Table 2: Summary of Study Characteristics and Key Findings on the Use of Liquid Biopsy Analysis in Patients with Thyroid Neoplasia

Reference	Biomarker Type(s)	Genes or Molecular Target (Diagnostic Assay Used)	Cases (Controls)	Sampling Time	Aim(s)	Results (prevalence of detection and/or estimate of diagnostic accuracy)
Aliyev et al. 2015[55]	mRNA	TSHR mRNA	528	Before surgery	Investigated the presence of TSHR mRNA in patients with PTmC (n=152) and association with clinicopathologic parameters.	Preoperatively, TSHR mRNA was detected in 46.1% (70/152) patients with PTmC compared to 80.0% (106/133) for patients with macroscopic PTC and 18.0% (41/228) in patients with benign thyroid disease. There was no difference between the TSHR mRNA-positive and -negative groups in any demographic or operative findings except for presence of symptomatic multinodular goiter. The prevalence of LN metastases whether tumors were <5 mm or ≥5 mm was not significant by TSHR mRNA-positive and -negative status.
Allin et al. 2018[56]	ctDNA	*BRAF, RET, NRAS, TP53, PTEN, APC, NOTCH1, HRAS, AILT1, NOTCH2, ARIDIA, KRAS, PIK3CA, DOCK2, VHL, CDKN2A, ATM*	51	Serial sampling (mean of five samples per patient)	(1) Detection of plasma ctDNA in TC subtypes (2) Compared ctDNA concentration with imaging, Tg, calcitonin, and CEA biomarkers.	NGS analysis of tumor tissue detected variants in 82.4% (42/51) cases. Overall, ctDNA was detected in 67.0% (28/42) of the patients. Of the 42 cases, the plasma ctDNA detection rate by histology with number of patients in study was: papillary (n=15) 53.0%; follicular (n=10) 60.0%; medullary (n=14) 79.0%; PDTC (n=2) 100.0%; ATC (n=1) 100.0%; total (n=42) 67.0%. Detection rate was highest in patients with metastatic disease (79.0%) compared to local recurrence (33.0%) or no macroscopic disease (0%). A significant positive correlation was found between ctDNA concentrations and Tg, calcitonin and CEA.

Bettegowda et al. 2014[9]	CTC, ctDNA	*KRAS, NRAS, BRAF*^{600}*, PIK3CA, EGFR*	4 (640)	Varied	Evaluated ctDNA mutations in detection of tumors.	In total, the study included 18 tumor types, stage I to IV. ctDNA was detected in 82.0% (112/136) of patients with solid tumors outside the brain. ctDNA was present in patients that had no detectable CTCs. There were no cases in which CTCs were detected, but ctDNA was absent. Detectable ctDNA significant varied with tumor type. Overall, ctDNA was detected in >75% of patients with advanced pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers. ctDNA was detected less than 50.0% of patients with primary brain, renal, prostate, and thyroid cancers. Among TC patients (n=4), one patient had detectable ctDNA yielding a sensitivity of 25.0%.
Busaidy et al. 2017[57] (Abstract)	ctDNA	*RET* M918T, *RET* V804M	16	After treatment	Examined ctDNA from advanced MTC patients with confirmed somatic *RET* M918T status by molecular testing of tissue.	Authors report emergence of *RET* V804M mutation after TKI exposure. Of the 13 patients treated with TKIs, *RET* V804M cfDNA was detected in 61.5% (8/13). In all cases, the amount of *RET* V804M cfDNA did not exceed *RET* M918T ctDNA levels.
Chae et al. 2016[58]	cfDNA	65 (Guardant360®, Guardant Health Inc., Redwood City, CA, US)	3	Varied (the median interval between collections of tissue biopsy and peripheral blood specimens was 89 days)	Identified concordance of genomic alterations obtained from tissue biopsies and plasma cfDNA in patients with advanced malignancy (n=28).	Two patients with thyroid tumors were included (one papillary and one poorly differentiated not otherwise specified). The authors do not separate diagnostic accuracy of cfDNA by cancer type, however, overall, compared to tissue-based biopsy, cfDNA assays had low sensitivity and high specificity, with a diagnostic accuracy range of 82.0–89.0%.

