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The AURORA pilot study for molecular screening of patients with advanced breast cancer—a study of the breast international group

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Several studies have demonstrated the feasibility of molecular screening of tumour samples for matching patients with cancer to targeted therapies. However, most of them have been carried out at institutional or national level. Herein, we report on the pilot phase of AURORA (NCT02102165), a European multinational collaborative molecular screening initiative for advanced breast cancer patients. Forty-one patients were prospectively enrolled at four participating centres across Europe. Metastatic tumours were biopsied and profiled using an Ion Torrent sequencing platform at a central facility. Sequencing results were obtained for 63% of the patients in real-time with variable turnaround time stemming from delays between patient consent and biopsy. At least one clinically actionable mutation was identified in 73% of patients. We used the Illumina sequencing technology for orthogonal validation and achieved an average of 66% concordance of substitution calls per patient. Additionally, copy number aberrations inferred from the Ion Torrent sequencing were compared to single nucleotide polymorphism arrays and found to be 59% concordant on average. Although this study demonstrates that powerful next generation genomic techniques are logistically ready for international molecular screening programs in routine clinical settings, technical challenges remain to be addressed in order to ensure the accuracy and clinical utility of the genomic data.

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INTRODUCTION

Several efforts have advanced our understanding of the alterations characterizing cancer genomes.1–2 Coupled to recent successes of targeted therapies in patients with molecularly profiled tumours3–4 and the decreasing costs of massively parallel sequencing, this has motivated several studies, albeit of limited size, to investigate the implementation of personalised molecular screening in the clinical settings.5–7

Most of these studies were focused on primary tumours and despite growing evidence that distant metastases may harbour additional molecular alterations absent from their matched primaries,8–12 genomic information about metastatic disease remains limited. Even though the clinical relevance of many of these alterations remains to be established, it is increasingly recognised that molecular profiling of advanced disease could help elucidate the biological underpinnings of phenomena such as distant recurrence and the emergence of de novo resistance to therapy.13 Lastly, in order to find applications in routine clinical practice, it is essential to assess the reliability and robustness of the chosen sequencing platform using orthogonal sequencing strategies.14,15

The Breast International Group launched AURORA—Aiming to Understand the Molecular Aberrations in Metastatic Breast Cancer, a pan-European molecular screening programme whose main goal is to deepen our knowledge of the genomic landscape of advanced breast cancer.16 Herein, we report on the pilot phase of this study whereby the primary objective was to investigate the feasibility with four European recruitment sites and central pathological and sequencing facilities. Secondary aims were to assess the concordance of somatic mutations between two targeted next generation sequencing (NGS) platforms and of somatic copy number aberrations (CNA) obtained from NGS and single nucleotide polymorphism (SNP) arrays.

RESULTS

Patient recruitment and logistics

A total of 41 patients provided informed consent and were enrolled in this pilot study between February 2013 and September 2014. Fig. 1a and b illustrates the study design and Table 1 summarises the clinical and pathological characteristics of the patients. Formalin fixed paraffin embedded (FFPE) biopsies of

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metastatic lesions and whole blood were prospectively collected from 35 (85%) of them. 20 (57%) patients had ER+ metastatic disease, 7 (20%) were HER2+ and 8 (23%) were triple negative breast cancer. Fig. 1c and d represent the distribution of the different anatomical sites and the breakdown of patients’ recruitment by participating centre. Following central pathological review, a tumour content below 10% was recorded for 8 (23%) patients whilst the median cellularity was 50% (range 10–85). One additional sample had an insufficient amount of extracted DNA whilst the median yield was 3.6 µg (range 0.075–59.2). Overall, real-time Ion Torrent sequencing results were obtained for 26 (74%) of the 35 patients (Fig. 1b). The median global turnaround time (TAT) from patient biopsy to sequencing results was 51 working days (range 16–146) whilst the median delivery TAT from sample reception at the sequencing facility to final NGS report was 9 working days (range 5–17) (Fig. 1e). There were no significant differences in TAT between the recruiting centres (Fig. 1f and g).

