TAN-POLARITY: A Tumour-Associated Neutrophil Polarisation Signal Framework for Biological Activity Assessment in Hepatocellular Carcinoma

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Disclaimer: This tool is intended solely to narrow the field of analytical focus and suggest directions for further investigation. It does not diagnose, treat, or make clinical decisions. All outputs require expert medical review. Numbers assigned to domains are evidence-informed estimates grounded in cited literature; they are not coefficients derived from a prospectively validated regression model. The scoring reflects signal prominence, not a prediction of clinical outcome.

Abstract

Tumour-associated neutrophils (TANs) in hepatocellular carcinoma (HCC) are not a monolithic population. Single-cell transcriptomic profiling across cancer types has resolved at least ten distinct neutrophil activation states, including angiogenic, antigen-presenting, inflammatory, and immunosuppressive subsets — with the angiogenic (VEGFA+SPP1+) subset linked to the worst patient outcomes and the antigen-presenting (HLA-DR+CD74+) subset associated with the most favourable survival signal. In HCC specifically, the balance between pro-tumour N2-polarised and anti-tumour N1-polarised TANs is shaped by contextual cues including disease aetiology (viral versus metabolic), local cytokine environment (TGF-β, GM-CSF, IFN-β), and neutrophil extracellular trap (NET) activity. We present **TAN-POLARITY**, an agent-executable composite scoring framework that integrates measurable features of the TAN axis in HCC — including circulating NLR, serum VEGF as a proxy for angiogenic TAN activity, TGF-β signalling evidence, CD10+ALPL+ immunosuppressive neutrophil signal, HLA-DR+ antigen-presenting neutrophil signal, NET activity markers, and HCC aetiology context — into a structured 0–100 Polarisation Signal Score (PSS). A higher PSS reflects a microenvironment more consistent with a pro-tumour, N2-dominant TAN configuration. A Monte Carlo uncertainty layer propagates measurement variability into a 95% confidence interval. Crucially, TAN-POLARITY does not

prescribe therapy. It generates a **TAN polarisation signal profile** that characterises which features are most consistent with N1-dominant, N2-dominant, or mixed TAN activity, maps these to their mechanistic implications for systemic therapy responsiveness, and flags biological axes warranting clinical attention. The framework is designed for research prioritisation, multidisciplinary discussion scaffolding, and transparent agentic clinical reasoning — not for point-of-care prescribing.

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1. Clinical Context and Justification

Neutrophils are the most abundant circulating leukocyte in humans, constituting 50–70% of all white blood cells. Their roles in infection and acute inflammation have been understood for decades. Their roles in cancer — and in HCC specifically — have been far more resistant to characterisation. The difficulty is biological: neutrophils are short-lived, transcriptionally sparse, and their function is highly context-dependent. For much of the past two decades, clinical practice in HCC used the neutrophil-to-lymphocyte ratio (NLR) as a prognostic surrogate without an accompanying mechanistic framework. A higher NLR has been associated with shorter overall survival across multiple HCC cohorts and treatment modalities, and pretreatment NLR ≥ 2.4 has been linked to poor survival in patients receiving tyrosine kinase inhibitor (TKI) plus immune checkpoint inhibitor (ICI) combination therapy. However, NLR alone does not answer the question that matters most for treatment orientation: is the neutrophil burden in this patient's tumour microenvironment functioning in a predominantly anti-tumour or pro-tumour capacity?

The past four years have produced evidence that reframes this question with new precision. A landmark 2024 study published in *Cell* integrated single-cell neutrophil transcriptomes from 17 cancer types and identified ten distinct neutrophil activation states, including a VEGFA+SPP1+ pro-angiogenic state linked to the worst patient outcomes pan-cancer and an HLA-DR+CD74+ antigen-presenting state associated with the most favourable survival signal. A 2025 paper in the *Journal of Experimental Medicine* identified a specific TAN subset — SiglecF-high TANs driven by c-Myc reprogramming — that is disproportionately prevalent in metabolic dysfunction-associated steatohepatitis (MASH)-related HCC compared to viral-related HCC. These cells suppress antigen presentation in tumour cells via TGF-β, promote cancer stemness, and directly attenuate checkpoint inhibitor responsiveness, explaining in part why non-viral HCC responds more poorly to immunotherapy than viral HCC. A separate 2023 paper in *Molecular Immunology* confirmed that immunosuppressive CD10+ALPL+ neutrophils promote resistance to anti-PD-1 therapy in HCC specifically by mediating irreversible T-cell exhaustion.

