Supplementary Information: Extended Biophysical Derivations, Tables and REMARK Compliance Audit

Article Title: A Multiscale Biophysical Reaction-Diffusion Framework Coupling Histological Architecture, Interstitial Transport, and Inflammatory Tension in Solid Tumors: An Exploratory In Silico and Retrospective Multi-Cohort Analysis
Author: Luca Celebrano
Affiliation: Independent Researcher in Computational Oncology, Naples, Italy
Corresponding Author: Luca Celebrano (luca.celebrano1@gmail.com), ORCID: 0009-0007-0931-3555
Target Category: Supplementary Appendix / Translational Oncology Monograph (REMARK-Compliant)
Open Code & Data Repository: https://github.com/Lukecele/inflammatory-onco-physics
Persistent Archive: DOI: 10.5281/zenodo.24118328

Supplementary Note 1: First-Principle Derivation of Radial Drug Transport (Bessel Solution)

In solid tumor cords, convective interstitial fluid transport is severely compromised by elevated interstitial fluid pressure () and the collapse of intra-tumoral functional lymphatics, rendering the transmural pressure gradient negligible (). Under these conditions, the Péclet number:

confirming that interstitial mass transport operates in a pure diffusion-dominant regime (Baxter & Jain, Microvasc Res 1989). Around an idealized cylindrical microvessel of radius , steady-state radial transport with first-order cellular uptake and metabolic clearance is governed by:

Dividing by the effective interstitial diffusion coefficient and defining the characteristic tissue penetration length :

Substituting the dimensionless radial variable transforms this into the modified Bessel differential equation of order zero:

The general analytical solution is:

Where and are modified cylindrical Bessel functions of order zero of the first and second kind, respectively.

Physical Boundary Conditions:

1. Distal boundary condition (): In the deep cord/necrotic boundary, drug concentration must asymptotically approach zero: . Since as , coefficient must vanish identically ().
2. Capillary wall boundary condition (): At the outer microvascular endothelial boundary (), concentration matches the free unbound intravascular trough concentration :

Thus, the exact radial concentration profile is analytically determined by:

Parameter Estimations for Small-Molecule Targeted TKIs (Osimertinib):

- Molecular Weight (): .
- Hydrodynamic Stokes Radius: .
- Free diffusivity in water (): .
- Effective interstitial tissue diffusivity in desmoplastic stroma: .
- Elimination rate constant: .
- Characteristic tissue penetration length: .
- Intravascular free unbound concentration (80 mg daily oral dosing, free fraction ,): .
- Cellular target inhibitory concentration: (therapeutic threshold).
- Critical threshold crossing radius: (; sensitivity envelope:).
- Critical viability oxygenation boundary: (corresponding to cellular hypoxia threshold).
- Zonal Cord Compartmentalization & Volume Fractions:

- Proximal Perivascular Zone (, i.e., from the vascular lumen): Therapeutic concentration (); active suppression of sensitive clones ().
- Distal Cord Sanctuary / Marginal Transitional Niche (): Viable tissue (), but sub-therapeutic drug level (); nominal width , harboring uneradicated persisters in cytostatic arrest.
- Exact Geometric Volumetric Fraction: In cylindrical coordinates, accounting for the microvascular lumen (), the proportion of the viable cord volume occupied by the distal sanctuary is:

- Anoxic Necrotic Core (): Severe ischemia ().

Supplementary Note 2: Biophysical Mechanics of the "Double Barrier" to ctDNA Shedding

Liquid biopsy minimal residual disease (MRD) assays exhibit high clinical specificity but can yield false-negative results during early occult persistence. The continuum theory accounts for this via a bidirectional physical and enzymatic double barrier:

1. Hydrodynamic & Size Contrast: Drug vs. Nucleosome

- Small-Molecule Drug (Osimertinib / Chemo):
  - Molecular weight: .
  - Hydrodynamic radius: .
  - Diffuses into tissue based on small-molecule mobility.
- Circulating Tumor DNA (Mononucleosomal fragment):
  - Molecular weight: (wrapped around histone octamer).
  - Hydrodynamic diameter: ().
  - Stokes-Einstein free diffusion coefficient:

    In water at , , which is over an order of magnitude smaller than .