Mutation detection from the Ion Torrent OncoDEEP clinical cancer panel and CNA using SNP arrays

Quality control metrics for the sequencing data are provided in Supplementary Figure S1. The target regions were covered on average at 1758X (494–3075X) sequencing depth. Non-synonymous somatic mutations were called from the OncoDEEP clinical cancer panel in exons covered by at least 100X sequencing depth and a fixed threshold of 10% variant allele fraction (VAF). In total, 128 unique genes harboured at least one mutation representing one third of the panel (Fig. 2a). The median number of mutations indexed per patient was 6 (range 0–35) and the overall mutation detection rate i.e., percentage of patients where at least one mutation could be indexed, was 96%. Only 8 (4.15%) of the 193 mutations had previously been described in release v.76 of COSMIC in any type of cancer whilst 178 (92.23%) mutations had never been reported in the literature. The most frequently mutated genes were PIK3CA (50%), TP53 (31%), SYNE1 (19%), and NF2 (15%).
Supplementary Tables S1 and S2 provide the actionable alterations from targeted NGS and SNP arrays and the corresponding copy number level as determined by SNP copies) which were missed by FISH assays owing to centromere 17 evidence of a correspondence between false negative calls were recorded for actional and biologically relevant alterations. 19 of 26 (73%) patients presented with at least one CNA and the CNA frequencies were comparable to those reported in the literature (Fig. 2d). This choice was guided by a low global Illumina specific false negative error rate on cluster 2 substitutions whilst maintaining a relatively low global Illumina specific false positive error rate on cluster 2 and 3 substitutions. Using this approach, a global concordance rate of 40.9% was achieved.

Substitutions in cluster 1 cannot be reconciled. There was a statistically significant difference in coverage between the Ion Torrent and Illumina NGS data for these substitutions (Fig. 3d). However, all but one of the substitutions were covered in excess of 100X in the Illumina NGS data and the residual coverage, which is the difference between the observed sequencing depth and the expected value at 99% statistical power to detect a mutation for given CCF and copy numbers, were positive for all except two substitutions (Fig. 3e). Similarly, two substitutions were called in cluster 3 which constitute Illumina specific false positives and were covered at 239X and 166X8 using the Ion Torrent NGS platform. In total, only 2.7% of the substitutions in cluster 2 were Illumina specific false positives using the Ion Torrent platform as standard whilst 5.41% were Illumina specific false negatives. There were no statistically significant differences in sequencing coverage between these groups of substitutions and the true positives called by both platforms (Fig. 3f). Furthermore, similar to cluster 1 substitutions, all the Illumina specific false negatives had positive residual coverage (Fig. 3e). For comparison, the sequencing coverage and the percentage of non-reference bases at these particular loci in the normal matched samples are shown in Supplementary Figure S6.

Comparison of CNA from targeted NGS and SNP arrays
The CNA obtained from the Ion Torrent sequencing were also compared to the profiles obtained from SNP arrays and Illumina

### Table 1. Clinical and pathological characteristics of patients and biopsies (n = 35)

| Characteristics                        | Number of patients (%) |
|----------------------------------------|------------------------|
| Age (years)                            |                        |
| Median                                 | 56                     |
| Range                                  | 23–73                  |
| ECOG performance status<sup>a</sup>    |                        |
| 0                                      | 16 (45)                |
| 1                                      | 17 (49)                |
| 2                                      | 2 (6)                  |
| Breast cancer subtype<sup>b</sup>      |                        |
| ER+                                    | 20 (57)                |
| HER2+                                  | 7 (20)                 |
| ER-/HER2-                              | 8 (23)                 |
| Number of prior lines of therapy       |                        |
| 0                                      | 6 (17)                 |
| 1                                      | 5 (14)                 |
| 2                                      | 4 (11)                 |
| 3                                      | 6 (17)                 |
| >3                                     | 14 (41)                |
| Number of metastatic sites             |                        |
| 1                                      | 8 (23)                 |
| 2                                      | 10 (28)                |
| 3                                      | 8 (23)                 |
| >3                                     | 9 (26)                 |

<sup>a</sup> ECOG; Eastern Cooperative Oncology Group, ER; oestrogen receptor evaluated by IHC, HER2; human epidermal growth factor receptor 2 evaluated by IHC and FISH. The breast cancer subtypes are based on the characteristics of the metastatic lesions.