These developments create both an opportunity and an obligation to articulate a more mechanistically grounded TAN assessment framework for HCC. TAN-POLARITY is designed

to fill that gap — providing a structured, executable, citable approach to characterising the TAN polarisation signal in an individual HCC case, and mapping it to the biological implications for current systemic therapy strategies.

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2. Biological Basis: Neutrophil Polarisation and the HCC Tumour Microenvironment

2.1 The N1/N2 Framework and its Limitations

The canonical N1/N2 classification describes anti-tumour (N1) and pro-tumour (N2) neutrophil phenotypes in broad parallel to the M1/M2 macrophage framework. N1 polarisation is promoted by IFN-β and pathogen-associated molecular pattern (PAMP) stimulation; it is characterised by a hypersegmented nuclear morphology, high surface expression of CD54 (ICAM-1) and CD95 (FasL), production of reactive oxygen species (ROS), hydrogen peroxide (H_2O_2), nitric oxide (NO), and TNF-α. N1 TANs can directly kill tumour cells and recruit cytotoxic T lymphocytes and NK cells. In early-stage HCC, N1-polarised neutrophils have been shown to capture and kill HCC cells by releasing NETs and targeting tumour cells through these cytotoxic mediators.

N2 polarisation is driven by TGF-β, IL-10, and GM-CSF. N2-polarised TANs express arginase-1 (ARG1), matrix metalloproteinase-9 (MMP-9), and VEGFA. They promote angiogenesis, facilitate tumour cell migration and invasion, suppress cytotoxic T-cell and NK-cell function, and drive EMT through FAM3C-mediated JNK-ZEB1/Snail signalling. In the established HCC microenvironment, N2-dominant TAN populations are the norm rather than the exception.

The binary N1/N2 classification is acknowledged to be an oversimplification. Single-cell transcriptomics has revealed that neutrophil polarisation exists as a spectrum of states rather than two fixed endpoints, and that the same cell may simultaneously express features associated with both N1 and N2 activity. In early-stage lung cancer, a subset of TANs with hybrid neutrophil and antigen-presenting cell (APC) properties has been described. In pancreatic tumours, immature and mature neutrophils converge into a terminally differentiated dcTRAIL-R1+ state. In HCC, TGF-β-driven SOX18 elevation promotes PD-L1 and CXCL12 upregulation, reinforcing the immunosuppressive N2 configuration. The N1/N2 framework remains clinically useful as a directional signal but should be understood as an approximation of a continuous functional landscape.

2.2 Pro-Tumour Mechanisms of N2 TANs in HCC

Four pro-tumour mechanisms are mechanistically well-supported in HCC:

Angiogenesis promotion. N2-polarised TANs are a significant source of VEGFA within the HCC tumour microenvironment. The VEGFA+SPP1+ TAN subset identified by pan-cancer single-cell profiling carries the strongest adverse prognostic association of any TAN state characterised to date, and was identified in the HCC cohort of that study (n=357). TANs also contribute pro-angiogenic signalling through MMP-9-mediated release of matrix-bound VEGF and through production of S100A9. In hypoxic tumour niches, dcTRAIL-R1+ TANs promote angiogenesis to enhance tumour oxygenation and nutrition.

T-cell suppression and immune evasion. Multiple mechanisms are described. GM-CSF and TNF in the HCC peritumoral area reprogramme neutrophils to enhance PD-L1 expression and suppress T-cell activity. CD10+ALPL+ immunosuppressive neutrophils in HCC mediate irreversible T-cell exhaustion, directly driving anti-PD-1 resistance. SiglecF-high TANs in MASH-related HCC suppress the antigen presentation machinery of tumour cells via TGF-β secretion. ARG1-expressing N2 TANs deplete local L-arginine, impairing T-cell receptor signalling and effector function. Peritumoral stromal neutrophils are essential for c-Met-elicited metastasis and further impair the anti-tumour immune response.

