Executive summary of tumour recognition

Alexander Young | Metastatic microsatellite-stable rectal cancer | 22 October 2026 | 1 of 2

HLA class I proteins display tumour peptides to CD8 T cells. Reprocessing Caris, Tempus and 18 public colorectal tumours supports relatively low TAP1 and HLA-A/B/C RNA in both case specimens; IRF1 depends strongly on transcript definition. Test whether impaired display and immune access are reversible in current tumour.

Specimens: Caris biopsy, March 2024 [C4]; Tempus/BostonGene treated rectal resection, October 2024 [C2] [C3] [C5]; Valius liver, October 2024 [C6]. Tumour content: BostonGene about 15%; Tempus 30% after microdissection [C13]. Percentiles use each provider's tumour reference. Research TPM (transcripts per million) is not a clinical percentile or tumour-cell-specific measurement. "Coding" means RNA transcripts annotated to encode protein; it is not a protein measurement. Sections 13–17 give results and reference comparisons. "Not reported" means unavailable, not absent.

Possible mechanism	Caris Rectal biopsy	Tempus Rectal resection	BostonGene Rectal resection	Valius Liver metastasis	Evidence for / against	Most useful discriminating test	Therapeutic options
Weak interferon response PTPN1/2 enzymes may restrain immune signalling.	IRF1 TPM: total 128; coding 18.8. TAP1 18.7. PTPN1/2 RNA detected; no established activating alteration.	IRF1 TPM: total 33.6; coding 2.8. TAP1 26.1. PTPN1/2 RNA detected; genes absent from DNA panel.	PTPN1 75th; PTPN2 37th. Low downstream IRF1 (2nd), TAP1 (1st), HLA-A/B/C (2nd/5th/3rd).	PTPN1/2 not reported in supplied single-cell summaries; bulk sequencing failed quality control.	Same-pipeline reference supports low TAP1/HLA-A/B/C in both samples. IRF1 is transcript-sensitive. Assay/cell mixture confound; PTPN1/2 causality unproven.	Interferon-γ alone versus plus AC484: measure activated STAT1, IRF1, surface HLA and immune-cell killing. Gene-specific suppression and tumour/immune-cell controls test whether PTPN1/2 cause the restraint.	ABBV-CLS-484 (AC484) ± PD-1 blockade: phase 1; colorectal benefit unproven. Registered expanded access needs current confirmation. [L1] [T1] [T2]
APC / PTPN13 Truncated APC may permit PTPN13 to suppress antigen display.	Two APC protein-truncating mutations, at residues 450 and 1411. PTPN13 RNA detected.	Confirms APC truncation at residue 450; second variant not listed. PTPN13 RNA detected.	PTPN13 93rd, with low IRF1 and HLA. Limited variant export does not overturn clinical APC calls.	APC/PTPN13 results not reported in supplied summaries; no usable bulk sequencing.	Specific genetic fit; mutations are unassigned to separate gene copies. PTPN13 activity and dependence remain unmeasured.	Compare PTPN13 suppression or APC11 peptide with delivery/inactive-peptide controls and AC484. Rescue of signalling, surface HLA and tumour killing would support this mechanism.	TAT-APC11, an APC-derived peptide with a cell-entry tag: preclinical; human safety/access unestablished. AC484 does not inhibit PTPN13. [L3]
PTEN / NRF2 PTEN loss may allow the stress regulator NRF2 to drive HLA destruction.	PTEN premature-stop mutation at residue 335. PTEN RNA 29th; protein loss untested.	PTEN mutation not listed, which is not proof of absence. PTEN RNA detected.	NRF2 97th; regulator KEAP1 12th; degradation adaptor NBR1 81st. PTEN RNA 98th; other NRF2 outputs mixed/low.	PTEN/NRF2 pathway results not reported in supplied summaries; no usable bulk sequencing.	PTEN loss unproven; high RNA does not establish functional protein. Low HLA RNA cannot demonstrate protein destruction.	Measure tumour PTEN protein, nuclear NRF2 and total versus surface HLA. Selective NRF2/NBR1 suppression restoring HLA and immune killing, with viability controls, would support causality.	No established pathway-specific rescue. NRF2 inhibitor ML385 is a laboratory probe; PI3K inhibitors or hydroxychloroquine are not validated substitutes. [L4]
Fixed HLA-display defect B2M (beta-2 microglobulin) supports stable HLA class I.	HLA-A/B/C 676/670/547 TPM. HLA-A loss of heterozygosity (allele-loss signal) not detected.	HLA-A/B/C 439/548/338 TPM; B2M RNA present. No validated B2M defect.	HLA-A/B/C low relative to reference; B2M RNA present. Tumour surface protein unmeasured.	B2M/HLA results not reported in selected summaries. Archived CEA target protein strongly positive. [C10]	Research-only B2M candidate: cysteine-to-arginine change at residue 100. Weak, read-end-biased evidence; no validated mutation or protein loss.	Independently sequence current tumour and normal DNA; assess both gene copies and tumour B2M/HLA protein. Test interferon reversibility and native tumour recognition before declaring irreversible loss.	If irreversible loss is confirmed: HLA-independent CEA-directed engagers/cells or natural killer approaches. Investigational; confirm current target protein. [L19] [L20]

Executive summary of immune access and function

Alexander Young | Evidence matrix continued | 2 of 2

Reading this page: Caris and BostonGene percentiles use different reference cohorts. Valius percentages mean cells with detectable RNA, not protein positivity; cell counts reflect sampled cells, not unbiased infiltration. Single-cell non-detection can reflect assay sensitivity. Tempus and BostonGene are two assays of the same historical resection. Sources: [C2] [C3] [C4] [C5] [C6] [C12].

Possible mechanism	Caris Rectal biopsy	Tempus Rectal resection	BostonGene Rectal resection	Valius Liver metastasis	Evidence for / against	Most useful discriminating test	Therapeutic options
Stromal exclusion Fibroblast signals may prevent immune cells reaching tumour.	Consensus molecular subtype 2 (canonical). TGFB1 36th; TGFB2 RNA detected in audit.	TGFB2 RNA detected. No stromal classification; TGFBR1 DNA coverage limited.	Fibrotic, non-inflamed portrait. TGFB2/3 98th; CXCL12 99th; fibroblast-associated ACTA2 99th and FAP 78th.	16,040 fibroblasts recovered; FAP RNA in 3.91% of fibroblasts. TGFβ/CXCL12 pathway results not reported.	Strong rectal stromal signal; exclusion unproven. Biopsy classification differs. Radiation fibrosis, smooth muscle and low purity confound interpretation.	Spatially map tumour, CD8 cells and fibroblasts; measure active TGFβ signalling (phosphorylated SMAD2/3). Improved migration and killing after TGFβ/CXCL12-pathway blockade would support exclusion.	Vactosertib + pembrolizumab; CXCR4 blocker plerixafor or CXCL12 blocker NOX-A12: early clinical research, no established colorectal regimen. [L8] [L9] [L10]
Adenosine suppression CD39/CD73 enzymes can produce an immune-inhibitory metabolite.	Adenosine receptor ADORA2A 24th. CD39/CD73 not reported in selected panel.	No comparable adenosine-pathway results reported or included in targeted RNA audit.	CD39 98th; partner enzyme CD73 63rd; receptor ADORA2A 51st.	CD39 RNA undetected in CD4/CD8 T cells; detected in 4.2% of natural killer cells. CD73 in 1.08% of malignant cells.	Liver cell-specific and rectal bulk RNA are not directly comparable. CD39 can mark useful T cells. Adenosine production and suppression remain unmeasured.	Localise CD39/CD73 and measure ATP-to-adenosine conversion. Enzyme blockade should reduce flux; enzyme or receptor blockade restoring immune killing would demonstrate functional suppression.	Etrumadenant-containing combination: phase 2 colorectal signal, individual drug contribution unresolved. Development paused; access uncertain. [L11] [L12] [T3]
Myeloid suppression Macrophages or neutrophils may inhibit anti-tumour immunity.	No quantitative suppressive-cell or spatial-infiltration assessment reported.	No quantitative suppressive-cell or spatial-infiltration assessment reported.	CSF1 91st; macrophage marker CD163 84th. Other markers low: CD68 14th and neutrophil-related CXCL8 7th.	11,273 macrophages and 6,066 neutrophils recovered. Suppressive function not measured.	Mixed rectal marker profile. Liver counts include resident cells; presence does not establish suppressive function.	Identify myeloid states and their location. Remove and add back patient myeloid cells in tumour–immune co-culture; restored killing on removal, lost on add-back, supports suppression.	Zanzalintinib + atezolizumab: phase 3 colorectal evidence, still investigational; broad vascular/myeloid effects do not prove this mechanism. [L13] [T4]
Insufficient useful immune cells Too few T cells may recognise and kill the tumour.	Microsatellite stable; mismatch-repair proteins retained. Mutation burden 5 per megabase.	Microsatellite stable; mutation burden 4.2 per megabase.	Non-inflamed portrait. Mutation burden 0.42 in low-purity tissue; CD8A 56th, CD8B 16th, killing-related PRF1 26th.	2,231 CD8 T cells and 1,732 natural killer cells recovered; recognition and killing untested.	Low mutation burden fits, but does not exclude useful antigens. Liver contains immune cells; function remains unknown. Assay differences preclude a mutation-burden trend.	Check current blood counts and immune-cell yield. Test patient T-cell recognition and killing of native tumour, with HLA controls; peptide binding alone is insufficient. Assess tumour entry and cell function separately.	Personalised vaccines or engineered T-cell receptors: early clinical research, requiring suitable antigens and intact HLA display. CEA-directed or natural killer approaches may bypass HLA. [L18] [L19] [L20] [L21]