We evaluated two established methods to call CNA from SNP arrays and obtained similar results (Fig. 2c and d) thereby attesting to the robustness of SNP arrays for the estimation of CNA. All the patients presented with at least one CNA and the CNA frequencies were comparable to those reported in the literature (Fig. 2d–g). For instance, 13 (72%) patients harboured a gain or an amplification of chromosome 8q where the MYC oncogene resides whilst 9 (50%) had a deletion of 17p where TP53 is located.

Two patients presented with a higher than expected number of mutations. The integration of the sequencing and CNA data obtained from these two outlier patients is shown in Supplementary Figure S2. In general, the distributions of VAF and cancer cell fractions (CCF) were high and tightly clustered, indicative of genuine somatic mutations. For the whole cohort, ERBB2 amplification status was assessed centrally using FISH whilst PTEN loss was evaluated by immunohistochemistry (IHC). These single assay data were compared with CNA obtained from the SNP arrays and the results are shown in Supplementary Figures S3 and S4. No false negative calls were recorded for ERBB2 amplification status comparing SNP arrays to FISH data. However, 7 of 18 patients (39%) effectively harboured supernumerary copies of ERBB2 (n ≤ 4 copies) which were missed by FISH assays owing to centromere 17 co-amplification or low level 17q polysomy. There was no concrete evidence of a correspondence between PTEN IHC staining results and the corresponding copy number level as determined by SNP array.

Actionable alterations from targeted NGS and SNP arrays
Supplementary Tables S1 and S2 provide the definitions of actionable and biologically relevant alterations. 19 of 26 (73%) patients harboured at least one actionable mutation, such as PIK3CAp.H1047R or ESR1p.Y537N, whilst 8 (31%) had only one such mutation and six (23%) had at least one biologically relevant mutation and none that were clinically actionable (Supplementary Figure S5). Comparatively, 16 of 18 (89%) patients for whom the SNP arrays were available harboured at least one actionable CNA, such as EGFR amplification, whilst 2 (11%) had only one such aberration and an equal number had at least one biologically relevant CNA and none that were clinically actionable. Using the combined information from the SNP arrays and the targeted sequencing, and focusing on the subset of strictly actionable alterations, 23 of 26 (88%) patients had at least one clinically actionable alteration whilst only 3 (12%) had only one such alteration. Overall, of the initial 7 patients without actionable mutations, four were found to harbour at least one actionable CNA.

Orthogonal cross-testing of substitution calls using Illumina NGS
The substitutions obtained from the Ion Torrent sequencing were compared to data generated from the Illumina NGS platform. The same DNA samples were sequenced and substitutions were called in overlapping regions using similar filtering criteria and a combination of three established mutation callers. Patient matched data were available for 19 (73%) patients and all three mutation callers were mostly consistent (Fig. 3a). All but one actionable substitution, NOTCH1p.D2082E in cluster 1 (see below), were concordant.

The substitutions were categorised into three clusters and the error rates of each mutation caller and several combinations thereof were benchmarked using this framework. Substitutions in clusters 1 and 3 are exclusive to one of the sequencing platforms and have 0% VAF in the alternate data, whereas substitutions in cluster 2 are those found by either or both NGS platforms and have non-zero VAF in both sequencing data. The definite calls were made for each substitution based on a majority vote of any two of the three mutation callers (Fig. 3b and c). This choice was guided by a low global Illumina specific false negative error rate on cluster 2 substitutions whilst maintaining a relatively low global Illumina specific false positive error rate on cluster 2 and 3 substitutions. Using this approach, a global concordance rate of 40.9% was achieved.