EMT promotion and invasive capacity. N2 TANs promote EMT through FAM3C-mediated JNK-ZEB1/Snail signalling. The VEGFA+SPP1+ TAN subset in HCC correlates with pathways related to cancer stemness, cancer proliferation, and EMT. In MASH-related HCC, SiglecF-high TANs specifically promote HCC stemness, proliferation, and migration through TGF-β secretion. IL-10/c-Met/STAT3 signalling through N2 TANs enhances distant metastasis and PD-L1 expression.

NET-mediated pro-metastatic functions. Neutrophil extracellular traps (NETs) — web-like chromatin structures decorated with antimicrobial proteins including myeloperoxidase (MPO), neutrophil elastase (NE), and citrullinated histone H3 (CitH3) — were originally characterised in infectious contexts but are now recognised as contributors to tumour biology. In the pro-tumour context, N2 TAN-derived NETs construct pre-metastatic niches by trapping circulating tumour cells (CTCs) and remodelling the ECM. In HCC, NETs promote tumour angiogenesis and have been implicated in resistance to immune checkpoint inhibition — particularly in the context of cirrhotic extracellular matrix, where elevated collagen type 1 (Col1) triggers immunosuppressive NET formation that attenuates anti-PD-1 therapy response.

2.3 Anti-Tumour Mechanisms of N1 TANs in HCC

Direct cytotoxicity. N1-polarised neutrophils kill HCC cells through ROS generation (superoxide, H_2O_2), NO production, TRAIL/FasL-mediated apoptosis, and antibody-dependent cellular cytotoxicity (ADCC). Leukotriene B4 (LTB4) drives ROS production via xanthine oxidase, enabling direct oxidative tumour damage.

Adaptive immune activation. N1 TANs recruit cytotoxic T lymphocytes and NK cells to the tumour site. The HLA-DR+CD74+ antigen-presenting neutrophil subset identified in the 2024 *Cell* pan-cancer atlas expresses MHC-II molecules and costimulatory molecules (CD80), can invoke both neoantigen-specific and antigen-independent T-cell responses, and carries the strongest favourable survival association of any TAN state across most cancer types studied. Critically, this antigen-presenting programme can be evoked by leucine metabolism and consequent histone H3K27ac modification — a finding with potential pharmacological implications.

Angiogenesis restriction. N1 TANs can inhibit pathological angiogenesis, restricting tumour vascularisation through mechanisms that partially counter the VEGFA secretory activity of N2 TANs.

NET-mediated anti-tumour activity. In the N1 context, NETs can ensnare tumour cells and deliver cytotoxic MPO and defensins directly, inducing tumour cell apoptosis through an NADPH oxidase 2-independent mechanism. The spatial localisation of NETs matters: N1 TAN-derived NETs tend to act at tumour margins, while N2 TAN-derived NETs are located nearer to the tumour vasculature and dormant cancer cells.

2.4 Disease Aetiology and TAN Polarisation in HCC

A key clinical finding emerging from the 2025 *Journal of Experimental Medicine* study is that TAN phenotype is not uniformly distributed across HCC aetiologies. SiglecF-high (pro-tumour) TANs are predominantly elevated in MASH-related HCC, where the microenvironment is enriched for linoleic acid and GM-CSF — factors that foster c-Myc-driven TAN reprogramming. Viral-associated HCC (HBV, HCV) shows comparatively lower SiglecF-high TAN infiltration. This biological distinction partially accounts for the observed aetiology-dependence of checkpoint inhibitor response in HCC clinical trials, where non-viral patients consistently demonstrate poorer responses to ICIs than viral patients. The aetiology signal is therefore not simply a proxy for something else; it encodes a genuine TAN biology difference that TAN-POLARITY captures as a distinct weighted domain.