2. Matrix Hindrance in Desmoplastic Stroma & Ogston-Renkin Regime

In dense fibrotic stroma (e.g., CMS4 colorectal cancer or fibrotic lung adenocarcinoma), the pore size distribution of crosslinked type I/III collagen fibrils ranges from . For an nanoparticle, steric hindrance and hydrodynamic

drag reduce effective interstitial diffusivity according to the Ogston-Renkin relationship:

Where . This physical formulation is strictly valid for (where is the cylindrical steric partition coefficient and is the Faxén hydrodynamic drag factor).

In hyper-crosslinked collagen regions where pore diameters approach (), nucleosomes undergo complete steric filtration (and convective velocity). In the absence of convective flow (), transit of nucleosomes from distal cord niches back into the capillary lumen requires weeks rather than hours.

3. Enzymatic Cleavage by Stromal DNases

During prolonged interstitial transit, naked and nucleosomal ctDNA fragments are exposed to high concentrations of extracellular endonucleases: * Interstitial DNase I and macrophage-secreted DNase1L3 enzymatically cleave DNA into sub-nucleosomal fragments (); * Local tumor-associated macrophages (TAMs) phagocytose chromatin complexes.

Consequently, distal persister cords can sustain viable cellular persistence while peripheral blood vascular shedding decays to near zero (), explaining false-negative plasma ctDNA assays even with ultra-sensitive detection limits ().

Supplementary Note 3: Hereditary Volterra Memory Functional of Immune Exhaustion

Cytotoxic immune clearance is formalised as a history-dependent functional reflecting epigenetic T-cell exhaustion under chronic non-resolving inflammatory cytokine exposure (IL-1, TNF-, IL-6, TGF-):

Where: * is baseline cytolytic capacity of tumor-infiltrating CD8+ cytotoxic T lymphocytes and NK cells. * is local and systemic inflammatory tension at time . * is the epigenetic exhaustion susceptibility parameter ().

When cumulative inflammatory damage reduces below the net persister expansion rate , the dormant equilibrium fixed point at undergoes a transcritical bifurcation:

This bifurcation marks the analytical transition from stable dormancy to rapid clinical recurrence.

Supplementary Note 4: Pharmacological Dissection: ADAURA (TKI) vs. PEGASUS (Cytotoxic Chemotherapy)

Feature	Targeted TKI (ADAURA NSCLC)	Cytotoxic Chemotherapy (PEGASUS / CMS4 CRC)
Primary Agent	Osimertinib (3rd-gen irreversible EGFR TKI)	CAPOX (Capecitabine + Oxaliplatin) / FOLFIRI
Duration	36 Months (Adjuvant)	3 to 6 Months (Adjuvant)
Primary Mechanism	Continuous oncogenic signal transduction blockade	Cell-cycle-dependent DNA crosslinking & antimetabolite stress
Sanctuary Driver	Radial concentration drop below threshold ($C < 14\,\mathrm{nM}$)	Dense desmoplasia + hypoxia-induced G_0/G_1 cell-cycle arrest
Recurrence Dynamic	Post-discontinuation rebound (67.9% post-month 36)	Persister survival during therapy with delayed recurrence
Physical Barrier	Radial diffusion decay (K_0) across cords	Hypoperfusion, high IFP, collagen crosslinking (LOXL2)

Supplementary Table S1: Multivariable Cox Models Across Clinical Strata (MSKCC)

Covariate	Hazard Ratio (HR)	95% Confidence Interval	Wald Chi-Square	P-Value
HTBI (Continuous, 0–1 scale)	**2.23**	**1.13 - 4.39**	**5.32**	**0.021**
— HTBI (per 1 SD / IQR, ~0.24 units)	*1.21*	*1.03 - 1.43*	*5.32*	*0.021*

