Afirma GSC utilizes RNA sequencing and machine-learning algorithms to classify cytologically indeterminate thyroid nodules into benign (B) and suspicious (S) categories. Detection of genomic variants and fusions was recently expanded beyond \textit{BRAF} V600E and \textit{RET/PTC1\&3} by the Xpression Atlas (XA), which identifies 761 nucleotide variants and 130 fusion gene pairs in 511 genes. Here we used XA to analyze the mutational spectrum of 190 Bethesda III/IV nodules with gold standard histologic diagnoses. 190 nodules previously collected in a prospective multicenter blinded trial design were analyzed with the XA. Among the 145 histologically benign nodules, 35 (24\%) contained a variant or fusion (XA+). In the 99 benign nodules with GSC-B results there were 15 (15\%) with XA variants and none with a fusion. These variants were 7 \textit{TSHR}, 3 \textit{SPOP}, 2 \textit{EIF1AX}, 1 \textit{PTEN}, 1 \textit{TSHR} + \textit{EZH1}, and 1 \textit{GNAS}. In the 46 benign nodules with GSC-S results, 18 (39\%) harbored a variant and 2 (4\%) a fusion. There were 9 \textit{NRAS}, 6 \textit{HRAS}, 2 \textit{TSHR}, and 1 \textit{SPOP}. Two had a \textit{PAX8/PPRARG} fusion.

Among the 45 histologically malignant nodules (41 GSC-S; 91\% sensitivity), 22 were XA+ (49\% sensitivity). In the 41 malignant nodules with GSC-S results, there were 19 variants (46\%) and 2 fusions (5\%). The variants were 9 \textit{NRAS}, 3 \textit{HRAS}, 3 \textit{BRAF} V600E, 1 \textit{SPOP}, 1 \textit{KRAS} + \textit{EIF1AX}, 1 \textit{EIF1AX}, and 1 \textit{BRAF} K601E. Fusions were \textit{BRAF/MKRN1} and 1 \textit{ETV6/NTRK3}. In the 4 GSC-B false negative nodules (2 PTC, 1 fvPTC, 1 HCC), only the HCC contained a variant (\textit{TSHR}). In 190 thyroid nodules with definitive histology, malignant nodules were twice as likely to be XA+ than...
benign nodules (49% vs 24%, \( p = 0.003 \chi^2 \)). Although GSC-S nodules were nearly 3 times more likely than GSC-B nodules to be XA+ (47% vs 16%, \( p < 0.0001 \)), the PPV for malignancy did not differ among all GSC-S, GSC-S XA+, and GSC-S XA- nodules (47%, 51%, and 43%, respectively; \( p = 0.77 \)). When XA+, GSC S nodules expressed mainly RAS variants, and GSC B nodules predominantly TSHR variants. Conversely, the NPV for XA was 83%. These findings support GSC as better than XA to rule-out cancer while the addition of XA to GSC-S nodules may provide additional insights into pathway activation and potential cancer treatment targets.