2.5 Key Proteins and Molecular Mediators of TAN Activity

The following proteins are central to TAN biology in HCC and form the molecular basis of domain scoring:

Protein / Factor	Function	TAN Polarity Association
VEGFA	Angiogenesis promotion, tumour vascularisation	Pro-tumour (N2 / VEGFA+SPP1+ subset)
TGF-β	N1→N2 polarisation master regulator; EMT driver; antigen presentation suppressor	Pro-tumour
GM-CSF (CSF2)	Drives SiglecF-hi TAN subset; enhances PD-L1 on TANs; T-cell suppression	Pro-tumour
ARG1	L-arginine depletion; impairs T-cell receptor signalling	Pro-tumour (N2)
MMP-9	ECM remodelling; VEGF release from matrix; invasion support	Pro-tumour
NE (Neutrophil Elastase)	IRS-1 degradation in tumour cells; PI3K/Akt activation	Pro-tumour
PD-L1	T-cell checkpoint suppression on TANs	Pro-tumour
c-Met	Hepatocyte growth factor receptor; TAN-mediated metastasis signalling	Pro-tumour
IL-10	Drives N2 polarisation; PD-L1 upregulation via c-Met/STAT3	Pro-tumour
FAM3C	EMT promotion via JNK-ZEB1/Snail in TAN-tumour crosstalk	Pro-tumour
S100A9	Angiogenesis; neutrophil recruitment; pre-metastatic niche formation	Pro-tumour
MPO	Oxidative tumour killing in NETs (N1 context)	Anti-tumour (N1)

CitH3 (citrullinated H3)	NET scaffold marker; context-dependent	Context-dependent
TNF-α	Direct tumour cytotoxicity (N1); peritumoral immunosuppression when sustained	Dual
IFN-β	Master inducer of N1 polarisation; reduces chemokine receptor expression	Anti-tumour (N1)
HLA-DR / CD74	MHC-II antigen presentation; T-cell activation	Anti-tumour (N1 / HLA-DR+ subset)
CXCL5 / CXCL8 (IL-8)	Neutrophil chemoattractant; tumour-directed recruitment	Context-dependent
LTB4 (Leukotriene B4)	ROS production via XO; tumour cell oxidative damage	Anti-tumour (N1)
SPP1 (Osteopontin)	Co-expressed with VEGFA in worst-prognosis TAN subset	Pro-tumour

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3. Scoring Architecture

3.1 Domain Selection and Weight Rationale

TAN-POLARITY integrates **nine domains** across four biological axes: the systemic neutrophil burden axis, the angiogenic/pro-tumour axis, the immunosuppressive axis, and the anti-tumour / N1 axis. Weights reflect the relative strength of published associations between each domain and N2-dominant TAN biology, prognostic impact, or documented mechanistic contribution in HCC or cross-cancer contexts where HCC data is supported. Weights are explicitly expert-informed estimates, not regression coefficients.

Directionality convention: All domains are scored such that a higher raw score (0–100) represents greater evidence of a pro-tumour (N2-dominant) polarisation signal. Anti-tumour features are scored in inverse — higher values of N1-promoting features reduce the pro-tumour contribution. The composite Polarisation Signal Score (PSS) therefore ranges from 0 (consistent with N1-dominant, immune-active TAN biology) toward 100 (consistent with N2-dominant, pro-tumour TAN biology).

Domain	Axis	Weight	Rationale
Neutrophil-to-lymphocyte ratio (NLR)	Systemic neutrophil burden	0.16	Extensively validated prognostic marker in HCC; elevated NLR associated with poor OS across curative and systemic treatment contexts (meta-analysis HR 1.55 for OS); pretreatment NLR ≥ 2.4 associated with poor survival in TKI+ICI therapy
Serum VEGF level	Angiogenic / pro-tumour	0.16	N2 TANs are a primary source of VEGFA in the HCC TME; VEGFA+SPP1+ TAN subset carries the worst pan-cancer survival signal; serum VEGF elevation reflects both tumour-intrinsic and TAN-derived angiogenic activity
TGF-β signalling evidence	Immunosuppressive / polarisation	0.14	TGF-β is the master regulator of N1→N2 polarisation; TGF-β-driven SOX18 elevation promotes PD-L1/CXCL12 upregulation in HCC; SiglecF-hi TAN TGF-β secretion suppresses antigen presentation; SM16 (TGF-β inhibitor) blocks N1→N2 conversion
HCC disease aetiology	Polarisation context	0.12	MASH-related HCC preferentially accumulates SiglecF-hi c-Myc-driven pro-tumour TANs (GM-CSF + linoleic acid driven); non-viral HCC has poorer ICI responses partly attributable to this TAN biology difference
CD10+ALPL+ immunosuppressive neutrophil signal	Immunosuppressive	0.12	CD10+ALPL+ neutrophils specifically drive irreversible T-cell exhaustion and anti-PD-1 resistance in HCC; a distinct and high-impact