First tests: confirm current nodal tumour content/viability; map HLA-I/B2M protein, CD8 cells and stroma; then test interferon-γ ± AC484 and the best-supported alternative in viable models. Include matched controls, measure immune killing and preserve tissue for independent confirmation.

Other data/access: blood RNA is not tumour expression; negative blood DNA with little tumour DNA cannot exclude a lesion. TrufflePig's input provenance is unresolved [C7]. PTPN12 and chromatin repression remain unproven possibilities [L5]. Prior botensilimab/balstilimab (CTLA-4/PD-1 blockade) and lack of trial-measurable disease limit access/applicability; adding AC484 to that combination is untested.

Immune evasion mechanisms and experimental treatment strategies

Alexander Young metastatic rectal cancer

22 October 2026 | Case evidence and research priorities

The leading hypotheses to test are impaired antigen presentation and a fibroblast-rich environment that may prevent effective immune-cell access. Reprocessing Caris, Tempus and all 18 GSE50760 primary colorectal tumours with one pipeline supports relatively low TAP1 and HLA-A/B/C RNA in both case specimens, consistent with part of BostonGene's finding. IRF1 is more uncertain and transcript-sensitive. Neither RNA abundance nor the available mutations establish PTPN1/PTPN2 as the cause or predict benefit from AC484.

This report connects the clinical course, pathology, sequencing, bulk and single-cell expression, and research RNA reanalysis to interventions and experiments. Sections 13–17 report the consistent Caris/Tempus pipeline, its limitations and public-reference comparisons. Historical molecular samples largely predate BOT/BAL by about two years; current nodal disease needs direct confirmation.

Priorities for investigation

Priority	Reason to investigate	Decisive next evidence
Antigen presentation and interferon response	BostonGene low ranks; same-pipeline public comparison supports low TAP1/HLA RNA in both case samples	Current tumour HLA-I/B2M protein; interferon response and functional rescue
Fibroblast and chemokine exclusion	Fibrotic classification; high TGFB2/3, CXCL12 and stromal markers	Spatial CD8 distribution; active signalling; migration and killing assays
Competing intracellular mechanisms	APC truncations with high PTPN13; PTEN R335* with mixed NRF2 clues	Protein and causal perturbation tests that distinguish pathways
Effector supply and alternative recognition	MSS/low TMB; unknown tumour-reactive repertoire; strong historical CEA staining	Native tumour recognition; current target protein and heterogeneity

These are priorities for testing, not a treatment ranking. The most useful initial package is contemporary pathology and spatial protein assessment, a carefully allocated tumour/normal DNA and RNA analysis, then a small interferon ± AC484 experiment if viable material permits.

AC484 has early human data and a registered expanded-access programme, but no established MSS colorectal cancer efficacy. Adding it to PD-1 inhibition has a mechanistic and trial rationale; adding it to botensilimab/balstilimab remains untested. Balstilimab already blocks PD-1. [L1] [L2] [T1] [T2]

Evidence language: "observed" denotes a reported or directly checked case finding; "hypothesis" denotes a mechanistic interpretation; "preclinical" denotes laboratory/animal evidence. Trial response rates are study results, not personal response estimates. Development and access status are dated 22 October 2026.

1 What the different specimens can establish

Specimen and timing	Evidence available	Main limitation
Initial rectal biopsy 27 March 2024	Caris DNA/RNA, pathology; full paired-read RNA re-quantification	Superficial biopsy; historical, before subsequent therapies
Treated rectal resection 22 October 2024	Tempus DNA panel/RNA; BostonGene WES/WTS and immune portrait	BostonGene about 15% tumour; Tempus reports 30% after microdissection. Exact RNA composition remains uncertain. [C13]
Resected liver metastasis 22 October 2024	IHC and 10x Flex single-cell summaries: 92,897 cells, 1,018 classified malignant	Only selected gene/cell-type summaries available; liver WES/WTS failed QC
Blood assays 2025 to 2026	ctDNA, germline/matched normal, Caris buffy-coat and cell-free RNA	Blood RNA is not tumour expression; low tumour fractions limit negative DNA evidence
Banked nodal tissue 6 October 2026	Processing inventory: 14 vials, 60 small fragments	Current tumour pathology, viable yield and molecular state not yet established

Sources: pathology, assay and molecular records [C1] [C2] [C3] [C4] [C5] [C6]; tissue inventory [C9]. TrufflePig input provenance is unresolved [C7]. The separate "Valius Pipeline" bulk-expression column cannot be assigned to liver because specimen mapping is unresolved.

Clinical context that changes interpretation

The cancer is pMMR/MSS, historically RAS/BRAF wild type. Caris TMB was 5 and Tempus 4.2 mutations/Mb. BostonGene's 0.42 estimate came from low-purity treated tissue; this is not evidence of a biological decline in TMB. Low TMB reduces the usual checkpoint-response rationale but does not exclude useful neoantigens. [C1] [C4] [C5]

The reported two-cycle BOT/BAL course ran from July to September 2026. September PET/CT documented nodal progression; reported ctDNA also rose. This establishes a reason to investigate resistance, without identifying its mechanism. Current reported treatment is irinotecan, panitumumab and capecitabine from 9 October. Prior anti-VEGF treatment is not documented in the reviewed chronology. [C8]

No active liver lesion was reported on the September scan, but prior liver metastases were resected. Outcomes from "no liver metastases" trial subgroups cannot be assumed to apply. The reported absence of RECIST-measurable disease is an access constraint; trial investigators would still need to adjudicate eligibility.