Substitutions in cluster 1 cannot be reconciled. There was a statistically significant difference in coverage between the Ion Torrent and Illumina NGS data for these substitutions (Fig. 3d). However, all but one of the substitutions were covered in excess of 100X in the Illumina NGS data and the residual coverage, which is the difference between the observed sequencing depth and the expected value at 99% statistical power to detect a mutation for given CCF and copy numbers, were positive for all except two substitutions (Fig. 3e). Similarly, two substitutions were called in cluster 3 which constitute Illumina specific false positives and were covered at 239X and 166X8 using the Ion Torrent NGS platform. In total, only 2.7% of the substitutions in cluster 2 were Illumina specific false positives using the Ion Torrent platform as standard whilst 5.41% were Illumina specific false negatives. There were no statistically significant differences in sequencing coverage between these groups of substitutions and the true positives called by both platforms (Fig. 3f). Furthermore, similar to cluster 1 substitutions, all the Illumina specific false negatives had positive residual coverage (Fig. 3e). For comparison, the sequencing coverage and the percentage of non-reference bases at these particular loci in the normal matched samples are shown in Supplementary Figure S6.
NGS Fig. 4a–c show the Log$_2$ ratios from the SNP array and both NGS platforms for a case patient. The segmented Log$_2$ ratios from the SNP array and Illumina sequencing clustered, as expected, into canonical copy number genotypes whilst the segmented Log$_2$ ratios obtained from the Ion Torrent NGS platform were spread between those canonical genotypes leading to relatively poor correlation in Log$_2$ ratio data space (Fig.4d and e). Nonetheless, this approach was applied to each copy number platform for 14 (54%) patients for whom all three data types were available and the segmented Log$_2$ ratios were compared genome-wide using the Spearman’s correlation. The distribution of correlation coefficients comparing the SNP array to the NGS data was bimodal for the Ion Torrent platform with 5 (29%) samples displaying a poor correlation of $\rho < 0.5$ (Fig. 5a). The median correlation coefficients were $\rho = 0.615$ and $\rho = 0.745$ for the Ion Torrent and Illumina NGS platforms respectively.

In order to further evaluate the ability of both NGS platforms to call CNA, the segmented Log$_2$ ratios were grouped into three categories i.e. deletion (−1), copy neutral (0), gain/amplification (1) and further compared using the accuracy which is the sum of concordant calls relative to the total number of aberrations. Fig. 5b shows the distribution of accuracy values evaluated genome-wide for each patient. The average accuracy of CNA call was 59.1% for the Ion Torrent and 74.0% for the Illumina NGS platforms. Both
distributions were bimodal with 6 (35%) and 5 (29%) patients showing low values of $\alpha < 0.5$ and $\alpha < 0.6$ for the Ion Torrent and Illumina NGS platforms, respectively. Lastly, the ability of both sequencing platforms to call CNA in particular genes of interest was evaluated on 15 clinically actionable or biologically relevant genes. For each gene, the CNA calls were pooled and the cohort-wise concordance measured as the accuracy of concordant calls. Fig. 5c contrasts the distribution of accuracy values observed across these 15 genes for both NGS platforms. We observed relatively high concordance rates using the Illumina NGS platform for genes frequently aberrant in breast cancers e.g., 90.1% (95% CI: 86.8–93.3) for MYC, 63.8% (95% CI: 61.4–66.2) for CCND1 and 57.4% (95% CI: 52.3–62.5) for ERBB2 whilst the corresponding values for the same genes were 29.5% (95% CI: 27.5–31.8), 40.2% (95% CI: 37.7–42.7) and 28.3% (95% CI: 25.6–30.9), respectively, using the Ion Torrent sequencing platform. There was a significant negative correlation between the accuracy and the number of aberrations per patient for the Ion Torrent data (Fig. 5d) but not for the Illumina NGS platform (Fig. 5e). There were no associations between the accuracy measured cohort-wise and the frequency of aberrations affecting the 15 genes of interest for either of the two NGS platforms (Fig. 5f and g).
DISCUSSION

The growing number of targeted anticancer therapies either approved or under clinical development has led to a wide interest in personalised treatment approaches in clinical practice. However, many of these cancer molecular screening initiatives have had limitations. For instance, several of these screening programs used different sequencing techniques and very often lacked orthogonal validation. Together with varying concepts of what constitutes an actionable mutation, this makes the results difficult to compare. The current study is a pilot phase undertaken to evaluate the feasibility of AURORA, a pan-European molecular screening programme for advanced breast cancer patients.

The success rate for the primary endpoint of 63% compares favourably with existing literature. However, many of these cancer molecular screening initiatives have had limitations. For instance, several of these screening programs used different sequencing techniques and very often lacked orthogonal validation. Together with varying concepts of what constitutes an actionable mutation, this makes the results difficult to compare. The current study is a pilot phase undertaken to evaluate the feasibility of AURORA, a pan-European molecular screening programme for advanced breast cancer patients.

