CIRCULATING SIGNALS AS PREDICTIVE TOOLS IN CUTANEOUS SQUAMOUS CELL CARCINOMA: READY FOR THE CLINIC?

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Abstract

Emerging evidence indicates that circulating tumor DNA, tumor cells, exosomal RNAs, immune ratios, soluble proteins, and epigenetic markers may serve as promising tools for disease monitoring, prognosis, and treatment response prediction in cutaneous squamous cell carcinoma (cSCC). This narrative review summarizes current research in the field. While these approaches could support personalized management and refine risk stratification, most studies are preliminary and limited by sensitivity, heterogeneity, and lack of standardization. Further prospective validation is needed before liquid biopsy can become part of routine care in cSCC.

Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer and its incidence is rising globally.[1] Although most tumors are effectively treated with local measures, up to 5% progress to regional or distant metastasis.[2] The wide biological heterogeneity of cSCC (ranging from indolent lesions to aggressive, treatment-refractory disease) creates a need for better tools to identify high-risk patients, monitor treatment, and guide therapeutic decisions. Blood-based biomarkers could potentially offer a minimally invasive approach to address these challenges.

We gathered evidence from studies investigating circulating signals in cSCC (Table 1). Circulating tumor DNA (ctDNA) was the most studied marker. It correlated with tumor burden and declined after surgery or radiotherapy, making it useful for treatment monitoring. However, its sensitivity was limited in small tumors. Advanced sequencing approaches such as cancer personalized profiling by deep sequencing (CAPP-seq) have detected mutations in cell free DNA (cfDNA) that mirror tumor tissue and can even precede radiological relapse. Similarly, circulating tumor cells (CTCs) and tumor microemboli were detectable in a proportion of patients with advanced disease, suggesting potential for prognostic applications. Case reports further demonstrated that ctDNA profiling may uncover actionable mutations that guide therapy, such as BRCA2 alterations responsive to poly (ADP-ribose) polymerase (PARP) inhibitors.[3-8]

Exosome-associated biomarkers represent another promising group. Studies show that exosomal circular RNAs, long non-coding RNAs, and micro RNAs (miRNAs) may contribute to tumor growth and drug resistance, and their serum detection could support early diagnosis or predict treatment resistance.[9-11] Broader miRNA profiling has identified differentially expressed molecules between precancerous actinic keratosis and invasive cSCC, suggesting a role in early detection of malignant transformation.[12]

Systemic immune and inflammatory markers derived from blood are particularly appealing given their accessibility. Ratios such as neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte (PLR) have been consistently associated with advanced disease, survival outcomes, and response to immunotherapy. More detailed immune profiling revealed elevated myeloid-derived suppressor cells, neutrophils, and T regulatory cells in aggressive disease, highlighting the immune dysregulation that supports tumor progression.[13-19]

Soluble proteins in serum have also been evaluated. Squamous cell carcinoma antigen (SCCA) was found to be elevated in advanced disease and declined during response to PD-1 blockade. Other

candidates include matrix metalloproteinase-13 (linked to invasiveness), complement factor H (a baseline predictor of progression under immunotherapy), and cytokines such as IL-8, which may serve as early pharmacodynamic markers of treatment efficacy. Serum metabolomic profiling has further suggested distinct metabolic signatures differentiating cSCC patients from healthy individuals.[20-28]

Finally, epigenetic and T-cell receptor–based approaches add a novel dimension. DNA methylation changes in circulating T cells, particularly of SERPINB9 and FOXP3, have been linked to increased risk and recurrence, especially in immunosuppressed transplant recipients. Functional immune signatures, such as hTERT-specific Th1 responses in cemiplimab-treated patients, have correlated with durable benefit, pointing toward future predictive assays.[29-31]

Collectively, these findings underscore the potential of liquid biopsy in cSCC for disease detection, risk stratification, and monitoring therapy. However, most studies remain exploratory, often with small cohorts, heterogeneous methods, and variable performance. Major challenges include low sensitivity in early-stage disease, lack of methodological standardization, and the biological diversity of cSCC. Before these biomarkers can be integrated into routine clinical practice, prospective validation in large, well-characterized patient cohorts is essential. Nonetheless, current progress signals an important step toward personalized, biomarker-guided management of cSCC.

Table 1: Summary of included studies.

Authors	Cohort / Design	Biomarker & Method	Key Findings
Fan et al. [3]	21 int/high-risk cSCC	ctDNA	78% positive in gross disease; cleared post-th → correlates with burden/ response
Kim et al. [4]	35 advanced cSCC	Personalized ctDNA	Sensitivity ~64%; reflects tumor stage, useful for monitoring
Sawamura et al. [5]	4 metastatic cSCC	cfDNA via CAPP-seq	Mutational profile mirrored tissue; VAF ↑ before progression
Chang et al. [6]	25 advanced cSCC, PD-1 th	ctDNA/tDNA NGS	8 gene muts in non-responders; PD-L1/TMB not predictive
Morosin et al. [7]	10 nodal mets	CTCs (IsoFlux™)	CTCs in 80%, CTMs 30% → possible prognostic value
Sun et al. [8]	Case (1 pt)	Liquid biopsy (BRCA2)	PARPi (fluzoparib) stabilized disease 5 mo → therapeutic relevance
Nguyen et al. [9]	5 H&N SCC (2 cSCC)	EV-DNA (LC-WGS)	Partial CNA concordance → limited utility
Zhang et al. (10)	5 cSCC vs 5 ctrls	Exosomal circRNA	circ-CYP24A1 ↑, linked to tumor size, SCCA → diagnostic/target