The newest reported blood lymphocyte values available for this analysis were low in May–June 2026, including $0.56 \times 10^3/\mu L$ on 16 June. They are not current immune-function measurements, but support checking present counts and PBMC yield before planning cellular assays. [C11]

2 Expression patterns that deserve explanation

The values below use one coherent comparison: BostonGene bulk RNA from the treated rectal resection against its colorectal adenocarcinoma reference. Percentiles describe relative RNA abundance, not the proportion of positive tumour cells or enzyme activity. The approximately 15% tumour fraction makes cell composition a major alternative explanation. [C2] [C3]

Pathway	Gene expression log2(TPM+1)	CRC percentile
Phosphatases	PTPN1 5.5; PTPN2 4.8; PTPN12 5.8	75; 37; 75
Alternative phosphatase	PTPN13 3.7	93
Interferon signalling	JAK1 6.4; JAK2 3.4; STAT1 5.8; IRF1 4.5	96; 93; 30; 2
Interferon receptors	IFNGR1 6.1; IFNGR2 6.2	46; 7
Classical HLA class I	HLA-A 9.1; HLA-B 9.0; HLA-C 8.3	2; 5; 3
Antigen processing	TAP1 4.4; TAP2 4.0; PSMB8 5.4; PSMB9 4.2	1; 12; 1; 1
Regulation and recruitment	NLRC5 2.6; CXCL9 2.2; CXCL10 1.3	11; 28; 3
PTEN and NRF2 context	PTEN 5.9; NFE2L2 6.9; KEAP1 5.5; NBR1 6.5	98; 97; 12; 81
Stromal ligands	TGFB2 2.8; TGFB3 5.2; CXCL12 7.1	98; 98; 99
Stromal and adenosine markers	ACTA2 11.3; ENTPD1/CD39 5.4	99; 98

What this pattern supports

BostonGene's low IRF1, antigen-processing and CXCL10 ranks are compatible with weak interferon output, reduced peptide presentation and poor recruitment. Caris/Tempus reanalysis against 18 identically processed public tumours strengthens the low TAP1/HLA observation; IRF1 remains transcript-sensitive. These findings cannot identify the cause or cell compartment, reproduce the vendor's precise percentiles, or establish absent HLA protein. Sections 13–17 give the comparisons and limitations.

What does not fit a simple PTPN explanation

PTPN2 is at the 37th percentile, so it is not unusually high by this comparison. No validated activating PTPN1/PTPN2 alteration or amplification has been established. NFKBIZ 83rd, SOCS3 96th and CD274 71st partly resemble exploratory AC484 cell-line associations, but HLA-E is 36th and those associations are not a clinical selection test. [C1] [C2] [L1]

Liver single-cell data contain CD8 and NK cells with relatively low reported T-cell exhaustion markers. TrufflePig's interferon score was near its reference baseline. These findings argue against claiming universal interferon silence or universal T-cell exhaustion across sites and time. [C6] [C7]

3 Antigen presentation and the PTPN hypotheses

A reversible interferon signalling brake

Interferon-γ can induce peptide display on HLA class I and attract immune effectors. PTPN1/PTPN2 inhibition may strengthen this response and immune-cell function. BostonGene's low downstream CRC-reference ranks justify testing this hypothesis. Research RNA comparisons cannot establish the cause of those ranks or predict drug response. Sparse IFNG RNA support and relatively low IFNGR2 leave low cytokine stimulation or receptor availability as alternatives, not measured defects. [C2] See sections 12–17.

AC484 acts on both PTPN1 and PTPN2. Preclinical effects include tumour interferon responses and T/NK-cell activity; some host-mediated activity persisted with tumour JAK1 or B2M defects. Therefore tumour-only assays cannot exclude all potential activity, and tumour target overexpression is not a prerequisite. [L1]

The clinical question is whether AC484 adds useful tumour recognition and killing beyond interferon stimulation alone. A positive signalling assay would be a reason for further investigation, not a validated prediction of clinical benefit. There is currently no case-specific evidence that this pathway explains progression on BOT/BAL.

APC and PTPN13 are a distinct possibility

APC V450fs and S1411fs truncate the protein before its C-terminal region; PTPN13 RNA is at the 93rd CRC percentile. A 2026 study linked APC loss to PTPN13-mediated STAT1 dephosphorylation, low IRF1/MHC-I and reduced CD8 infiltration. APC-derived 11-amino-acid peptides disrupted the PTPN13–STAT1 interaction in experimental systems. This gives the case a more specific genetic connection to PTPN13 than to an activating PTPN1/PTPN2 lesion. [C1] [C2] [L3]

Nevertheless, the two APC variants have not been fully phased, and PTPN13 activity is unmeasured. APC11 formulations remain preclinical. AC484 does not target PTPN13; a shared downstream pathway does not make the drugs interchangeable. Wnt inhibition is also not an established substitute for this mechanism. [L3]

PTEN and loss of surface HLA through degradation

The reported PTEN R335* variant, high NFE2L2/NRF2 RNA and low KEAP1 percentile justify a second hypothesis. A 2026 MSS-CRC study describes noncatalytic PTEN support of KEAP1; loss permits NRF2/NBR1-linked degradation of MHC-I. This predicts a protein-trafficking or degradation defect, which low HLA RNA alone cannot diagnose. [C1] [C2] [L4]

PTEN RNA itself is high, but this neither proves functional protein nor excludes loss of function. R335* does not establish complete PTEN loss. NRF2 outputs are mixed: SQSTM1, SLC7A11 and GPX4 are only 18th, 12th and 25th percentiles. Confirm PTEN protein, nuclear NRF2 and surface versus total HLA before prioritising this model. ML385 is a research probe; PI3K/AKT inhibitors and hydroxychloroquine are not established clinical substitutes for the reported mechanism. [C2] [L4]

4 Innate sensing and epigenetic repression

What the new PTPN12 paper changes

The 21 October 2026 Nature Communications paper proposes that PTPN12 restrains endogenous double-stranded RNA sensing through TRIM28-Y370 dephosphorylation, chromatin repression and cytoskeletal regulation. Genetic depletion restored innate immune responses and improved anti-PD-1 activity in mouse colorectal cancer models. [L5]

PTPN12 RNA is present at the 75th CRC percentile in the case, but there is no measurement of TRIM28-Y370 phosphorylation, transposable-element activation, cytosolic double-stranded RNA or dependence on the relevant sensing machinery. Its involvement is plausible and unproven. [C2]

A crucial translational distinction is that doxycycline in the paper switched on an engineered shPTPN12 gene-silencing construct. It was not a selective PTPN12 inhibitor. No clinically developed selective PTPN12 inhibitor or matching registered trial was identified in the reviewed sources. This is currently a laboratory collaboration or drug-development hypothesis, not an immediately available treatment route. AC484 targets different phosphatases. [L5]

Could chromatin repression be reversible

Low antigen-processing and interferon-response transcripts can arise through epigenetic regulation. There is no case methylome or chromatin assay establishing that cause. A focused epigenetic experiment becomes more useful if viable malignant cells retain the relevant genes but fail to restore expression despite an intact early interferon signal.

Clinical experience cautions against equating pathway changes with patient benefit. Azacitidine plus pembrolizumab produced a 3% response rate in 30 refractory CRC patients and stopped early for insufficient efficacy, despite measurable biological effects. [L15]

CAPability-01 compared sintilimab/chidamide with or without bevacizumab. The triplet produced 44% responses in 25 patients versus 13% in 23 on the doublet, but there was no standard-care comparator and two treatment-related deaths occurred. Prior PD-1/PD-L1 treatment was excluded. It supports further study of a combination, not a conclusion that epigenetic priming will reverse this case's post-BOT/BAL resistance. [L16] [T6]

Experiments that would separate these explanations

First establish whether interferon produces early STAT1 activation, then RNA induction, then surface HLA. A block between these stages is more informative than another baseline expression panel. If the PTPN12 model remains compelling, use controlled genetic perturbation and test double-stranded RNA, type I interferon and dependence on the proposed sensing pathway. If chromatin repression remains likely, select locus-level methylation or accessibility assays around the deficient programme. These are research tests, not established treatment-selection assays.