Condello et al. 2018[59]	ctDNA	*BRAF*^{V600E}	59	Before surgery	Evaluated the presence of circulating *BRAF*^{V600E} in plasma of patients with thyroid nodules and PTC causing distant metastases.	In total, 37.3% (22/59) primary tumors had a *BRAF*^{V600E} mutation and none of the corresponding plasma samples were found positive for *BRAF*^{V600E} mutation in either rtPCR or dPCR analysis.
Cote et al. 2017[60]	ctDNA	*RET* M918T	75	Serial sampling	Examined levels of ctDNA in MTC patients with *RET* M918T positive tissue biopsy.	In total, 50 patients were *RET* M918T positive by tissue biopsy. Of those, *RET* M918T in cfDNA was detected 32.0% (16/50). Calculated sensitivity and specificity based on patients with mutation detected in plasma by DM status is 34.5% and 82.4%, respectively.
Dent et al. 2016[61]	CTC	EpCAM, cytokeratins, NIS, CD45	6 (12)	During treatment	Evaluated assay for detection and characterization of CTCs in multiple tumor types.	Over half (4/6) of the patients with TC had detectable CTCs (MTC, FTC, PTC, and FVPTC). The highest number of CTCs detected (118) were in a patient with known metastatic PTC.
Ehlers et al. 2018[62]	CTC	Staining for EpCAM+, CD45- cells	67 (15)	After treatment	Examined CTC in TC subtypes and correlate with tumor status.	CTC in TC increased over normal controls (2.4 ± 3.1 CTC versus 0.2 ± 0.4 CTCs, $p<0.0001$). CTC number correlated with initial tumor stage. There was no difference in TC subtypes or metastatic status. Tumor-free patients still had positive CTCs.

Iyer et al. 2017a[63] (Abstract)	ctDNA	*BRAF*^{V600E}	16	Baseline imaging and clinical follow-up	Examined concordance of ctDNA levels with changes in tumor burden measured by imaging throughout treatment in *BRAF* positive ATC patients.	At diagnosis, 10 patients were stage IVC and six were stage IVB. A total of 46 ctDNA samples paired with imaging. Concordance in plasma ctDNA levels and imaging were 94.0% (24.5/26) with regression, 100.0% with stable disease (3/3), and 47.0% with progressive disease (8/17). Overall, 71.0% of the ctDNA results were predictive of response to treatment.
Iyer et al. 2017b[64] (Abstract)	cfDNA	*BRAF*^{V600E} (Guardant360®, Guardant Health Inc., Redwood City, CA, US)	35	Not specified	Compare *BRAF* mutation detection by ddPCR to Guardant360 testing and tumor tissue testing in ATC patients.	Tissue *BRAF* mutation was identified in 48.5% (17/35) of the patients. *BRAF* mutation detection by ddPCR was 91.0% concordant with tissue findings with a sensitivity of 94.0% and specificity 89.0%. Guardant360 was 89.0% concordant with tissue with a sensitivity of 72.0% and specificity 100.0%. ddPCR was 85% concordant, but not significantly different, with Guardant360.
Janku et al., 2016[65]	ctDNA	*BRAF*^{V600E} (Idylla Systems, Biocartis US, Inc., Jersey City, NJ, US)	10	Serial sampling	Evaluated presence of plasma *BRAF*^{V600E} ctDNA verses tissue *BRAFV*^{600E} in patients with progressing advanced TC.	Of patients with advanced TC, 80.0% (8/10) had *BRAF*^{V600E} in tissue and 50.0% had positive ctDNA for *BRAFV*^{600E}.
Kim et al. 2014[66]	plasma DNA	*BRAF*^{V600E} (PNA Clamp™ BRAF Mutation Detection kit, Panagene, Daejeon, Korea)	72 patients with PTCs, 5 patients with benign pathology	At initial surgery	Evaluated the presence of *BRAFV*^{600E} in plasma DNA in advanced and aggressive PTC patients.	Overall, 68.1% (49/72) PTC tumor samples were determined to harbor the *BRAF* mutation. Only 6.1% (3/49) of these cases also detectable *BRAF* mutation in plasma. All three of the patients with *BRAF* mutation-positive plasma were stage IVC.