			pro-tumour subset with HCC-specific evidence
NET activity markers (MPO, CitH3, NE)	Context-dependent / pro-tumour in N2	0.12	Cirrhotic ECM induces immunosuppressive NET formation attenuating aPD-1 therapy; N2 TAN NETs construct pre-metastatic niches; elevated circulating NET markers (cf-DNA, CitH3, MPO-DNA complexes) associated with HCC progression and poor outcomes
HLA-DR+ / antigen-presenting neutrophil signal	Anti-tumour / N1 axis	0.10	HLA-DR+CD74+ TANs represent the anti-tumour neutrophil subset with the strongest favourable survival signal across 17 cancer types including HCC (n=357); leucine-evocable antigen-presenting programme activates neoantigen-specific T-cell responses; **scored inversely**
GM-CSF / c-Myc TAN reprogramming signal	Pro-tumour / aetiology-linked	0.08	GM-CSF promotes SiglecF-hi TAN polarisation; c-Myc regulon enrichment in pro-tumour TANs; GM-CSF+TNF expression in peritumoral HCC enhances PD-L1 and T-cell suppression
IFN-β / N1-promoting cytokine signal	Anti-tumour / N1 polarisation	0.00*	IFN-β is the master N1 inducer; type I IFN therapy increases N1 markers and cytotoxic features; **scored inversely; weight 0.00 in current version** pending validated measurement approach

*IFN-β domain is included in the biological framework as a directional signal but is not weighted in the composite score in the current version due to the absence of a standardised serum measurement that maps reliably to intratumoral TAN N1 polarisation. Future iterations should incorporate this domain as validated assays become available.

Total active weight: 1.00 (0.16 + 0.16 + 0.14 + 0.12 + 0.12 + 0.12 + 0.10 + 0.08 = 1.00)

3.2 Composite Score Calculation

Each domain receives a raw subscale score (0–100) based on measured or estimated inputs. The Polarisation Signal Score (PSS) is:

PSS = Σ (domain_raw_score × domain_weight)

Capped at 100. A higher PSS is more consistent with a pro-tumour, N2-dominant TAN configuration. A lower PSS is more consistent with an anti-tumour, N1-active TAN configuration. The PSS does not determine treatment and does not predict clinical outcome. It characterises the biological signal prominence in the TAN axis.

3.3 Monte Carlo Uncertainty Layer

Continuous inputs (NLR, serum VEGF, NET marker quantification) carry real-world measurement variability. To represent this honestly, 5,000 Monte Carlo simulations perturb each continuous input with Gaussian noise (coefficient of variation 10–15%, reflecting typical inter-laboratory variability) and recompute the PSS. The 2.5th and 97.5th percentile outputs form the reported 95% confidence interval.

This CI reflects input measurement uncertainty, not model validation uncertainty. The model has not been prospectively calibrated or externally validated. The CI is a tool for intellectual honesty, not a statistical guarantee. Where categorical inputs (aetiology, HLA-DR+ signal level) are uncertain or not clearly assignable, the analyst should explicitly document this and treat the score as indicative rather than precise.

3.4 TAN Signal Profile

Beyond the PSS, TAN-POLARITY generates a **TAN Signal Profile** — a structured characterisation of which biological axes are most elevated, mapped to the mechanistic implications for systemic therapy responsiveness:

TAN Axis	Pro-Tumour Signal Features	Mechanistic Implications
Angiogenic (N2)	VEGF↑, NLR↑, VEGFA+SPP1+ signals	Consistent with angiogenic TAN activity; potential VEGFR/VEGF targeting relevance (sorafenib, lenvatinib, bevacizumab)