5 Stromal exclusion and metabolic suppression

Fibroblasts and TGF beta signalling

BostonGene classifies the treated rectal specimen as fibrotic and immune non-inflamed. TGFB2/3 are 98th percentile, TGFBR1/2 and ACTA2 99th, FAP 78th and COL3A1 79th. Together these make stromal exclusion a high-priority hypothesis. Rectal smooth muscle, radiation fibrosis and low tumour purity could produce part of the pattern. Active signalling and the location of T cells relative to malignant nests remain unknown. [C2] [C3]

TGFβ blockade restored immune control in CRC models. Human translation is less secure: vactosertib plus pembrolizumab produced 12.6% responses in 103 efficacy-evaluable CRC patients, missing its prespecified 25% threshold. Prior checkpoint therapy was excluded. It is a mechanistically relevant research option with unproven applicability after BOT/BAL. [L7] [L8]

CXCL12 and restricted immune trafficking

CXCL12 is 99th percentile and CXCR4 72nd. This provides an additional reason to test fibroblast-derived chemokine barriers. Plerixafor produced immune changes in paired human CRC/pancreatic biopsies, and NOX-A12 plus pembrolizumab has small early-phase experience. Neither establishes an effective CRC regimen. Plerixafor's availability for stem-cell mobilisation does not establish suitability for this use. [C2] [L9] [L10]

A useful test would localise CXCL12 and assess whether blocking the ligand/receptor restores directed immune migration through autologous fibroblast-conditioned matrix and increases killing. Tumour-only organoids omit the physical barrier.

CD39 and adenosine

ENTPD1/CD39 is 98th percentile, while CD73 is 63rd and ADORA2A 51st. The pathway is not uniformly elevated. CD39 can also identify tumour-reactive T cells, so its cellular source matters. Test enzyme activity and ATP-to-adenosine flux with functional immune rescue, rather than inferring metabolic suppression from one transcript. [C2] [L11]

ARC-9's etrumadenant/zimberelimab/FOLFOX/bevacizumab regimen improved outcomes over regorafenib in a randomized phase 2 study. The multi-drug regimen cannot isolate the adenosine antagonist's contribution, prior checkpoint treatment was excluded, and further etrumadenant development is paused. [L12] [T3]

Myeloid and vascular hypotheses have mixed support

CSF1 91st and CD163 84th suggest macrophage-related biology, but CD68 is 14th. CXCL8, S100A8 and S100A9 are low relative to the CRC reference, weakening an empirical CXCR1/2 strategy. HIF1A is 83rd, yet VEGFA 15th, SLC2A1 1st and LDHA 3rd; TrufflePig's hypoxia score has unresolved provenance. Protein, spatial and functional evidence should precede claims of dominant hypoxia or suppressive myeloid activity. VEGFB at 99th is not a predictive bevacizumab biomarker. [C2] [C7]

6 Effector supply and alternative recognition

Are suitable immune effectors present

MSS/low-TMB biology may limit spontaneous priming, but the case contains candidate mutant peptides. Neither prediction nor expression proves presentation or a useful T-cell repertoire. A personalised vaccine needs retained, expressed antigens and functional recognition of native tumour. The reported JLF design/manufacturing work and Invoke research design do not yet establish a vaccine-induced antitumour response. [C1]

The liver single-cell report recorded 2,231 CD8 cells and 1,732 NK cells. CD8 PDCD1, LAG3 and TIGIT detection was 16.6%, 27.8% and 15.5%; NK TIGIT was 45.6%. These selected RNA markers cannot classify all effectors as exhausted or competent. Liver tumour CD274 was undetected in the selected summary, while non-malignant cells showed some expression; dropout and site differences prevent a categorical PD-L1 conclusion. [C6]

Personalised TCR-T produced responses in three of seven patients in a small pMMR-CRC report, establishing feasibility. Vaccine studies also establish immune-response feasibility in selected solid tumours, without validating this case's vaccine design or efficacy. Conventional TCR/CD8 approaches still require the relevant tumour peptide–HLA complex. [L18] [L21]

If restoring HLA is insufficient or impossible

Surface-antigen CAR cells and antibody-based T-cell engagers can bypass classical tumour peptide–HLA recognition. Archived liver CEA protein is a stronger target clue than RNA alone: H-score 300, with 100% 3+ staining. Current nodal membrane expression and heterogeneity need confirmation. CEA-CD3 cibisatamab provides early clinical CRC proof of concept, not an established available treatment. [C10] [L19]

A2B530 is not automatically an option: its HLA-based safety gate requires germline HLA-A*02 heterozygosity and tumour-specific loss. The case's predicted A*02:01/A*02:01 does not meet that configuration. Clinical HLA confirmation is appropriate, but there is no positive eligibility biomarker for this product. [C1] [T7]

NK and some γδ T-cell approaches offer alternatives to conventional HLA restriction, but low HLA does not guarantee susceptibility. An early nine-patient CAR-NK CRC peritoneal-metastasis study had no objective responses. AC484 plus expanded Vδ1 cells has CRC laboratory and ex vivo recruitment evidence, without treated-patient efficacy. Current stress ligands, trafficking and suppressive context would still need testing. [L20] [L6]

Other surface targets are conditional

Liver malignant-cell RNA detection was 24.75% for B7-H3 and 19.94% for B7-H4, with heterogeneity across cell states. These are leads for current membrane-protein testing. ADCs may deliver cytotoxic payloads independently of HLA; activity would not prove that checkpoint-mediated immune suppression was reversed. DB-1311 requires measurable disease, and the ifinatamab CRC cohort excludes prior irinotecan/topoisomerase-I therapy. [C6] [T9] [T10]

7 Therapeutic evidence and development status

The table separates biological relevance from human evidence. None of these data establishes a safe, effective add-on to the current chemotherapy/anti-EGFR regimen or a rescue combination with BOT/BAL.

Approach	Human evidence and status	Implication for this case
AC484 ± PD-1	Phase 1. Preliminary pooled RCC cohort: 22 patients, ORR 18%, disease control 41%. Separate EAP record. [L2] [T1] [T2]	Relevant functional test; no established MSS-CRC efficacy or validated biomarker. EAP availability needs confirmation.
Vactosertib + pembrolizumab	Phase Ib/IIa CRC ORR 12.6%; missed response target. No confirmed recruiting route here. [L8]	Fits TGFβ hypothesis; prior ICI excluded, so post-BOT/BAL benefit unknown.
Zanzalintinib + atezolizumab	Phase 3 OS 10.9 vs 9.4 months; HR 0.80 versus regorafenib. FDA decision due 3 March 2027. [L13] [T4]	Strongest late-phase CRC evidence among these immune-modulating combinations; prior PD-1/PD-L1 excluded.
Etrumadenant combination	Phase 2 PFS 6.2 vs 2.1 months and OS 19.7 vs 9.5 versus regorafenib; development paused. [L12] [T3]	Multiple active drugs confound mechanism; prior ICI excluded; not a readily available option.
Epigenetic priming + PD-1	Azacitidine trial weak; CAPability triplet signal in a small trial with serious toxicity. [L15] [L16]	No case epigenetic biomarker; CAPability excluded prior ICI.
TCR or vaccine strategies	Early human proof of concept; personalised manufacture and target validation required. [L18] [L21]	Must prove native tumour recognition and relevant HLA presentation; NCI TCR trial requires measurable disease. [T8]
CEA or other surface-target approaches	CEA-CD3 phase 1 evidence; CAR/NK and B7 ADC programmes remain investigational. [L19] [L20] [T9]	Current target density, heterogeneity, safety constraints and eligibility matter more than historical RNA alone.
APC11, NRF2 probes, selective PTPN12 inhibition	APC11 and ML385 are preclinical; no clinical selective PTPN12 inhibitor identified. [L3] [L4] [L5]	Research perturbations, not available treatment substitutes.

Safety context: vactosertib/pembrolizumab had grade ≥3 treatment-related events in 18.3% of the full 120-patient CRC/gastric cohort, with one possibly related death. ARC-9 had grade ≥3 treatment-emergent events in 82% versus 49%. These different measures should not be compared directly. [L8] [L12]

ORR = objective response rate; PFS = progression-free survival; OS = overall survival; HR = hazard ratio; EAP = expanded-access programme; ICI = immune checkpoint inhibitor. Cross-trial comparisons cannot establish which therapy would work best for an individual.