The median global TAT of 51 working days was due to unforeseen delays either between patient consent and surgical biopsy or between collection of biological samples and shipment to the central pathology laboratory. In part, due to the above findings, these parameters are now rigorously monitored in the parent programme where the global TAT does not exceed 20 working days (personal communication). 73% of the patients harboured one or more actionable mutations and this increased to 88% when considering the underlying CNA. These numbers are encouraging on account of the availability of alterations that can be theoretically targeted despite limited clinical trial based evidence of benefit to patient survival from targeted therapy in breast cancer. Yet, the decision to prioritise a particular molecular target for trial allocation given several such alterations within the same patient constitutes a considerable challenge for which specific algorithms and combined expertise will be required in the future.

Regarding the secondary endpoint and considering clinically actionable mutations, our results are reassuring. All but one substitution was positive using both the Ion Torrent and Illumina sequencing platforms. Furthermore, from a pragmatic viewpoint, 5 (26%) sample pairs showed complete agreement. However, no single mutation caller was able to reproduce globally with 100% concordance the results obtained in real-time from the Ion Torrent NGS platform. The Illumina specific false negative and false positive rates over somatic substitutions with non-zero VAF in both data types can be reasonably contained whilst substitutions
private to either sequencing technology despite adequate coverage in both data types were more frequent in the Ion Torrent data. Thus, from a technical perspective, our results are somewhat sobering and whilst the higher background error rate of the Ion Torrent NGS technology could be a contributing factor, the small number of samples and mutations assessed here do not allow for a full exploration of the possible causes of these discrepancies.

Massively parallel sequencing using targeted gene panels has emerged as a technology that can be applied within a clinically meaningful TAT to identify known biomarkers of resistance or sensitivity to a given drug. In the broader context of AURORA, all the results are reported to investigators unaltered. However, our analysis shows the major limitations of this approach outside known mutation hotspots and in anticipation, the AURORA study design allows biobanking of frozen and residual FFPE material for later research purposes using alternative high-throughput technologies such as whole genome or exome sequencing.

To the best of our knowledge, no other studies have compared prospectively CNA obtained from targeted sequencing data to SNP arrays in a clinical setting. Our results show that the gold

![Fig. 5](https://example.com/fig5.png)

**Fig. 5** Comparison of copy number profiles from SNP arrays and targeted sequencing data. a distribution of Spearman’s correlation coefficient comparing the segmented Log₂ ratios from the SNP arrays and the NGS data across patients with all three data types. The segmented Log₂ ratios were further categorised into copy number aberration calls i.e., −1, 0 and +1 and compared patient wise between the SNP arrays and the NGS data. The resulting distributions of accuracy values are depicted in b. For a small set of 15 clinically actionable or biologically relevant genes, the accuracy values comparing SNP arrays and NGS data were computed using all available samples and displayed individually for each gene in c, d, e correlation of accuracy values measured genome-wide for each patient as in b vs. the number of aberrations as determined by the corresponding NGS platform. f, g correlation of accuracy for each of the 15 clinically actionable or biologically relevant genes vs. the frequency of aberrations for the same genes measured using the SNP array. In c, each value of accuracy was generated by 100 bootstrap replicates. The values displayed represent the mean of the replicates and the error bars represent the standard error of this estimate.
standard FISH assay for \textit{ERBB2} is fully concordant with SNP arrays. However, the Illumina NGS platform outperforms the Ion Torrent technology when compared to the same SNP arrays. This is not unexpected since the Illumina NGS panel accommodates intergenic targets which, combined with the fact that hybrid capture methods for sequencing library preparation often carry off-target reads, increases the effective number of regions where copy numbers can be evaluated. Several algorithms\textsuperscript{22, 23} take advantage of this, benefiting downstream CNA calling and translating into higher concordance with the denser SNP arrays. The combination of assays, such as mutation profiling and assessment of CNA, into one cost effective package is an attractive idea. Together with a low requirement of input DNA, as is often obtainable from clinical samples, makes for a compelling argument in favour of the Ion Torrent NGS platform. However, given the poor agreement with solid benchmarks such as SNP arrays, the results presented here do not support, at least for now, the targeted Ion Torrent NGS platform as a truly multipurpose assay in a clinical setting. Within AURORA, central pathology results reported to investigators in real-time include \textit{ERBB2} IHC as well as FISH when applicable. This is clinically meaningful since \textit{ERBB2} gene amplification remains, to date, the sole approved CNA biomarker in breast cancer. Sub-studies undertaken in the framework of the main AURORA programme include real-time SNP arrays and retrospective whole genome sequencing to alleviate the technical limitations of identifying CNA using the Ion Torrent technology and will allow the study of the clonal evolution of breast cancer.