Immunosuppressive (N2)	CD10+ALPL+↑, ARG1 evidence, TGF-β↑, PD-L1 on TANs	Consistent with checkpoint inhibitor attenuated response; TAN-mediated T-cell exhaustion pathway prominent
Aetiology-driven N2	MASH/metabolic aetiology, GM-CSF↑, SiglecF-hi features	Consistent with the MASH-specific immunosuppressive TAN programme; non-viral ICI response attenuation biology
NET-mediated pro-metastatic	Elevated NET markers, cirrhotic ECM, Col1↑	Consistent with NET-driven premetastatic niche and ICI resistance via cirrhotic-ECM pathway
Anti-tumour (N1)	HLA-DR+ signal, low NLR, IFN-β context	Consistent with antigen-presenting TAN activity; more permissive immune microenvironment for checkpoint inhibitor engagement

3.5 Score Categories

PSS	Category	Interpretation
< 20	LOW (N1-consistent)	TAN axis more consistent with anti-tumour, immune-active features; relatively favourable TME signal from neutrophil axis
20–39	MODERATE	Mixed TAN polarisation signal; neither N1 nor N2 axis clearly dominant; evaluate individual domain contributions
40–59	HIGH (N2-leaning)	TAN axis more consistent with pro-tumour features; multiple N2-associated signals present
≥ 60	VERY HIGH (N2-dominant)	Strong convergent pro-tumour TAN signal; angiogenic, immunosuppressive, or NET-mediated axes likely prominent

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4. Demonstration Scenarios

Scenario 1 — Viral HCC (HBV) with moderate NLR and early immune-active features

Patient: HBV-related HCC; NLR 2.8; serum VEGF 220 pg/mL; no clinical or radiological evidence of TGF-β pathway activation; MASH absent, viral aetiology; no documented CD10+ALPL+ neutrophil enrichment on prior biopsy TME profiling; NET markers: MPO-DNA complexes mildly elevated, CitH3 negative; HLA-DR+ neutrophil signal: present on flow cytometry; GM-CSF not elevated in clinical data.

Domain scores:

- NLR (2.8): raw 35 → weighted 5.6
- VEGF (220 pg/mL): raw 40 → weighted 6.4
- TGF-β: clinically absent: raw 5 → weighted 0.7
- Aetiology (HBV/viral): raw 10 → weighted 1.2
- CD10+ALPL+: not elevated: raw 0 → weighted 0.0
- NET markers: mildly elevated: raw 25 → weighted 3.0
- HLA-DR+ (present, inversely scored): raw 25 → penalty: raw pro-tumour contribution offset → effective contribution 7.5 (inverse)
- GM-CSF: not elevated: raw 5 → weighted 0.4

Composite PSS: ~24.8 / 100 [MODERATE] 95% CI (estimated): [21.4, 28.5]

TAN signal profile:

- Angiogenic axis: **LOW-MODERATE** (VEGF mildly elevated, NLR in moderate range)
- Immunosuppressive axis: **LOW** (TGF-β absent, CD10+ absent)
- Aetiology-driven N2: **LOW** (viral aetiology — SiglecF-hi programme not typically dominant)
- NET axis: **MILD** (MPO-DNA mildly elevated, no CitH3)
- Anti-tumour (N1) signal: **PRESENT** (HLA-DR+ neutrophils detectable)

Interpretive note: This profile's TAN axis is more consistent with a mixed but N1-leaning biology. The viral aetiology, detectable HLA-DR+ antigen-presenting neutrophil signal, and absent immunosuppressive TAN markers are features more consistent with a TAN microenvironment less antagonistic to checkpoint inhibitor engagement. The moderate NLR and VEGF elevation indicate that the pro-angiogenic axis is not absent. These are mechanistic observations only — not treatment recommendations.

Scenario 2 — MASH-related HCC with elevated NLR and TGF-β pathway activity

Patient: MASH-related HCC; NLR 5.4; serum VEGF 380 pg/mL; TGF-β pathway active (elevated serum TGF-β1, radiological fibrosis grade F4, SOX18 expression elevated on molecular panel); MASH aetiology confirmed; CD10+ALPL+ neutrophil enrichment documented on prior immunohistochemistry; NET markers: MPO-DNA complexes elevated, CitH3 positive; HLA-DR+ neutrophil signal: absent by flow cytometry; GM-CSF elevated in the peritumoral environment (reported on biopsy).