8 Access and practical treatment distinctions

AC484 has an access lead that needs current confirmation

NCT04777994 was labelled Recruiting on retrieval, with the last update posted 4 June 2026. It requires RECIST-measurable disease; listed expansion cohorts are not an MSS-CRC-specific cohort. An old recruiting label is not confirmation of an open, suitable slot. The current listed central contact is Calico Clinical Operations, info@calicolabs.com. [T1]

A separate individual expanded-access record, NCT06188975, lists Available, but was last updated 3 January 2024 and gives no detailed eligibility criteria. It is a concrete route for the treating clinician to investigate, not confirmation of current UK acceptance, drug supply, eligibility or combination access. The sponsor must confirm who now manages requests. [T2]

The report of activity after anti-PD-1 treatment concerns clear-cell renal cancer, not colorectal cancer. It is preliminary, small and pooled across AC484 monotherapy and combination cohorts. It cannot establish that AC484 will reverse resistance to BAL or that adding BOT is beneficial. [L2]

Angiogenic treatments need a separate conventional oncology review

Zanzalintinib inhibits VEGFR, MET and TAM kinases. STELLAR-303 supports clinical activity of its atezolizumab combination, without isolating the immune mechanism. Grade 3 or worse treatment-related events were 60% versus 37% with regorafenib; five versus one treatment-related deaths occurred. Prior PD-1/PD-L1 exposure was excluded. [L13] [L14]

The verified US regulatory record remains investigational, with FDA review extended to March 2027. Exelixis has a physician-requested early-access policy, including named-patient consideration outside the US; this is discretionary, not assured supply or a verified UK route for Alex. [T4] [T5]

Absence of documented prior anti-VEGF therapy also makes ordinary CRC sequencing worth reviewing independently of immune hypotheses. For example, SUNLIGHT established survival benefit for trifluridine/tipiracil plus bevacizumab over trifluridine/tipiracil alone. That evidence does not support simply adding bevacizumab to the current panitumumab-containing regimen. [C8] [L24]

Checkpoint combinations are not interchangeable

LEAP-017's lenvatinib/pembrolizumab regimen missed its prespecified survival endpoint. The published BOT/BAL signal in selected MSS-CRC patients does not imply benefit from repeating it after progression. The case's limited reported course and lack of formal response adjudication leave clinical details to resolve, but do not supply a mechanism-specific rationale for an untested triple combination. [L25] [L17]

The most useful sponsor discussion would state prior checkpoint exposure, current therapy, the imaging/RECIST situation and the proposed biological rationale, then ask about current access and permitted partners. An ex vivo result could support that discussion but would not replace safety, eligibility and benefit–risk review.

9 First experiments and preservation of tissue

Stage 0 Establish what can safely be allocated

The processing report lists 14 vials containing 60 fragments of approximately 1–2 mm, with no banked PBMC or plasma vials. Fragment count is not viable tumour yield. The nodal location is patient-reported; the storage report itself does not establish histology, malignant fraction or post-thaw viability. [C9]

Obtain the pathology/procedure record and confirm identity, preservation, handling and any diagnostic blocks held elsewhere. Agree one coordinated allocation plan with the receiving laboratory and any prospective cell-therapy programme. Use a limited representative assessment, preserve an untouched reserve, and avoid consuming all fragments in parallel exploratory assays.

Stage 1 Locate the barrier in current tissue

A core pathology and multiplex protein panel should identify malignant cells, HLA-ABC/B2M, CD8 cells, fibroblasts and myeloid cells. Add PTEN, nuclear NRF2, PTPN13, PD-L1 and pSMAD2/3 according to material and the first findings. PTPN1/PTPN2 localisation can assign cell compartments but does not measure enzyme activity. Use validated antibodies and internal non-malignant positive controls.

Finding	Meaning	Next step
Low tumour surface HLA-I/B2M	Supports a presentation barrier; not its cause	Interferon response and current DNA/RNA analysis
CD8 outside tumour nests near active fibroblasts	Supports spatial exclusion	Migration/co-culture tests with stromal perturbation
HLA retained and CD8 within nests	Weakens global presentation loss/exclusion	Test native recognition, effector function and local suppression
Low PTEN with nuclear NRF2 and low surface HLA	Supports the degradation hypothesis	Compare total versus surface HLA and selective pathway perturbation

Stage 2 Validate genetic and antigenic prerequisites

Profile current tumour DNA with suitable matched normal, native RNA and clinical high-resolution HLA typing, with explicit purity and coverage review. Assess HLA/B2M, interferon sensing, antigen processing and retention of vaccine/TCR targets. Ensure PTPN1/PTPN2 are actually covered if those genes are a question; the historical Tempus DNA panel did not include them. [C1] [C5]

Independently test B2M C100R if it would influence decisions, and interpret any positive result with clonality, copy number and tumour protein. Resolve APC variant/transcript phase before assuming both frameshifts generate the proposed peptides. Low-input tumour enrichment may be more valuable than immediately committing scarce tissue to a large single-cell assay.

10 Functional testing of interferon and AC484

Stage 3 Start with a small interpretable comparison

If current material is viable, compare vehicle, interferon-γ, AC484 and the combination. A specialist laboratory should select exposures and establish target engagement; this is a proposed research design, not a dosing protocol. Identify malignant and immune cells in every readout and include viability, vehicle and responsive assay controls.

Measure in causal order: early tumour pSTAT1, later IRF1/antigen-processing RNA, surface HLA-I/B2M and CXCL9/10 secretion. Then test recognition and actual killing by autologous immune cells. A molecular change or cytokine increase alone is not the desired endpoint.

Result	Interpretation	Follow-up
Interferon alone restores output	Reversible pathway; low local stimulation is plausible	Test whether sufficient native tumour recognition follows
AC484 adds to interferon	Drug-responsive restraint is plausible	Confirm engagement and PTPN1/2 attribution; test killing
pSTAT1 rises but HLA does not	Downstream transcription, processing or trafficking remains limiting	Separate RNA from total/surface protein; test competing pathways
HLA rises but killing does not	Presentation may be insufficient, or effectors/antigens are missing	Native antigen validation, TCR repertoire and suppression assays
No tumour response with valid controls	Weakens direct tumour sensitisation under tested conditions	Review receptor/JAK integrity; test immune-cell compartment separately

Stage 4 Establish causality and the relevant compartment

If the pharmacological result is convincing, compare PTPN1 and PTPN2 suppression individually and together, with non-targeting and rescue controls. Add PTPN13 perturbation when the protein/context findings justify it. Concordant independent perturbations strengthen attribution; a drug response by itself does not identify which phosphatase is responsible.

Separate tumour-only pretreatment, immune-only exposure and mixed exposure, with washout/carry-over controls. Use HLA blocking within separate CD8/NK arms: HLA blockade can itself disinhibit NK cells. Allogeneic donor cells can introduce unrelated recognition and should not be mistaken for autologous antitumour specificity.

Add PD-1 blockade only after an interpretable recognition/killing system exists. Testing botensilimab requires relevant immune and Fc-mediated interactions, including appropriate myeloid/Treg context; tumour-only organoids cannot adequately model BOT/BAL. An ex vivo rescue prioritises a hypothesis but remains an unvalidated clinical predictor. Organoid/lymphocyte methods provide feasibility precedent, with uncertain success in this banked MSS specimen. [L22]

11 Targeted follow-up and decision rules

Stage 5 Test the alternatives supported by the first results

Hypothesis	Focused experiment	Evidence that would strengthen it
APC and PTPN13	PTPN13 perturbation ± APC11 research peptide; matched delivery and rescue controls	Restored STAT1/IRF1/HLA and killing beyond control effects
PTEN and NRF2	PTEN protein; total/surface HLA; NRF2 or NBR1 perturbation with viability controls	Surface presentation restored with a degradation-specific pattern
TGFβ or CXCL12 exclusion	Spatial protein; tumour/immune/CAF cultures and migration ± focused blockade	Infiltration and killing improve when the implicated barrier is removed
CD39 and adenosine	Localise CD39/CD73; controlled ATP-to-adenosine flux; functional blockade	Measured flux and suppression reverse with target engagement
Suppressive myeloid cells	Phenotyping plus autologous suppression, depletion and add-back	Removal restores function and add-back restores suppression
PTPN12 and innate sensing	Genetic perturbation; TRIM28, dsRNA/type I IFN; sensor-dependence controls	Causal restoration of sensing and downstream antitumour function

Stage 6 Validate the proposed immune recognition strategy

Paired nodal single-cell RNA and T-cell receptor alpha/beta sequencing can assign HLA/interferon programmes to malignant cells and link expanded T-cell clones to their functional-state signatures. It can distinguish widespread from subpopulation-specific presentation deficits and prioritise candidate reactive clones. Preserve an uncultured aliquot and confirm preservation-compatible paired-receptor recovery. Low RNA may reflect dropout; expanded clones may be bystanders. Spatial exclusion needs tissue imaging, and receptor specificity or dysfunction needs native-tumour recognition tests. Collect current blood immune cells if suitable.