Overall, our study contrasts the benefits and pitfalls of personalised molecular screening for patients with advanced cancer involving the combination of multiple high-throughput genomic techniques. In view of these results, greater effort is being devoted to improving the concordance of mutation calls between different NGS platforms and to harmonising CNA calls with SNP arrays in the ongoing AURORA programme. With recent findings on the clinical utility of circulating tumour DNA\textsuperscript{71, 24} and its implementation into the AURORA study design, it becomes imperative to thoroughly evaluate the technical feasibility of profiling circulating biomarkers to support clinical decision making.

\section*{Methods}

\subsection*{Patients and samples}

Patients were enrolled in four European centres (Institut Jules Bordet, Belgium; Hospital Vall d’Hebron, Spain; Sana Klinikum Offenbach, Germany; and Dundee Cancer Centre, United Kingdom). The study was approved by the respective ethical committees from the named institutions and was performed in accordance with relevant guidelines and procedures. Patients considered eligible for this study were those (1) with histologically proven distant metastatic or locally recurrent invasive breast cancer (2) with an favourable prognosis as assessed by a multidisciplinary board and (3) with clinical and laboratory parameters safe for tumour biopsy, (4) for whom FFPE tumour tissue from a locally recurrent or distant metastatic or locally recurrent invasive breast cancer (2) with an favourable prognosis as assessed by a multidisciplinary board and (3) with clinical and laboratory parameters safe for tumour biopsy, (4) for whom FFPE tumour tissue from a locally recurrent or metastatic lesion and whole blood for research purposes were available. The samples were centralised in real time at the European Institute of Oncology (Milan, Italy) where H&E slides were reviewed by a board-certified pathologist for the evaluation of cellularity. At the central laboratory, IHC for ER, PR, HER2, \textit{Ki67} and \textit{PTEN} were performed using the 

\section*{Bioinformatics analyses}

\textit{Mutation calling from Ion Torrent targeted gene screen}. Sequence reads from the tumour and matched normal samples were aligned against the human genome reference version hg19/GRCh37 using the Ion Torrent TMAP aligner with default parameter settings. Mutations were called from the resulting BAM files using the Torrent Suite variant caller (Life Technologies) with the default settings of the ‘Somatic High Stringency’ pipeline and cross-checked using the NextGEne software (Softgenetics) using the ‘Ion Torrent’ predefined pipeline. Germ line mutations were filtered by extracting variants found in the matched normal sample from those called in the corresponding tumour sample. The resulting somatic mutation calls were further filtered to exclude variants (1) that were not sequenced in both sense with a minimum ratio of 10/90\%, (2) with less than 100 read depth, and (3) variant allele fractions lower than 10\% in the tumour sample. For an alpha list of target genes (Supplementary Table S5),
mutations occurring below 10% VAF were accepted. Manual evaluation was done for indels using IGV.25

**Mutation calling from Illumina targeted gene screen.** Sequence reads from the tumour and matched normal samples were pre-processed according to the GATK best practices. The raw reads were aligned against the human genome reference hg19/GRCh37 using the BWA aligner.26 Duplicate reads were marked using Picard following which the data were filtered to keep only properly paired and mapped reads with mapping quality greater than 60. Sequence reads around potential indels were locally realigned and the base quality scores were recalibrated using GATK.27 Mutations were called from the resulting BAM files using MuTect2,28 SomaticSniper29 and Strelka30 in matched tumour/normal mode using the default parameter settings for each of the aforementioned algorithms. The resulting somatic mutation calls were further filtered to include only variants (1) occurring in regions common to both Ion Torrent and Illumina sequencing panels, (2) having a VAF above 10% in the tumour sample, and (3) having a read depth greater than 50X. Similar to the Ion Torrent NGS, mutations occurring in the alpha list (Supplementary Table S5) were accepted if they filtered to include only variants (1) occurring in regions common to both Ion Torrent and Illumina sequencing panels, (2) having a VAF above 10% in the tumour sample, and (3) having a read depth greater than 50X. Similar to the Ion Torrent NGS, mutations occurring in the alpha list (Supplementary Table S5) were accepted if they occurring below 10% VAF were accepted. Manual evaluation was done for indels using IGV.25

