Real-world transcriptomic biomarkers as replacement for immunohistochemistry and FISH studies in breast cancer

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Introduction

HER2 amplification and hormone receptors are binary biomarkers for selecting breast cancer therapy and predicting outcomes. In the era of antibody-drug conjugates (ADC), a relatively low HER2 expression level is adequate for targeting tumor cells. We explored the potential of RNA profiling, determined by next generation sequencing (NGS), to provide more flexible clinical biomarkers as compared with immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH)

Methods and Materials

Patients' characteristics

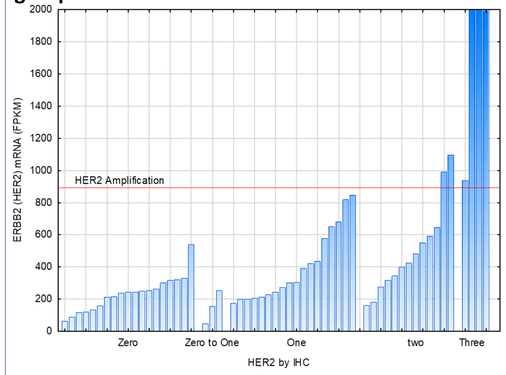
Age median (range)	52 (26-84)
Race	Asian: 4 African American: 11 White: 23 Hispanic or Latino : 8 Other or unknown :11
Stage	I : 3 II : 13 III: 14 IV: 20 Unspecified: 7
Grade	I: 2 II : 20 III: 37 IV : 4 Unspecified: 4
Tumor	T1: 10 T2: 12 T3: 14 T4: 8 Unspecified: 13
Node	N0: 14 N1: 26 N2: 4 N3: 4 Unspecified: 9
Metastasis	M0: 31 M1: 19 Unspecified: 7

Clinical and laboratory data from 57 breast cancers collected by the COTA real-world data company were used to study biomarker levels as detected by routine clinical transcriptomic tests. HER2 (*ERBB2*), estrogen receptor alpha (*ESR1*), and androgen receptor (*AR*) mRNA levels were compared with IHC and FISH results.

The samples were selectively enriched for 1408 cancer-associated genes. cDNA was generated from the cleaved RNA fragments using random primers during first- and second-strand synthesis. The sequencing adapters were ligated into the resulting double-stranded cDNA fragments. The coding regions of expressed genes were captured from this library using sequence-specific probes to create the final library. Sequencing was performed using an Illumina Novaseq (Illumina, San Diego, CA, USA). A minimum of ten million reads per sample was obtained in a single run, and the read length was 2 × 75 bp. An expression profile was generated from the sequencing coverage profile of each sample using the Cufflinks software. Expression levels were measured as fragments per kilobase of transcripts per million.

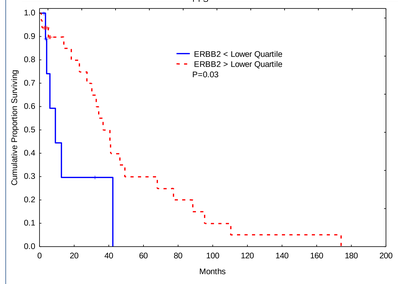
Results

Marked variation in *ERBB2* (HER2) mRNA between IHC groups



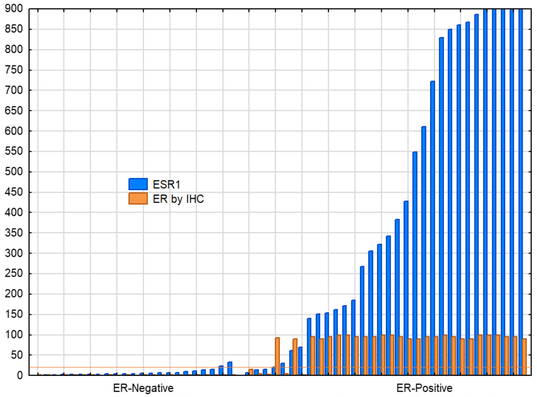
The RNA level did not differ significantly between score of zero and zero-to-one (P=0.32) or between a score of one and two (P=0.31). There was a significant difference between zero and one (P=0.03) and between two and three (P=0.007).

IHC Score	Valid N	Mean	Median	Minimum	Maximum	Quartile Range	Std. Dev.
Zero	19	231	243	63	537	170	110
Zero vs one	3	151	155	45	253	208	104
Zero-to-one vs one	18	397	302	172	845	365	222
One vs two	13	495	423	159	1094	273	284



Patients with very low *ERBB2* levels (< 211 FPKM, lower quartile) had significantly shorter PFS than patients with higher *ERBB2* mRNA levels, irrespective of whether they were amplified or not.

Marked variation in *ESR1* mRNA within ER-positive breast cancers



Four cases classified by IHC as positive had RNA levels below 22 FPKM (mean +2 standard deviation), and most likely should have been classified as negative. One case classified as negative by IHC showed *ESR1* mRNA expression at 33 FPKM, and most likely should have been classified as positive based on the overall pattern of expression.

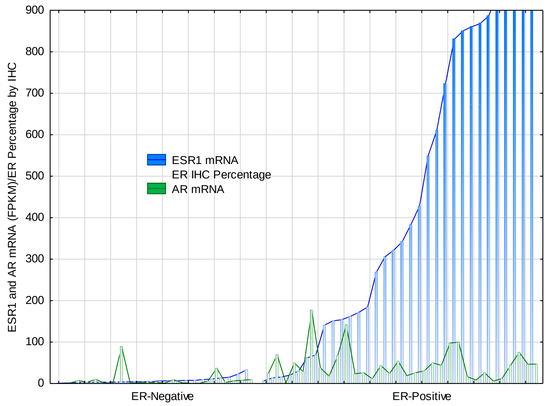
IHC Classification	Valid N	Mean	Median	Min	Max	Quartile Range	Std. Dev.
ER-Negative	23	7.62	4.62	0.18	32.97	6.26	7.56
ER-Positive	32	464	332	6.53	1170	709	395
ER-Positive >90%	20	531	362.46	20.09	1131	689	377

Conclusions

These findings suggest that RNA analysis using NGS can be an alternative to IHC and FISH to evaluate breast cancer biomarkers and provides continuous data that can determine cut-off points and should be explored in predicting ADC response.

Androgen receptor correlation with *ESR1* levels

A dynamic relationship between the ER and AR has been reported in breast cancer. The two receptors form heterodimers and bind to the same DNA sequence, thereby activating specific pathways. Therefore, these two genes transcriptionally regulate each other. There was no significant correlation between *ESR1* and *AR* (r^2 = 0.01, P=0.23).



Patients with breast cancer expressing high levels of both *ESR1* and *AR* had significantly better overall PFS than patients with low levels of both *ESR1* and *AR*.