For vaccine or TCR candidates, test mutant versus wild-type sequences, HLA restriction and native autologous tumour recognition. Peptide-loaded targets, tetramers or ELISpot alone are insufficient. Include normal-cell/cross-reactivity controls appropriate to the proposed product. Immunopeptidomics can directly support presentation when material permits, but a negative result is insensitive.

If viability is poor, preserved DNA/RNA and histology can still answer some questions; specialist TCR-discovery methods from biopsy RNA are a research fallback, not a confirmed clinical service. If classical presentation is irreversibly lost, prioritise current CEA or other membrane targets and functional NK/CAR/engager testing instead. [L23]

A practical decision rule

Advance a mechanism when current-cell evidence, target engagement and improved recognition or killing agree. A failed controlled perturbation weakens that mechanism in the tested compartment; a tumour-only failure does not exclude immune-cell benefit. Preserve uncertainty when poor viability, missing stromal architecture or lack of antigen-reactive effectors makes the test uninformative. Avoid a broad combination screen before these distinctions are clear.

12 Sequencing audit and limits of negative evidence

What the raw RNA checks add

Targeted alignment review independently supports transcript presence in the initial Caris rectal biopsy and Tempus resection RNA. PTPN1 had 1,614 and 1,025 accepted exon-overlapping alignment records respectively; PTPN2 had 654 and 242. PTPN13 had 386 and 1,083. IFNG support was sparse. These are alignment counts, not unique molecules, TPM, malignant-cell expression or a valid cross-sample differential analysis.

That earlier alignment audit used primary mapped, QC-passing, non-flagged-duplicate records with MAPQ ≥20, NH=1 where available and unstranded union-exon overlap. Caris used GRCh38/GENCODE 37; Tempus used GRCh37/GENCODE 19. The supplied Tempus BAM lacked NH, pairing and duplicate flags. The new analysis in sections 13–14 instead uses the original paired FASTQs and a common transcript reference, resolving the BAM-pairing limitation for RNA abundance estimation.

Mutations do not yet identify a proven immune escape lesion

Reported pathogenic APC truncations and PTEN R335* are plausible mechanistic context. SMARCA4 D1177Y has discordant classifications and does not establish chromatin-remodeller loss. JAK2 N1108S and the listed common TAP2 changes are benign/likely benign rather than demonstrated interferon or antigen-processing defects. Low-quality IFNGR1 candidates are unvalidated. [C1]

The B2M C100R research candidate is especially important to avoid overcalling. Support in embedded alignments was alignment-sensitive and concentrated near read ends. The full Caris RNA retained two supporting records with one shared read/query name after the stricter read-end filter. That is weak residual evidence, not zero evidence and not a validated somatic mutation or proof of B2M loss. Independent current-tissue testing is needed if it will affect a decision.

No functional PTPN1/PTPN2 activating alteration or amplification has been established in the reviewed calls. This is not proof that the genes are wild type in every clone: the Tempus DNA panel omitted them, BostonGene did not report copy number in the low-purity sample, and negative plasma assays had very low or undetectable tumour fractions. A generic copy-number field of 2 cannot resolve those limitations. [C1] [C5]

Blood and research datasets need compartment labels

Caris blood RNA contained PTPN1/PTPN2 at 8.26/34.94 TPM in buffy coat and 10.84/35.74 TPM in cell-free RNA. These establish blood-fraction expression, not tumour upregulation. Selected blood VCF checks found no validated protein-altering explanation in the audited pathway set. The public Natera identity-SNP VCF is not a comprehensive somatic call set.

Liver single-cell summaries cover selected targets, so an unlisted gene is missing data rather than zero expression. The TrufflePig model is not an independent replicate until its input is traced. Conclusions should therefore remain anchored to specimen, assay, time and cell compartment.

The patient retains supporting alignment audits, reproducible RNA results, input checksums, commands and scripts. These are research analyses, not a certified clinical diagnostic assay.

13 Consistent RNA quantification across the two samples

All five genes are detected in both specimens. Comparison with 18 public colorectal tumours processed identically supports relatively low TAP1 and HLA-A/B/C RNA in both Caris and Tempus. Tempus total estimates are also broadly compatible with BostonGene's reported abundance. This supports further investigation of antigen presentation, without validating BostonGene's precise percentiles. IRF1 requires separate interpretation because transcript allocation is unstable. Sections 15–17 provide the reference comparisons.

All original paired reads were reprocessed with the same Salmon 2.8.0 software and GENCODE 37 transcript reference. TPM means transcripts per million: estimated RNA abundance after transcript-length and library-size adjustment. Gene totals include all annotated transcript types. [Q1] [Q2]

Gene and role	Caris biopsy TPM (range)	Tempus resection TPM (range)	BostonGene approx. TPM	BostonGene CRC percentile
IRF1 Interferon regulator	127.8 (103.1–181.6)	33.6 (15.8–63.3)	21.6	2
TAP1 Peptide transporter	18.7 (16.5–20.6)	26.1 (23.6–28.0)	20.1	1
HLA-A Peptide display	675.6 (664.3–692.6)	439.3 (424.8–446.7)	547.7	2
HLA-B Peptide display	670.2 (650.8–682.7)	548.3 (531.6–568.9)	511.0	5
HLA-C Peptide display	546.7 (530.0–559.7)	338.3 (324.8–350.7)	314.2	3

Ranges: 2.5th–97.5th percentiles from 30 technical bootstrap resamples, conditional on the reference and model; not biological confidence intervals. BostonGene TPM was reconstructed as 2^x − 1 from its rounded log2(TPM+1) values, so is approximate. Its vendor pipeline and reference percentiles were not recomputed. [C2]

What is supported and what remains uncertain

Tempus versus BostonGene: HLA-A/B/C are 0.80, 1.07 and 1.08 times the BostonGene abundance; TAP1 is 1.30 times and IRF1 1.55 times. This is broad numerical compatibility, not formal assay agreement; IRF1 allocation is uncertain. Both concern the same resection/block context. Tempus reported 30% tumour after microdissection versus BostonGene's approximately 15%; exact RNA aliquot composition is unverified. [C13]

Caris versus Tempus: total IRF1 RNA is 3.8 times higher in the earlier biopsy; HLA-A/B/C are also higher, whereas TAP1 is lower. A higher value than another specimen does not establish normal expression. Sampling date, treatment, cell composition and library preparation prevent attributing these differences to therapy. IRF1 transcript sensitivity is examined below. [Q3]

Clinical implication: investigate antigen display and interferon responsiveness in the current node, but do not select a PTPN inhibitor from these RNA values alone. Tumour-cell HLA/B2M protein and interferon ± AC484 functional rescue remain more decisive than bulk RNA abundance. Public-reference context and transcript sensitivity are detailed in sections 15–17.