**Copy number aberration analysis using SNP arrays.** The raw intensity values were normalised to obtained Log_{2} ratios, B Allele Frequencies (BAF) and genotyping calls (AA/AB/BB) using Affymetrix Power Tools. We used release NA.33 of the NetAffx library for the reference model and annotation. We computed the Median Absolute Deviation and the Median Auto-Correlation from the Log_{2} ratios as quality control metrics and used a threshold of 0.40 and 0.45, respectively, to discard failed arrays. We used two parallel approaches involving (a) allele specific copy number analysis using heterozygous SNP probes and (b) total copy number analysis using the full set of 200K markers and parameters from (a) to control for the cancer cell fraction and genomic mass. From the BAF and genotyping calls, only informative SNP probes displaying heterozygous genotype (AB) and 0.1 < BAF < 0.9 were kept for analysis at (a). The Log_{2} ratios and BAF were smoothed using the median absolute deviation and segmented jointly using a multitrack segmentation algorithm from the library copynumber31 to determine common breakpoints. Estimates for the cancer cell fraction and genomic mass were obtained using GAP32 and segmented by penalised least square segmentation35 and CNA calls were obtained using the default clustering algorithm. The pool of normal samples were marked using Picard following which the data were filtered to keep only properly paired and mapped reads with mapping quality greater than 60. Sequence reads around potential indels were locally realigned and the base quality scores were recalibrated using GATK.27 Mutations were called from the resulting BAM files using MuTect2,28 SomaticSniper29 and Strelka30 in matched tumour/normal mode using the default parameter settings for each of the aforementioned algorithms. The resulting somatic mutation calls were further filtered to include only variants (1) occurring in regions common to both Ion Torrent and Illumina sequencing panels, (2) having a VAF above 10% in the tumour sample, and (3) having a read depth greater than 50X. Similar to the Ion Torrent NGS, mutations occurring in the alpha list (Supplementary Table S5) were accepted if they occurring below 10% VAF were accepted. Manual evaluation was done for indels using IGV.25

**Statistical analyses** Integration of the VAF of mutations indexed by the Ion Torrent NGS platform and CNA profiles from the matched SNP arrays to compute the Log-Likelihood and individual CCF of each mutation was done using ABSOLUTE.33 Similarly, the sequencing depth required to achieved 99% statistical power to call a given base was computed using ABSOLUTE with an error rate \( E = 2E-03 \) and an FDR = 5E-07. The frequencies of aberration calls and the percentage accuracy were obtained by bootstrapping. The values displayed are mean estimates and confidence intervals are standard errors. The concordance of mutation calls for substitutions was evaluated using the simple matching coefficient. All correlations were measured using the non-parametric Spearman's \( \rho \) coefficient and all statistical hypothesis tests were done using the non-parametric Wilcoxon's rank sum test. \( P \) values were two sided and paired or unpaired as appropriate. Unless otherwise stated, all computations were done in R/Bioconductor.

**Data availability** The sequencing and SNP arrays data have been deposited at the European Genome-Phenome Archive (http://www.ebi.ac.uk/ega/) which is hosted by the European Bioinformatics Institute, under accession number EGAD00001000870.

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**AUTHOR CONTRIBUTIONS** M.P., Sh.L. and C.S. designed the study. M.M. and D.B. analysed the data, interpreted the results and wrote the manuscript. P.A., A.I., F.R., Y.B., D.F., D.Z. and C.D. were involved in data interpretation. P.A. and C.S. designed the study. M.M. and D.B. analysed the data, interpreted the results and wrote the manuscript. P.A., A.I., F.R., Y.B., D.F., D.Z. and C.D. were involved in data interpretation. P.A., S.L., A.T., J.C., S.S., S.V. and M.O. were involved in ethics submission, patient consent and recruitment. G.V. performed the central histopathological review of the samples. J.L. and P.C. generated the sequencing data. All the authors read and approved the final manuscript.

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