Authors	Cohort / Design	Biomarker & Method	Key Findings
Wang et al. (11)	30 pts + models	Exosomal lnc-PICSAR	Associated with cisplatin resistance → resistance marker
Dańczak-Pazdrowska et al. (12)	13 AK, 2 cSCC	Serum miRNA seq	26 miRNAs diff.; hsa-miR-101-3p common → early detection potential
Hock et al. (13)	31 RTR, 22 CKD, 14 cSCC, ctrls	MDSC/DC ratios	↑ MDSCs esp. in transplant pts → prognostic/therapeutic target
Seddon et al. (14)	168 pts	Neutrophils/G-MDSC	↑ linked to thick tumors, poor OS
Rollison et al. (15)	327 immunocompetent	Tregs (CCR4hi)	High levels + UV exposure → ↑ SCC risk
Di Raimondo et al. (16)	51 cSCC	CBC (NLR, RDW)	Higher in advanced cases → simple prognostic tools
Maeda et al. (17)	222 primary cSCC	NLR	High NLR → poor DSS, SLN positivity
Strippoli et al. (18)	30 elderly la/mcSCC, cemiplimab	NLR, PLR	Low PLR + low/high NLR → better ICI response
Lai et al. (19)	93 cSCC	FOXP3+ OX40+ Tregs	Correlated with metastasis; OX40 blockade reversed suppression
Fatica et al. (20)	60 SCC (11 cSCC)	SCCA (Kryptor)	Sensitivity low (18%), specificity >95%
Wang H. et al. (21)	77 cSCC, 50 ctrls	Serum MMP-13	AUC 0.87–0.94; correlates with size/invasion
Rudhart et al. (22)	55 cSCC	CYFRA 21-1	Not prognostic
Geidel et al. (23)	104 high-risk/adv, cemiplimab	Serum CFH	High CFH → advanced stage; low CFH → longer PFS
Vanni et al. (24)	43 la/mcSCC, cemiplimab	cfDNA, exosomal PD-L1, soluble checkpoints	Low baseline markers → durable response; high IFN-γ/sCTLA-4 → poor outcome
Komulainen et al. (25)	60 transplant SCC	ctDNA (NGS)	Detection ↑ in advanced disease (85% vs 25%) → burden/th monitor
Tan et al. (26)	199 cSCC/BCC, ctrls	Serum NLRP1	Low NLRP1 → advanced TNM, mets, recurrence, mortality

Authors	Cohort / Design	Biomarker & Method	Key Findings
Fukumoto et al. (27)	6 cSCC, 9 melanoma, ctrls	Serum metabolomics	Distinct metabolite signatures separate SCC/ melanoma from controls
Esposito et al. (28)	Cemiplimab pts	Serum IL8, PD1+ Tregs	↓ in responders; potential early response marker
Peters et al. (29)	KTRs (discovery + validation)	SERPINB9 methylation	High methylation before onset & recurrence → risk marker
Sherston et al. (30)	58 KTRs	FOXP3 TSDR	Demethylated FOXP3-TSDR ↑in cSCC → accurate Treg marker
Scala et al. (31)	Advanced cSCC, cemiplimab	CD4+ T cells (ELISpot, RNA-seq)	hTERT-specific Th1 profile → predicts durable response

Legend: AK = actinic keratosis; AUC = area under the curve; BCC = basal cell carcinoma; CBC = complete blood count; cfDNA = cell-free DNA; circRNA = circular RNA; cSCC = cutaneous squamous cell carcinoma; ctrls = controls; CTC = circulating tumor cell; CTM = circulating tumor microemboli; EV = extracellular vesicle; H&N SCC = head and neck squamous cell carcinoma; ICI = immune checkpoint inhibitor; IL8 = interleukin-8; KTRs = kidney transplant recipients; la/mcSCC = locally advanced / metastatic cutaneous squamous cell carcinoma; LC-WGS = low-coverage whole-genome sequencing; lncRNA = long non-coding RNA; miRNA = microRNA; MDSC = myeloid-derived suppressor cell; NLR = neutrophil-to-lymphocyte ratio; NLRP1 = NOD-, LRR- and pyrin domain-containing protein 1; OS = overall survival; PARPi = poly (ADP-ribose) polymerase inhibitor; PBMC = peripheral blood mononuclear cell; PD-1 = programmed cell death protein 1; PD-L1 = programmed death-ligand 1; PLR = platelet-to-lymphocyte ratio; PFS = progression-free survival; pts = patients; RTR = renal transplant recipient; SCCA = squamous cell carcinoma antigen; SLN = sentinel lymph node; tDNA = tumor DNA; TMB = tumor mutational burden; Treg = regulatory T cell; TSDR = treg-specific demethylated region; th = treatment; VAF = variant allele frequency.

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