BREAST CANCER

Identification of tumor-specific alterations in plasma of breast cancer patients

MATERIALS AND METHODS

For this prospective observational study, we collected 92 plasma samples from 84 advanced HR+/HER2+ breast cancer patients before starting 1st (51 samples) or 2nd (41 samples) line treatment. For eight patients, two blood samples from both time points were available. The study was approved by the ethics committee of the University Hospital Graz (ethical approval number 21-227 ex 09/15), and written informed consent was obtained from all patients. Samples were analyzed as follows (Figure 1): using SIMSeq-Seq (SSS) and the AVENO-cDNA Expanded Kit, enriching for 77 clinically relevant cancer genes, PIK3CA-mutation detection and variant allele frequencies (VAF) were compared between the two methods. Additionally, nMafSeq was used to estimate the tumor fractions in plasma samples.

RESULTS

Of the initial 92 samples, three failed with the SSS assay, leaving 89 samples from 82 patients for a head-to-head comparison of variants covered by both assays. Remarkably, we observed an excellent concordance rate of 97.8% (82/89 samples) between the AVENO and the SSS assay in detecting PIK3CA hot spot mutations, confirming the high sensitivity of the panel sequencing assay in detail, one variant was missed in one sample by the SSS assay, while the AVENO assay failed to detect a variant in another sample (Table 1). Across both assays, 39 variants were detected in 36 samples (40.4%), although two samples were slightly below the LOD (limit of detection) of the SSS assay. Notably, both assays consistently identified concurrent double mutations in three distinct samples. 53/89 samples (59.6%) had no PIK3CA variants detected by either assay. Beyond the overlapping variants, the AVENO assay detected 10 additional alterations in the PIK3CA gene (Table 2).

The high concordance rate between the two assays was also confirmed by excellent agreement (Cohen’s kappa = 0.95, 95% CI 0.89–1.00, Figure 2A). Furthermore, Spearman rank correlation demonstrated a strong, positive association between the VAF measured by the AVENO assay (SSS) and the ratio of the same mutations in the AVENO assay, yielding a rho value of 0.91 (95% CI 0.94–0.99, p < 0.001, Figure 2B). This robust correlation highlights the remarkable consistent performance and high reliability of both assays in quantifying PIK3CA mutations.

The median –score from nMafSeq analyses was 2.54 (0.07–10.756) percent; 1.06–5.415) and 39/89 (44%) samples had ≥3.3, indicating elevated tumor fractions (V%). An elevated –score was a strong predictor for worse progression-free survival (HR 95% CI 2.44–4.29, 1.24–2.416, p = 0.008, Figure 3).

In our study, the AVENO cDNA Expanded Kit demonstrated comparable sensitivity and concordance rates to the SSS assay in detecting PIK3CA hot spot mutations in advanced breast cancer patients. Furthermore, we demonstrated a significant advantage of panel sequencing over a single-gene approach that interrogating multiple genes can indicate a true negative PIK3CA result if other variants present with a high VAF. Moreover, other actionable targets or mechanisms of resistance can be captured simultaneously, thus potentially improving the efficacy and precision treatment of metastatic breast cancer patients.

Beyond PIK3CA alterations, important potential targets including ESR1 and BRCA1, could be identified. ESR1 mutations were identified in 21% overall and are associated with endocrine resistance. Of note, our patients were treated with endocrine treatment regardless of cDNA results, i.e., ESR1 and BRCA1 may potentially represent resistance mechanisms to CDK4/6 inhibitors. Although BRCA1 alterations were identified in this study as variants of unclear significance, their role in responsiveness to CDK4/6 inhibitors should be further evaluated. As shown in Figure 4, no alterations could be identified in eleven variant line samples compared to two initial line samples.

Furthermore, we have repeatedly shown that elevated cDNA fractions are associated with worse prognosis and shorter PFS, which could be confirmed in the present study.

To our knowledge, this is the first study to confirm that the AVENO cDNA Expanded Kit can be used with comparable sensitivity as SSS for the detection of PIK3CA mutations to identify patients who are candidates for alpelisib treatment and, in the same analyses, reveal additional potential molecular markers.

REFERENCES