Konda et al. 2017[67] (Abstract)	ctDNA	*BRAF*^{V600E}	37	On day one of dabrafenib ± trametinib treatment cycle.	Evaluated the correlation between *BRAF*^{V600E} mutation levels in ctDNA in radioactive iodine refractory DTC patients.	At the time of reporting, 54.1% (20/37) patients had detectable *BRAF*^{V600E} mutation in the cfDNA sample. Of these patients, 35.0% (7/20) had detectable mutation at baseline, and all 7 (100.0%) had undetectable *V600E* levels by cycle 3 of treatment with 5/7 patients having partial response and 2/7 stable disease as best response.
Li et al. 2017[68]	cfDNA	5hmC markers	46 (90)	Before treatment	Evaluated presence of 5hmC in plasma cfDNA and tumor tissue among cancer patients, patients with benign disease, and healthy individuals.	Sensitivity for thyroid cancer was 28.0%. The ability of 5hmC biomarkers to distinguish cancer patients from healthy individuals with high sensitivity and specificity was observed in colorectal and gastric cancers only.
Li et al. 2018[69]	CTC	EpCAM, TSHR	25	After confirmation of positive TgAb and undetectable serum Tg and before imaging	Evaluated the clinical role of CTCs in PTC patients with positive serum TgAb and undetectable serum Tg.	After CECs testing, 25 patients were classified into a recurrence group (n=7) and remission group (n=18) based on biopsy or imaging studies. The median numbers of EpCAM+-CECs and TSHR+-CECs were significantly increased in the recurrence group compared to the remission group. For EpCAM+-CECs using a cut-off value for EpCAM+-CECs of 10 cells/mL, the assay showed a sensitivity 85.7% and specificity of 100.0% in predicting recurrence. For TSHR+-CECs using a cut-off value of 10 cells/mL, the assay showed a sensitivity 85.7% and specificity of 77.8% in predicting recurrence.

Lin et al. 2015[70]	CTC	EpCAM, TSHR	48 (17)	Not specified	Analyzed the number of CTCs from control subjects and PTC patients who were disease-free or with distant metastatic disease.	The median CTC (EpCAM+) for healthy controls was 6/mL, 12/mL for disease-free PTC, and 91/mL for distant metastasis. The median number of CTC with TSHR was 9, 16, 100 cells/mL for controls, disease-free PTC and distant metastasis PTC patients. A cut-off value of 33 TSHR+-CECs/mL yielded a sensitivity and specificity of 72.7% and 96.2%, respectively in distinguishing patients with distant metastasis from disease-free status patients A cut-off value of 22 EpCAM+-CECs/mL yielded a sensitivity and specificity of 86.4% and 92.3%, respectively in distinguishing patients with distant metastasis from disease-free status patients A cut-off value of 21 TSHR+-CECs/mL yielded a sensitivity and specificity of 86.4% and 100.0%, respectively in distinguishing patients with distant metastasis from healthy controls. A cut-off value of 16 EpCAM+-CECs/mL yielded a sensitivity and specificity of 90.9% and 100.0%, respectively in distinguishing patients with distant metastasis from healthy controls.
Lin et al. 2018[71]	CTC	EpCAM, TSHR, and PDPN	128	Four to six weeks after surgery or radioactive iodide therapy	In a long-term follow-up study, evaluated whether the number of CTCs expressing EpCAM, TSHR, or PDPN is related to remission and DSM.	The mean follow-up was 7.8 (SD: 5.8) years. The number of EpCAM+, TSHR+, and PDPN+ CECs was statistically higher in the non-remission group than in the remission group. The accuracy of the assay was 80.4% (EpCAM+), 76.6% (TSHR+) and 77.3% (PDPN+) to determine the status of remission from non-remission using the cut-off points of 40, 47 and 14 (cells/mL), respectively. The number of EpCAM+, TSHR+, and PDPN+ CECs was statistically higher for patients in the