Domain scores:

- NLR (5.4): raw 80 → weighted 12.8
- VEGF (380 pg/mL): raw 70 → weighted 11.2
- TGF-β: active: raw 85 → weighted 11.9
- Aetiology (MASH): raw 80 → weighted 9.6
- CD10+ALPL+: documented: raw 80 → weighted 9.6
- NET markers: elevated + CitH3+: raw 75 → weighted 9.0
- HLA-DR+ (absent, inversely scored): raw 0 → weighted 0 (no N1 offset)
- GM-CSF: elevated: raw 70 → weighted 5.6

Composite PSS: ~69.7 / 100 [VERY HIGH] 95% CI (estimated): [63.2, 75.9]

TAN signal profile:

- Angiogenic axis: **HIGH** (VEGF 380, NLR 5.4)
- Immunosuppressive axis: **HIGH** (TGF-β active, CD10+ALPL+ documented)
- Aetiology-driven N2: **HIGH** (MASH — SiglecF-hi TAN programme biology relevant)
- NET axis: **HIGH** (MPO-DNA elevated, CitH3 positive — pro-metastatic and ICI-attenuating configuration more consistent)
- Anti-tumour (N1) signal: **ABSENT** (HLA-DR+ neutrophils not detected)

Interpretive note: This profile presents convergent pro-tumour TAN signals across angiogenic, immunosuppressive, aetiology-specific, and NET-mediated axes. The MASH context, elevated GM-CSF, TGF-β pathway activity, and absent HLA-DR+ antigen-presenting neutrophil signal are features more consistent with an N2-dominant TAN microenvironment. The documented CD10+ALPL+ neutrophil enrichment is specifically associated with anti-PD-1 resistance in HCC literature. Taken together, these features identify a TAN axis profile warranting careful biological scrutiny in any discussion of immune checkpoint inhibitor strategy — though no conclusion about therapy suitability is drawn here.

Scenario 3 — Advanced HCC with cirrhotic ECM and mixed signals

Patient: HCV-related HCC (SVR achieved, inactive viral); advanced cirrhosis F4; NLR 4.1; serum VEGF 310 pg/mL; TGF-β pathway: elevated, attributed primarily to cirrhotic ECM rather than active inflammatory aetiology; aetiology: formerly viral, now cirrhosis-dominant; CD10+ALPL+: not documented on molecular panel; NET markers: highly elevated (MPO-DNA, CitH3 positive, consistent with cirrhotic-ECM NET induction pattern); HLA-DR+ signal: low-moderate; GM-CSF: mildly elevated.

Domain scores:

- NLR (4.1): raw 62 → weighted 9.9
- VEGF (310 pg/mL): raw 58 → weighted 9.3
- TGF-β: elevated (cirrhotic-ECM driven): raw 65 → weighted 9.1
- Aetiology (formerly viral, cirrhosis-dominant): raw 40 → weighted 4.8
- CD10+ALPL+: not documented: raw 0 → weighted 0.0
- NET markers: highly elevated, CitH3+: raw 85 → weighted 10.2
- HLA-DR+ (low-moderate, inversely scored): raw 15 → partial offset → effective contribution 3.0
- GM-CSF: mildly elevated: raw 30 → weighted 2.4

Composite PSS: ~47.7 / 100 [HIGH] 95% CI (estimated): [42.0, 53.8]

TAN signal profile:

- Angiogenic axis: **MODERATE-HIGH**
- Immunosuppressive axis: **MODERATE** (TGF-β elevated but cirrhosis-attributed rather than MASH/GM-CSF specific)
- Aetiology-driven N2: **MODERATE** (SVR achieved; cirrhosis now the dominant contextual driver)
- NET axis: **VERY HIGH** (consistent with cirrhotic-ECM-induced immunosuppressive NET pattern documented in HCC; most prominent domain in this profile)
- Anti-tumour (N1) signal: **LOW** (HLA-DR+ low-moderate; partially offsetting)

Interpretive note: The most prominent feature in this profile is the NET axis, whose elevation appears more consistent with the cirrhotic-ECM-mediated NET induction pattern described by Shen et al. (2024) than with a de novo MASH-driven TAN reprogramming. This distinction has mechanistic relevance: cirrhotic-ECM-induced NETs attenuate anti-PD-1 response through a collagen type 1-dependent mechanism, and this pathway may be a more tractable intervention target than the GM-CSF/SiglecF-hi axis prominent in Scenario 2. These observations are speculative and hypothesis-generating only.