14 RNA methods and quality limitations

Both samples used a full-transcriptome GENCODE 37 index (GRCh38.p13, k=31), selective alignment, automatic library orientation, sequence/GC-bias correction and 30 bootstraps. Transcript TPM was independently summed to genes and checked against Salmon's gene output. Each sample totals approximately one million transcript TPM. Both estimates converged without quantification errors. [Q1] [Q2]

Quality measure	Caris	Tempus
Original read pairs examined	14,875,459	13,837,280
Final assigned fragments / input pairs	81.76%	89.12%
Detected strand convention	Reverse (ISR)	Forward (ISF)
Mapped fragments incompatible with convention	6.11%	5.53%
Orphan mappings / all input pairs	3.38%	9.61%
Malformed records or mismatched mate IDs	0	0

The different strand conventions were inferred and applied per library. Both runs warned that over 5% of mapped fragments disagreed with the inferred convention; incompatible fragments were excluded. This and the orphan mappings warrant caution about library-specific bias. Original reads were not newly trimmed or deduplicated. Tempus identifier tags resemble molecular barcodes, but their chemistry was not confirmed, so no barcode correction was attempted.

Gene RNA is not equivalent to functional protein

The primary table sums all annotated RNA forms. Summing only protein-coding forms gives IRF1 18.8 TPM in Caris and 2.8 in Tempus, versus totals of 127.8 and 33.6. The whole-transcriptome denominator is unchanged. This tests transcript allocation, not protein loss or abnormal splicing. HLA-C also has substantial retained-intron assignment. Tumour-cell IRF1 protein and targeted assays for coding RNA could clarify the discrepancy.

Coding-transcript component in TPM	Caris	Tempus
IRF1	18.8	2.8
TAP1	12.7	23.8
HLA-A	665.4	435.4
HLA-B	589.1	425.0
HLA-C	393.3	173.0

Limitations: no genomic decoys or personalised HLA alleles were included; HLA homology and polymorphism can bias mapping. Exact RNA library/capture protocols remain incompletely specified. Technical bootstrap ranges omit these systematic effects and differences in cell mixture. Histological tumour-cell percentages are not RNA fractions and cannot convert bulk TPM into tumour-cell TPM. No formal differential-expression test or clinical percentile was calculated.

Reproducibility materials include original-input checksums, reference/software hashes, commands, QC and comparison tables. Public scripts and provenance accompany this report; private original inputs and released derivatives are distinguished. [Q9] The original paired Tempus FASTQ export was used; the older unpaired BAM export was not used for this quantification.

15 Public colorectal expression references

Public data were available and queried: 375 unique primary TCGA colorectal tumours (283 colon, 92 rectal), excluding normal tissue and repeat participants. The rectal subset is shown separately. [Q4] [Q5]

Gene	Public CRC reference Median TPM (middle 50%) 375 tumours	Rectal subset Median TPM (middle 50%) 92 tumours
IRF1	51.8 (35.1–82.3)	51.5 (36.0–68.7)
TAP1	85.3 (55.1–129.0)	78.1 (56.3–105.2)
HLA-A	1038.3 (722.1–1509.7)	1052.8 (775.1–1413.5)
HLA-B	1243.3 (846.0–1801.5)	1260.7 (927.7–1810.9)
HLA-C	707.2 (436.4–1001.5)	658.5 (494.2–858.8)

How the case compares with this public distribution

The case's all-transcript Tempus estimates fall below the lower quartile of both TCGA distributions for all five genes. This numerical placement uses different processing pipelines. IRF1 lies close to the combined lower quartile, has a wide technical range and is sensitive to transcript definition. The comparison therefore provides exploratory context rather than independent validation.

For Caris, TAP1 and HLA-A/B fall below the combined lower quartile, HLA-C lies between the lower quartile and median, and total IRF1 exceeds the upper quartile. High total IRF1 does not establish an intact interferon response: its protein-coding component is much smaller and requires comparison with the same transcript definition.

Why this is not independent clinical percentile validation

Toil uses STAR/RSEM with GENCODE 23; the case uses Salmon/GENCODE 37. Public log2(TPM + 0.001) values were inverted as 2^x − 0.001. Selection required legacy Xena HiSeqV2 disease membership and Toil expression availability. MSS status, purity, treatment and library preparation were unmatched. These differences can alter ranks; no patient percentile was assigned. [Q3] [Q4] [Q5]

Comparison using the same computational pipeline

All 18 GSE50760 primary tumours were processed with the case's Salmon/GENCODE pipeline (sections 16–17). TAP1 and HLA-A/B are below the lower quartile of both public references in both case samples. Caris HLA-C is below that threshold in GSE50760 but not TCGA, illustrating reference dependence. Total IRF1 is high in Caris and lower in Tempus, with substantial technical/transcript uncertainty. Laboratory preparation and cell mixture remain unmatched. [Q7]

Assay-matched percentiles would help, but Tempus confirmed on 22 October 2026 that it provides raw TPM rather than broader percentiles outside selected research-use-only Hub calls. [C14] Public-reference queries/checksums are retained; no patient data were submitted to the public server.

16 Colorectal reference processed with the same pipeline

All 18 prespecified primary tumours from GSE50760 were reprocessed with the same Salmon 2.8.0 software, GENCODE 37 transcript reference and settings as Caris and Tempus. One primary sample per person was included; matched normal colon and liver metastases were excluded. [Q7]

The reference comprises stage IV colorectal cancers with synchronous liver metastases: 17 microsatellite-stable and one MSI-high cancer. It provides an exploratory comparison after harmonising computational processing. [Q8]

Gene	Reference TPM Median (middle 50%)	Caris biopsy TPM and count below	Tempus resection TPM and count below
IRF1	58.0 (46.5–73.2)	127.8 TPM 17/18 below	33.6 TPM 1/18 below
TAP1	76.4 (50.9–99.2)	18.7 TPM 0/18 below	26.1 TPM 0/18 below
HLA-A	1752.5 (1152.1–1961.6)	675.6 TPM 1/18 below	439.3 TPM 0/18 below
HLA-B	1571.0 (1315.3–1904.9)	670.2 TPM 0/18 below	548.3 TPM 0/18 below
HLA-C	1045.9 (763.4–1161.3)	546.7 TPM 2/18 below	338.3 TPM 0/18 below

TPM means transcripts per million. The primary analysis sums all annotated transcript types. “Below” counts reference tumours with a lower value than the patient sample; a small count places the patient near the low end. These counts are not clinical percentiles. The middle 50% spans the reference 25th–75th percentiles.

Placement within this reference

TAP1 and HLA-A/B/C are below the reference lower quartile in both case samples. In Tempus, all four are below the lowest reference point estimate; in Caris, TAP1 and HLA-B are below it. These lower-quartile placements persist after removing any one reference patient.

Total IRF1 is high in Caris, with 17 of 18 reference tumours lower. Tempus IRF1 lies near the low end, but its technical range (15.8–63.3 TPM) crosses the reference lower quartile (46.5 TPM). Its low point estimate is therefore less secure than the TAP1/HLA pattern. The coding-transcript comparison changes Caris IRF1 interpretation, as shown next.

What the comparison can establish

IRF1 regulates interferon responses, TAP1 transports peptides, and HLA-A/B/C display peptides to T cells. The results support investigating antigen presentation, but bulk RNA cannot identify the responsible cell type or demonstrate impaired protein function.

Eighteen research samples cannot validate BostonGene’s precise 1st–5th percentile estimates. Results depend on the reference population as well as laboratory preparation and tissue composition. Clinical interpretation must consider the TCGA comparison, transcript sensitivity below and current tumour functional testing.

17 Transcript sensitivity and reference quality

This sensitivity analysis sums only transcripts annotated as protein-coding, retaining the same whole-transcriptome TPM denominator in every sample. The reference is recalculated using that same measure. It estimates a component of gene RNA abundance; it does not measure protein or prove abnormal splicing.

Gene	Reference TPM Median (middle 50%)	Caris biopsy TPM and count below	Tempus resection TPM and count below
IRF1	41.8 (32.7–54.6)	18.8 TPM 0/18 below	2.8 TPM 0/18 below
TAP1	70.5 (46.9–87.6)	12.7 TPM 0/18 below	23.8 TPM 0/18 below
HLA-A	1711.9 (1106.3–1941.5)	665.4 TPM 1/18 below	435.4 TPM 0/18 below
HLA-B	1522.6 (1280.8–1829.2)	589.1 TPM 0/18 below	425.0 TPM 0/18 below
HLA-C	1006.7 (725.3–1092.1)	393.3 TPM 0/18 below	173.0 TPM 0/18 below

Genes whose placement below the lower quartile changes when only protein-coding transcripts are summed: Caris: IRF1; Tempus: none of the five genes. Each comparison uses the corresponding reference measure.