						DSM group compared to the patients who survived. CECs counts were able to distinguish mortality from survival status with an accuracy of 69.5% (EpCAM+), 67.2% (TSHR+), and 68.5% (PDPN+) using cut-off points of 27, 25 and 9 (cells/mL), respectively.
Lubitz et al. 2016[72]	RNA	*BRAF*^{V600E}	48 (22)	Before surgery or treatment	Compared circulating *BRAF*^{V600E} levels with *BRAF* mutation status from surgical pathologic DNA-based tissue assays in patients with PTC.	Median serum *BRAF* levels were 3pg with benign disease, 17.3pg for tissue BRAF+ PTC and 2pg for tissue *BRAF*-PTC. Cut-off values of 5, 10 and 20 *BRAF*^{V600E} thresholds in predicting *BRAF* tissue results yielded assay sensitivities of 62.5%, 50.0%, and 43.8%, respectively and specificities of 71.1%, 86.8%, and 92.1%, respectively.
Lupo et al. 2018[73]	cfDNA and ctDNA	*BRAF*, *CTNNB1*, *EGFR*, *FOXL2*, *GNAS*, *KRAS*, *NRAS*, *PIK3CA*, *TP53* (Pathway Genomics, San Diego, CA, US)	56	Before tissue pathology and FNAB	Evaluated ctDNA detection in determining thyroid malignancy in patients with thyroid nodules.	FNAB results determined 13 patients with malignant disease and 43 patients with benign lesions. There were no significant differences in cfDNA concentrations between patients with and without detectable ctDNA, and no significant difference in cfDNA concentrations between patients with benign lesions and those with malignancies. The sensitivity was 7.7% and specificity 95.4% between detection of ctDNA and pathologic diagnosis and molecular testing from FNAB results.
Patel, 2015[74]	ctDNA	*BRAF*^{V600E}	61 (55 for surgery; 6 PTC for RAI)	Thyroid nodules before and	Investigated detection of *BRAF*^{V600E} in	Of 55 surgical patients, 13 (21.3%) were benign, 26 (42.6%) classical PTC, 13 (21.3%) non-classical PTC, and three (4.9%) FTC.

				after surgery and before RAI	ctDNA to distinguish between benign and malignant thyroid nodules.	In total, 14.8% (9/61) of patients had detectable *BRAF*^{V600E} in the preoperative ctDNA sample. All the patients with detectable *BRAF*^{V600E} ctDNA had classical PTCs and none of the benign thyroid nodules had detectable *BRAF*^{V600E}. Of the paired pre- and post-surgery samples (n=37), there was a significant association in the decline of *BRAF*^{V600E} ctDNA levels. There was no significant correlation found between *BRAF*^{V600E} ctDNA levels and tumor stage, nodal metastases, and ETE among patients with a final diagnosis of PTC.
Porter et al. 2018[75] (Abstract)	ctDNA	70 (Guardant360®, Guardant Health Inc., Redwood City, CA, US)	88	Samples taken retrospectively	Evaluated concordance between tumor and cfDNA sample NGS analysis in HNSCC patients.	The most common mutations identified by ctDNA analysis were *TP53* (51.0%) *PIK3CA* (25.0%), *NOTCH1* (14.8%), and *ARID1A* (14.8%). Of the 29 matched tumor samples, *TP53* (48.0%) and *PIK3CA* (24.0%) were reported with the highest frequency. Of the thyroid cancer patients, 75% of thyroid cancer patients had actionable mutations (sample size not reported).
Pupilli et al. 2013[76]	cfDNA	*BRAF*^{V600E} (Taqman®, Thermo Fisher Scientific Inc., Waltham, MA, US)	103 patients with nodular goiter (49 healthy subjects; 16 patients with non-nodular thyroid diseases)	Before and after surgery in TC patients.	Investigate the role of *BRAF*^{V600E} mutated allele in cfDNA as a marker for the diagnosis and follow up of PTC.	The percentage of circulating *BRAF*^{V600E} was significantly different between TC patients and controls and throughout different cytological categories of ultrasound-assisted fine-needle aspiration. Patients with a histopathological diagnosis of PTC showed significantly higher percentage of circulating *BRAF*^{V600E} compared to those with benign histology. Using cut-off value of 2.65% yielded 80.0% sensitivity and 65.0% specificity for the diagnostic performance of circulating *BRAF*^{V600E}. A second blood draw, taken 3–6 months after surgery, showed a significantly lower

