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Clinical Validation of the Afirma Genomic Sequencing *BRAF* V600E Classifier

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INTRODUCTION

Several DNA variants have a high positive predictive value (PPV) for thyroid cancer, including *BRAF* V600E. The Afirma Genomic Sequencing Classifier (GSC) identifies, by RNA sequencing and machine learning algorithms, genomically benign thyroid nodules among those with indeterminate cytology to prevent unnecessary diagnostic surgery with high NPV, and modest PPV. Additional cassettes within the GSC aim to detect the molecular signatures of specific neoplasms with high PPV that further alter patient care (Figure 1). Here we report the clinical performance of an embedded *BRAF* V600E classifier.

METHODS

452 FNA samples were used in algorithm training to build an RNA-based classifier (Table 1). The classifier is comprised of 12 expression-based sub-models that are further improved with inclusion of variant features from RNA-seq. 9,880 genes, of which 1,042 have the largest impact on classification performance.

RESULTS

The final locked classifier was blindly validated on 264 independent FNAs. There is no single accepted gold standard test or mutated allele frequency to define *BRAF* V600E status. We used castPCR™ to define this "truth". Samples with <5% *BRAF* V600E allele frequency were defined as negative; those with ≥5% as positive. Compared with *BRAF* status by castPCR DNA sequencing, the classifier had 100% Positive Percent Agreement (PPA) [62/62 *BRAF* V600E positive samples called by *BRAF* V600E classifier when compared to castPCR result; CI 94.2-100%] and 99.0% Negative Percent Agreement (NPA) [200/202 *BRAF* V600E negative samples called by *BRAF* V600E when compared to castPCR result; CI 96.5-99.9%] (Table 2). One "false positive" had a castPCR allele frequency of 4.77% and was histologically PTC, and the other had a castPCR allele frequency of 0% with a double mutation on DNA sequencing at nucleotide positions 1798 (T>A) and 1799 (T>A) with 27% allele frequency, demonstrating that it is a true positive.

CONCLUSIONS

The preoperative genomic identification of thyroid nodules with a virtually 100% chance of malignancy may influence patient and physician decisions regarding the need for surgery, and potentially refine the extent of thyroid surgery prior to the initial operation. This information may improve patient care by avoiding delays in diagnosis and/or the need for a completion thyroidectomy.

TABLE 1.
Training Set Composition

Group	*BRAF* Negative 0%	*BRAF* Marginal (>0% to <2.5%)	*BRAF* Positive (≥2.5% to ≤100%)	Pseudo *BRAF* Negative*	Sum
Core	87	15	107	0	**209**
ENHANCE	0	0	0	243	**243**
Sum	**87**	**15**	**107**	**243**	**452**

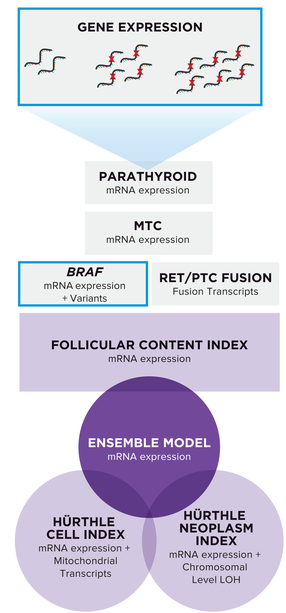
* Pseudo *BRAF* Negative: either no *BRAF* variant found in >45x depth on RNA-seq, or Afirma GEC benign (Afirma GEC benign and *BRAF* Positive are mutually exclusive.)

TABLE 2.
Independent Validation Performance

		BRAF Truth Label	
		Positive (castPCR≥5%)	Negative (castPCR<5%)
***BRAF* V600E Classifier Result**	**Positive**	62	2
	Negative	0	200

Sensitivity	Specificity
100% (94.2-100%)	99% (96.5-99.9)

FIGURE 1.
Afirma Genomic Sequencing Classifier (GSC) Integrated Workflow



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