) (E) for PrRs with TP53WT and no LOH at the TP53 locus, TP53WT and LOH at the TP53 locus, and TP53MUT and LOH at the TP53 locus. There are no PrR samples with TP53MUT and no LOH at the TP53 locus.
73 Patients with DNA extractions
192 samples (73 blood, 67 baseline and 62 surgery)

202 samples hybridised
Illumina OmniExpressExome-8 v3

Allele-Specific Copy number Analysis of Tumours (ASCAT) SCNAs Analysis

5 blood samples failed QC and replaced with germline genotype predictions

2 tumour samples failed QC
(2 baseline samples, 1 Good and 1 Poor)

Good Responders (GdRs): 28
Ki67% change after AI 2wks > 75%

Baseline only: 6
(1 with technical replicate)

Surgery only: 6

Pairs: 16
(2 with baseline, 1 with surgery technical replicates)

Poor Responders (PrRs): 28
Ki67% change after AI 2wks < 60%

Baseline only: 8

Surgery only: 3

Pairs: 17
(3 with baseline, 1 with surgery technical replicate)

Controls: 17
No AI treatment

Baseline only: 2

Surgery only: 4

Pairs: 11
(2 with surgery technical replicates)

202 samples hybridised
Illumina OmniExpressExome-8 v3

Allele-Specific Copy number Analysis of Tumours (ASCAT) SCNAs Analysis

5 blood samples failed QC and replaced with germline genotype predictions

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(3 with baseline, 1 with surgery technical replicate)

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Pairs: 11
(2 with surgery technical replicates)
Supplemental Figure 2. Percent of representative tumors from each of the 73 patients with gains (A), losses (B) or LOH (C) for each of 47807 bedtool segments separated by chromosome. Segments generated by integrating all ASCAT output segments for all tumor samples with bedtools multiintersect tool. For patients with multiple tumor samples, representative tumor was randomly chosen. Plot (D) showing percent of tumors with gains (red), losses (blue) and LOH (green) for each segment from Supplemental Figures 1B-D. Venn diagram (E) showing the overlap between gains, losses and LOH of ASCAT output segments from representative tumors of all 73 patients.
Supplemental Figure 3B
Supplemental Figure 3. ASCAT estimates of SCNA gains (red) and losses (blue) relative to tumour ploidy for GdRs (A), PrRs (B) and Control samples (C) for baseline and surgery samples. Green bars represent regions with LOH. Label to right of figure shows sample ID and timepoint of biopsy (B – Baseline and S – Surgery). Samples with baseline and surgery pairs or replicate samples are grouped together at top of figures.
Supplemental Figure 4. Percentage of 33 AI treated pairs with gains relative to tumour ploidy (A) in baseline (dark red) and surgery (pink) samples, with losses relative to tumor ploidy (B) in baseline (dark blue) and surgery (light blue) samples, and with LOH (C) in baseline (dark green) and surgery (light green) samples. Difference in the percentage of SCNA gains (D), losses (E) and LOH (F) between surgery and baseline samples (% surgery SCNA - % baseline SCNA).
Supplemental Figure 5

A. Boxplot showing percent of genome with SCNAs that are exclusive to one sample from a pair of baseline and surgery samples or from technical replicate samples taken from the same timepoint. B. Boxplot showing GI (percent of genome with SCNAs) for samples in which both samples in the baseline-surgery pair were observed to have exclusive SCNAs that covered at least 10% of the genome (Both), samples in which only one sample in the pair was observed to have exclusive SCNAs that covered at least 10% of the genome (Single) or none of the samples in the pairs had exclusive SCNAs covering more that 10% of the genome (None).

Supplemental Figure 6

A. ASCAT estimates of SCNA gains (red) and losses (blue) relative to tumour ploidy and regions with LOH (green) in paired set of core-cuts in which the SCNAs exclusive to the baseline and surgery sample are greater than the SCNAs shared between the pair of samples. B. Comparison of B or Minor allele frequencies (BAF) for baseline and surgery biopsy samples showing the tumor biopsies are from the same individual as there is a very high concordance for alleles to be either homozygous or heterozygous in both samples.
Supplemental Figure 7. Boxplots showing the percent of each chromosomal arm with gains (A), losses (B) and LOH (C) for GdRs (green) and PrRs (red). Significance of difference between PrRs and GdRs based on Mann-Whitney tests (one sided) after multiple correction (FDR BH) is also shown.
Supplemental Figure 8. Difference in the percentage of SCNA gains, losses and LOH (A) between PrR and GdR samples (% PrR SCNA - % GdR SCNA). Boxplot showing percent of genome with SCNAs (B), gains (C), losses (D) and LOH (E) for GdRs, PrRs that are HER2 negative and PrRs that are HER2 positive. Percentage of samples with gains relative to tumour ploidy for PrRs (dark red) and GdRs (pink), with losses (light blue – GdR, dark blue – PrR) and with LOH (light green GdR, dark green – PrR) at 47807 bedtool segments generated from POETIC SCNA analysis with HER2 positive samples removed. Difference in the percentage of SCNA gains, losses and LOH (G) between PrR and GdR samples (% PrR SCNA - % GdR SCNA) including (red) or excluding (light blue) HER2 positive samples.
Supplemental Figure 9. Boxplots showing the percent of each chromosomal arm with gains (A), losses (B) and LOH (C) for GdRs (green) and PrRs (red) for HER2-negative tumors. Significance of difference between PrRs and GdRs based on Mann-Whitney tests (one sided) after multiple correction (FDR BH) is also shown.
Supplemental Figure 10. Percentage of samples with LOH (light green GdR, dark green – PrR) for chromosome 17 (A) and difference in the % of samples with LOH between PrRs and GdRs (B). HER2 positives samples were removed from the PrR samples.
Supplemental Figure 11. Breast cancer-specific survival (BCSS) survival plots for METABRIC LumA stratified by TP53 mutation (A) and GI (B). Samples in the top 25% of GI for LumA tumors (>35% of genome with SCNAs) were defined as having high GI (red). Kaplan-Meier survival curve analysis was performed in R (version 3.2.3) with survfit function from the survival package and survival plots generated with ggsurvplot function from the survminer package.