5. Explicit Limitations

The following limitations are not caveats appended for completeness — they are central to the correct use of this tool.

This tool does not diagnose, treat, prescribe, or eliminate therapeutic options. Its sole purpose is to characterise the TAN polarisation signal in HCC and make this biological reasoning explicit, structured, and auditable in a research or multidisciplinary discussion context.

The model is not externally validated. Domain weights are evidence-informed estimates anchored to cited literature, not regression coefficients derived from a prospective cohort. The tool cannot compute sensitivity, specificity, or positive/negative predictive value.

The N1/N2 framework is a useful approximation, not a molecular truth. Neutrophil biology as understood through single-cell transcriptomics is far more complex than the binary framework suggests. The TAN polarisation spectrum is continuous, context-dependent, and may be spatially heterogeneous within a single tumour. Scores should be understood as directional signals, not precise phenotypic assignments.

The Monte Carlo confidence interval reflects input measurement noise, not model performance. A narrow CI does not imply that the model is correct; it implies that the score is stable across small perturbations of the continuous inputs. Categorical inputs (aetiology, CD10+ALPL+ signal, HLA-DR+ signal) carry additional uncertainty that is not propagated in the current CI calculation.

Biomarker availability varies substantially. CD10+ALPL+ neutrophil quantification, HLA-DR+ neutrophil flow cytometry, and NET marker panels are not universally available in clinical practice. Missing domains should be treated as uninformative (using conservative default scores) and noted explicitly in the output interpretation. The NLR and serum VEGF are the most accessible inputs and form the empirical anchor of the framework.

HCC aetiology scoring carries important caveats. The MASH-specific TAN biology finding (SiglecF-hi TANs) is established in preclinical models and supported by human cohort data but has not yet been prospectively validated as a treatment-modifying biomarker. The aetiology domain should not be treated as more precise than the mechanistic basis warrants.

Serum VEGF as a TAN angiogenic proxy has limitations. Serum VEGF reflects contributions from tumour cells, stromal cells, platelets, and circulating cells — not TANs exclusively. It is used here as the most accessible proxy for the VEGFA+SPP1+ TAN axis, not as a direct TAN

measurement. Where tumour-tissue VEGFA expression data or single-cell TAN profiling is available, those data should be considered more directly informative than serum VEGF.

All outputs require expert medical review. This framework is designed for use by clinicians and researchers who can contextualise its outputs within the full clinical picture, including performance status, hepatic reserve (Child-Pugh score), prior therapy, contraindications, and local institutional practice. It is not appropriate for use without such oversight.

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6. Why This Framework Exists

Three concrete research and clinical use cases motivate TAN-POLARITY:

Biological profile mapping. When discussing an HCC case in a multidisciplinary tumour board, the TAN axis is rarely characterised with mechanistic precision. TAN-POLARITY provides a structured vocabulary for documenting which TAN-related features are most prominent — shifting the discussion from "the NLR is high" to a richer mechanistic account of what that might mean in context.

Research prioritisation. For investigators designing HCC biomarker studies, the framework identifies which TAN axis domains are most likely to differentiate patient subgroups with distinct immunotherapy outcomes, and which domains (e.g., CD10+ALPL+ signal, HLA-DR+ neutrophil quantification) are most under-characterised relative to their mechanistic importance.

Agentic auditability. In AI-mediated clinical tooling, safety-relevant reasoning should not live in hidden prompts. An explicit, executable, open framework allows domain weights to be challenged, updated, and calibrated as the rapidly evolving TAN biology field produces new evidence. The current framework reflects the literature through early 2026 and should be treated as a living document.

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7. References

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TAN-POLARITY is an agent-executable research framework. It is not a medical device, a validated clinical prediction tool, or a substitute for specialist oncological assessment. The neutrophil biology of HCC is evolving rapidly; domain weights and signal assignments should be reviewed as new evidence emerges.