						percentage of *BRAF*^{V600E} in cfDNA than the presurgical sample (n=19).
Qiu et al. 2018[77]	CTC	EpCAM expression and aneuploidy (NE-iFISH)	72 (30)	Before treatment	Investigated how CTCs correlate with clinicopathological factors and prognosis in DTC patients with DM.	Most, 86.1% (62/72), of the patients with DTC had detectable CTC (mean 7.31/7.5 mL), as did 26.7% of healthy controls (mean 0.4pg/7.5 mL, range 1–3). The mean number of CTCs in patients DM^{+} DTC (10.19) was significantly higher than in DM^{-}DTC (mean 2.6) and control group (mean 0.4). Using a cut-off ≥5 CTCs to predict DTC DMs, yielded a sensitivity of 64.3% and specificity of 83.8%.
Ried et al. 2017[78]	CTC	ISET methodology (Rarecells Diagnostics, Paris, France)	5	Varied	(1) Compared CTC detection for screening in patients with an increased risk of cancer (2) Evaluated CTC count to cancer treatment effectiveness in cancer patients.	No TC was diagnosed in the screening group. The CTC count was <3 among the five patients with stage I TC. In the group of subjects with no cancer diagnosis at the time of sampling (n=265) CTC were detected in half of the patients screened (n=132). Among these subjects, follow-up testing/imaging indicated cancerous lesions in 20% (n=24) within 0.5–10.0 months (mean = 3.5 months).
Salvianti et al. 2017[79]	cfDNA	*APP* gene (67 and 180 base pair amplicons with integrity index 180/67)	97 (49)	After FNAB of thyroid nodules.	Evaluated the presence of longer and shorter circulating DNA strands, their ratio, and FNAB results (some confirmed by surgery).	Patients affected by nodular thyroid diseases showed a significantly higher concentration of total cfDNA than healthy individuals. Comparing healthy subjects with patients with cytological diagnosis of thyroid carcinoma AUROC showed a significant agreement of 0.765, 0.982, and 0.796 for cfDNA quantity by 67 bp amplicon, cfDNA quantity by 180 bp amplicon and integrity index, respectively. Comparing patients with benign nodules to patients with cytological diagnosis of thyroid carcinoma AUROC showed a significant

						agreement of 0.699 with diagnosis for cfDNA integrity.
Sandulache et al. 2017[80]	ctDNA	50 genes for tissue assay and 70 genes for ctDNA assay (Guardant360®, Guardant Health Inc., Redwood City, CA, US)	23	During treatment evaluation	Evaluated concordance between tumor and ctDNA sample NGS analysis in patients with ATC.	Mean and median follow-up times for the cohort were 142 and 102 days, respectively. The concordance was 72.0% between tissue mutations and those in ctDNA in patients with distant and/or locoregional disease without treatment prior to study (n=12). There was 100% concordance for the presence of *BRAF*^{V600E} and *NRAS* mutations. Patients with surgery or treatment previous to study (n=7), had 6.0% concordance and there was no concordance between tissue and ctDNA in patients without active disease at the time of the study.
Willms et al. 2016[81]	taMPs	EpCAM+, EpCAM+CD147+, and CD147+	43 patients with thyroid nodules (included in study as control group)	Serial sampling	Evaluated diagnostic performance of detection of taMPs in various cancer types.	EpCAM+ taMPs were significantly elevated in thyroid nodules patients compared to healthy controls.
Winkens et al. 2014[82]	CTC	EpCAM (maintrac® blood test, Bayreuth, Germany)	28	Before therapy and at three time points post RAI	(1) Investigated the number of CTCs expressing EpCAM in patients with DTC after RAI (2) correlated CTC changes with serum Tg and clinical response.	There was no correlation between radiotherapy activity and changing levels of CTC at any of the time points. There was no significant correlation between the clinical or serum Tg evidence of response and the percent change of CTC at any time point.