Coding-transcript IRF1 estimates in both case samples are below all reference point estimates, despite high total IRF1 in Caris. This reinforces the need to distinguish RNA forms. Coding-only technical intervals were not calculated; this finding does not establish loss of IRF1 protein or abnormal splicing.

Methods and quality checks

All 18 libraries completed and converged, covering 559,072,411 original read pairs (23.2–42.6 million per sample). Assigned fragments ranged from 86.1–90.6% of input pairs; inferred library type: unstranded (IU). All used the same transcript index as the patient runs, automatic library orientation, sequence/GC-bias correction and 30 technical bootstrap resamples. Independent checks confirmed TPM normalisation and gene aggregation.

Source FASTQs were checked against archive file sizes and MD5 checksums; processed pair counts matched archive metadata. Independent record-level checks covered the first 10,000 pairs per reference sample. The study used poly-A-selected TruSeq v2 libraries and paired-end HiSeq2000 sequencing. [Q8]

Limits that remain after identical computation

Laboratory preparation, RNA integrity, prior treatment, anatomical site and tumour/immune-cell composition remain unmatched. Individual purity and treatment details are insufficiently verified. A rectal-only reference was not established; one reference tumour was MSI-high.

IRF1 allocation among transcript isoforms and HLA homology/polymorphism remain relevant; genomic decoys and personalised HLA alleles were not included. Technical bootstraps do not capture these systematic effects or biological variability. Quartile placement and removal of one reference patient at a time use point estimates; technical and reference-sample uncertainty are not fully propagated. No differential-expression significance test or calibrated clinical percentile was calculated.

Reproducibility materials retained with the analysis include source metadata, verified run manifests, quantification outputs and comparison tables. Only the complete 18-patient result set is used here. [Q9]

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Case records and mechanistic studies

[C1] Case molecular summary and report provenance
YoungCRC case dataset, reviewed 22 October 2026.

[C2] BostonGene expression and colorectal reference percentiles
Kandinsky gene explorer; use BostonGene columns and their specimen provenance.

[C3] BostonGene molecular portrait
Treated rectal resection collected 22 October 2024; report 30 September 2026.

[C4] Caris and Tempus molecular reports
Initial rectal biopsy and treated rectal resection respectively. Tempus report linked separately as C5.

[C5] Tempus molecular report
xT DNA panel and separate RNA profiling; not whole-exome DNA sequencing.

[C6] Valius liver single-cell report
October 2024 liver specimen; selected target and cell-type summaries.

[C7] TrufflePig research analysis
Input specimen and pipeline provenance require resolution before treating it as an independent replicate.

[C8] Clinical treatment and imaging chronology
Includes reported BOT/BAL course and September 2026 nodal progression.

[C9] Banked tissue processing report
6 October 2026; 14 vials containing 60 fragments; no documented PBMC stock.

[C10] Archived liver CEA immunohistochemistry
CEACAM5 H-score 300, 100% strong staining.

[C11] Laboratory observations and blood count chronology
Available lymphocyte counts in the case record; historical values, not a current immune-function assay.

[C12] Valius liver single-cell gene and cell-type data
Companion expression summary; RNA-detection fractions and recovered-cell counts, not protein positivity or unbiased infiltration.

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[L9] CXCR4 inhibition in human pancreatic and colorectal cancer
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[L10] CXCL12 inhibition with NOX-A12 and pembrolizumab
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[L11] CD39 and CD103 identify tumour-reactive CD8 T cells
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[L12] Etrumadenant combination in previously treated metastatic colorectal cancer
ARC-9 randomized phase 2 study, 2026. PMID 41870279.

[L13] Zanzalintinib plus atezolizumab in refractory metastatic colorectal cancer
STELLAR-303 phase 3, Lancet, 2025. PMID 41130252.

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Protocol description includes exclusion of prior PD-1 or PD-L1 therapy.

[L15] Pembrolizumab plus azacitidine in chemotherapy-refractory colorectal cancer
Phase 2 study, 2022. PMID 34991708.

[L16] Sintilimab and chidamide with or without bevacizumab
CAPability-01 randomized phase 2, 2024. PMID 38438735.

[L17] Botensilimab plus balstilimab in relapsed or refractory MSS colorectal cancer
Nature Medicine, 2024. Phase 1 study.

[L18] Personalized neoantigen-reactive TCR T cells in metastatic colorectal cancer
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[L21] Personalized RNA neoantigen vaccine autogene cevumeran
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[L22] Tumour organoid and lymphocyte co-cultures
Cell, 2018. Feasibility in selected cancer settings; not a validated response predictor.

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[L24] Trifluridine tipiracil plus bevacizumab in refractory metastatic colorectal cancer
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[T2] AC484 individual expanded access NCT06188975
Registry lists Available; last update posted 3 January 2024.

[T3] Arcus second-quarter 2026 pipeline update
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[T4] FDA review extension for zanzalintinib
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[T5] Exelixis access to investigational therapies
Physician-requested early access policy, including named-patient consideration.

[T6] CAPability-01 NCT04724239
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[T7] EVEREST-1 A2B530 trial design
Sponsor scientific poster. Requires germline HLA-A*02 heterozygosity and tumour-specific loss.

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[T10] Ifinatamab deruxtecan IDeate-PanTumor02 NCT06330064
MSS CRC cohort excludes prior topoisomerase I therapy including irinotecan.

Registry and sponsor sources can lag real recruitment, supply and territorial availability. Record dates are provided so the treating team can verify an actionable route. Trial exclusions described in the body are material examples, not a full eligibility assessment.

References

RNA quantification and source records

[Q1] Salmon quantification and output documentation
Salmon 2.8.0; transcript estimates, gene aggregation and bootstrap files. Official documentation.

[Q2] GENCODE human release 37
GRCh38.p13 comprehensive chromosome transcript reference used for both patient samples.

[Q3] Misuse of RPKM or TPM normalization when comparing across samples and sequencing protocols
Zhao et al., RNA, 2020. TPM does not remove library-composition or sequencing-protocol differences.

[Q4] UCSC Xena Toil TCGA gene TPM metadata
Public tcga_RSEM_gene_tpm dataset: RSEM, log2(TPM + 0.001), GENCODE v23; downloaded 22 October 2026.

[Q5] UCSC Xena public cohort processing methods
TCGA/TARGET/GTEx Toil recomputation and dataset documentation.

[Q7] Public colorectal RNA sequencing dataset GSE50760
18 patients with primary colorectal tumour, normal colon and synchronous liver metastasis; public SRA reads, SRP029880.

[Q8] GSE50760 original study and laboratory methods
Kim et al., A nineteen gene-based risk score classifier predicts prognosis of colorectal cancer patients. Molecular Oncology, 2014; 8:1653–1666. Poly-A selected TruSeq v2 RNA; 18 patients, 17 MSS and one MSI-high.

[Q9] Reproducibility materials for the immune-evasion analysis
Public scripts, comparison tables, quality checks and source provenance accompanying this report. Original patient inputs and public derivatives are distinguished.

[C13] Tempus Scientific Affairs confirmation of sample quality and tumour content
Patient-held Tempus Scientific Affairs correspondence, 11 July 2025: 30% tumour after microdissection; DNA/RNA sequencing QC passed. Exact RNA aliquot composition was not independently remeasured. This correspondence is not publicly available.

[C14] Tempus Scientific Affairs reply on expression percentiles
Patient-held correspondence, 22 October 2026: raw TPM can be provided, but broader gene-expression percentiles are not available outside selected research-use-only Hub expression calls. This correspondence is not publicly available.