Quantitative RT-PCR (2-gene assay)
As a companion diagnostic tool, a single-tube triplex qPCR assay was developed and optimized in a GMP format for testing a response rate in the Phase 2 patient population. Taqman-based RT-PCR assays were designed for 3 markers: *RASGRP1* (guanine nucleotide exchange factor that activates RAS), A*PTX* (aprataxin involved in DNA excision repair) and HMBS (used as an internal control). Sequences of Taqman primer probe sets are listed in Table 1 (Supplemental Files). JY cellular RNA (Stratagene) was used as external normalization controls. A 2-gene ratio was calculated according to the algorithm described in ***Statistical Design and Analysis*** of ***Patients, Materials, and Methods*** section of the article.

Single-tube multiplexed RT-PCR in a GMP format
50ng of total RNA was used as a target input in a triplex qRT-PCR which was carried out on an Applied Biosystems Prism 7500 Sequence Detection System in a 25µL reaction. RNA samples (including normalization control RNAs) were thawed on ice and diluted to10 ng/µl. The qRT-PCR was carried out using reagents from the Veridex BLN RT-PCR Kit (GeneSearch Breast Lymph Node (BLN) Test Kit, IVD, Cat #2900004): GeneSearch BLN Enzyme Mix, IVD, P/N 7700040 and BLN Base Master Mix, P/N 7700031. 25× probe-primer master mix was prepared comprising: 400 nM *RASGRP1* Forward and Reverse primers and 200 nM FAM-labeled *RASGRP1*; 400 nM *APTX* Forward and Reverse primers, and 200 nM Gold 540-*APTX*, and 400 nM *HMBS* Forward and Reverse primers and 200 nM Cy5-*HMBS* (Table 1). Each reaction consisted of 9 µL of BLN Base Master Mix, 10 µL of 2.5× BLN Enzyme mix and 1 µl of 25× primer/probe mix. 5 µl of total RNA from patient samples or normalization controls (Universal or JY RNA) was added to 20 µl of RT-PC master mix. The qRT-PCR assays were carried out using the following cycling parameters: 1 min at 95°C for denaturation step; 30 minutes at 58°C (RT reaction); 5% ramp to 70°C and incubation for 2 min followed by 40 cycles at 95°C for 15 seconds (denaturation) and 60°C for 1 minute (annealing/extension).

Table S1. Sequences of TaqMan assays used for the 2-gene assay

Description	Sequence Name	Sequence (5' to 3')
HMBS Upper primer	HMBS_454_ U19	CCTGCCCACTGTGCTTCCT
HMBS Lower primer	HMBS_ 520_L 20	ATCATGAGGGTTTTCCCGCT
HMBS TaqMan Probe	HMBS_ 477_P 24	5'- Quasar 670-GCTTCACCATCGGAGCCATCTGCA-BHQ2-3'
RASGRP1 Upper primer	RASGPR1_287_U21	CTGGACGATCTCATTGACAGC
RASGRP1 Lower primer	RASGPR1_368_L22	CTTGCAACAGTTGGTTACTTCG
RASGRP1 TaqMan Probe	RASGPR1_310_P31	5'-FAM-CATTCAATCTTTTGATGCAGATGGAAACCTG-BHQ1-3'
APTX Upper primer	APTX_825_ U22	CGCTTCCGATTGGGCTAC
APTX Lower primer	APTX_892_L22	AGAATCAAAATCCTGGCTGATC
APTX TaqMan Probe	APTX_846_P28	5'-CAL Fluor Gold 540-CACGCCATTCCGAGTATGAGCCATGTAC-BHQ1-3'