Xu et al. 2016[83]	CTC	EpCAM (Veridex's CellSearch®, Menarini Silicon Biosystems, Inc. Huntington Valley, PA, US)	42 (10 history of DTC but NED)	Not specified	Assessed CTC levels to predict overall survival in patients with metastatic DTC (n=14) and metastatic MTC (n=18).	Overall, 72.2% (13/18) metastatic patients with MTC had detectable CTCs. One metastatic DTC patient, who had an aggressive tall cell variant PTC and lung metastases, had 3 CTCs/7.5 mL and one other metastatic DTC patient had 1 CTCs/7.5 mL detected. Using the cut-off of ≥5 CTCs/7.5 mL, the sensitivity and specificity to distinguish metastatic disease patients from NED controls were 20.0% and 100.0%, respectively. The cut-off to distinguish metastatic disease patients from NED controls was more than or equal to 1 CTC/7.5 mL, yielding a sensitivity of 41.0% and specificity of 90.0% using AUROC curve analysis.
Zane et al. 2013[84]	ctDNA	$ctDNA_{ALU244/ALU83}$, *SLC5A8* and *SLC26A4* hypermethylation, and $BRAF^{V600E}$	181 (19)	Before surgery	Evaluated diagnosis and prognosis of thyroid cancer using ctDNA in patients with ATC (n=9), MTC (n=58), SMFC (n=5), FA (n=23), and PTC (n=86).	$ctDNA_{ALU244/ALU83}$: Using a cut-off of 11.741 ng/mL, the diagnostic power of $cfDNA_{ALU83}$ showed 94.7% specificity and 73.5% sensitivity. Using a cut-off of 4.412 ng/mL, the diagnostic power of $ctDNA_{ALU244}$ showed 67.0% sensitivity and 100.0% specificity. There was no correlation observed between cfDNA and clinical features studied. Median plasma $ctDN_{ALU244/ALU83}$ were significantly correlated with the histological type of TC. *SLC5A8* and *SLC26A4* methylation: The amount of ctDNA was significantly increased in the methylation positive ctDNA group compared to the group with no methylation events. $BRAF^{V600E}$*:* Of the 46 $BRAF^{V600E}$ positive tissue samples, no $BRAF^{V600E}$ mutations were found in corresponding ctDNA samples.

Zheng et al. 2018[85] (Abstract)	CTC	NIS expression (CanPatrol™, (SurExam, Guangzhou, China)	202	Before I-131 treatment	Monitored CTC counts and epithelial-mesenchymal transition subtypes in parallel with Tg assay, I-131 whole body scintigraphy, and cervical color ultrasound assay in patients with DTC.	CTCs were detected in 69.3% (140/202) of the samples. The rates of NIS mRNA expression-positive CTCs were 41.0%, 61.2%, 65.3% from epithelial CTCs, biophenotypic CTCs, mesenchymal CTCs, respectively.

Abbreviations: ATC = anaplastic thyroid carcinoma; CEA = carcinoembryonic antigen; CD45 = cluster of differentiation 45; CECs = circulating epithelial cells; cfDNA = cell free DNA; CTCs = circulating tumor cells; ctDNA = circulating tumor deoxyribonucleic acid; ddPCR = droplet digital polymerase chain reaction; DM = distant metastases; dPCR = digital polymerase chain reaction; DSM = disease specific mortality; DTC = differentiated thyroid cancer; EpCAM = epithelial cell adhesion molecule; ETE = extra-thyroidal extension; FA = follicular adenomas FFPE formalin fixed paraffin-embedded; FNAB = fine-needle aspiration biopsy; FTC = follicular thyroid cancer; FVPTC = follicular variant of papillary thyroid cancer; HNSCC = head and neck squamous cell carcinoma; ISET = Isolation-by-Size-of-Epithelial-Tumor; LN = lymph node; MTC = medullary thyroid carcinoma; NE-iFISH = negative enrichment with immunofluorescence and in situ chromosomal hybridization; NED = no biochemical or radiographic evidence of disease; NGS = next-generation sequencing; NIS = sodium iodide symporter; PDPN = podoplanin; PDTC = poorly differentiated thyroid cancer; PTC = papillary thyroid carcinoma; PTmC = papillary thyroid microcarcinoma; RAI = radioactive iodide; ROCAUC = receiver operating characteristic area under the curve; rtPCR = real time polymerase chain reaction; SD = standard deviation; SMFC = synchronous medullary and follicular thyroid cancers; tamps = tumor associated microparticles; TC = thyroid cancer; Tg = thyroglobulin; TgAb = anti-thyroglobulin antibody; TKIs = tyrosine kinase inhibitors (cabozantinib or vandetanib); TNM = tumor, node, metastasis (classification of malignant tumors); TSHR = thyroid-stimulating hormone receptor; TSHR mRNA = thyroid stimulating hormone receptor messenger ribonucleic acid; WGS = whole-genome sequencing.