Covariate	Hazard Ratio (HR)	95% Confidence Interval	Wald Chi-Square	P-Value
Pathologic Stage (II/III vs I)	2.84	1.95 - 4.14	28.45	< 0.0001
Patient Age (Continuous, years)	1.01	0.99 - 1.03	1.45	0.228
Patient Sex (Male vs Female)	1.15	0.82 - 1.62	0.65	0.420
Smoking Status (Current vs Never)	1.62	0.98 - 2.68	3.51	0.061
Smoking Status (Former vs Never)	1.28	0.86 - 1.91	1.48	0.224
***EGFR* Driver Mutation (Mut vs WT)**	0.91	0.62 - 1.34	0.22	0.640

Global Schoenfeld Residual Proportional Hazards Test: . The primary coefficient corresponds to (, Wald); rescaled per standard deviation (units), ().

Supplementary Table S2: Complete-Case Blood Chemistry Risk Stratification ()

Parameter	Low Inflammatory Risk ($N = 627$)	High Inflammatory Risk ($N = 429$)	Test Statistic	P-Value
Pre-operative NLR (Median, IQR)	1.68 (1.31 - 2.05)	3.42 (2.65 - 4.88)	Mann-Whitney U	— *(Stratifying variable)*

Parameter	Low Inflammatory Risk ($N = 627$)	High Inflammatory Risk ($N = 429$)	Test Statistic	P-Value
Serum CRP (mg/L, Median, IQR)	1.10 (0.50 - 2.30)	8.90 (4.50 - 18.20)	Mann-Whitney U	*— (Stratifying variable)*
Serum Albumin (g/dL, Mean ± SD)	4.28 ± 0.35	3.88 ± 0.46	Student's t	< 10^-15
5-Year Overall Survival (%)	**74.7%**	**51.6%**	**Log-Rank** $\chi^2 = 69.96$	**5.94 × 10^-17**
Adjusted Cox HR (TNM-adjusted)	**1.000 (Reference)**	**1.474 (1.165 - 1.865)**	**Wald** $z = 3.24$	**0.00121**

Note: Mann-Whitney U tests for NLR and CRP reflect descriptive separation across the predefined stratification criteria (NLR vs ; mGPS vs); independent prognostic validation is provided by the Log-Rank and multivariable Cox proportional hazards models adjusting for Pathologic Stage and Grade.

Supplementary Table S3: Multi-Cohort Solid Epithelial Validation & Falsification Controls ()

Cohort Identifier	Cancer Type	Sample Size (N)	Primary Endpoint	Hazard Ratio (95% CI)	P-Value	Hypothesis Status
Discovery (MSKCC)	Lung Adenocarcinoma	604	Recurrence-Free Survival	2.23 (1.13 - 4.39)	0.021	Significant (Independent)
PLOS ONE Complete	Surgical Mixed Solid	1,056	Overall Survival	1.47 (1.17 - 1.87)	0.00121	Significant (Independent)
TCGA-	Colorecta	594	Overall	1.68 (1.18	0.004	Validated

Cohort Identifier	Cancer Type	Sample Size (*N*)	Primary Endpoint	Hazard Ratio (95% CI)	P-Value	Hypothesis Status
COAD	l Adenocarcinoma		Survival	- 2.39)		Solid Epithelial
TCGA-PAAD	Pancreatic Ductal Adenoca	184	Overall Survival	1.74 (1.13 - 2.68)	0.012	Validated Solid Epithelial
TCGA-ESCA	Esophageal Adenocarcinoma	182	Overall Survival	2.24 (1.39 - 3.61)	0.0008	Validated Solid Epithelial
TCGA-CHOL	Cholangiocarcinoma	36	Overall Survival	2.89 (1.06 - 7.89)	0.038	Validated Solid Epithelial
GSE31210	External Lung Adenoca	226	Overall Survival	1.89 (1.11 - 3.22)	0.018	Validated Solid Epithelial
TCGA-LAML	Acute Myeloid Leukemia	173	Overall Survival	0.95 (0.63 - 1.43)	0.810	Correct Negative Control
TCGA-TGCT	Testicular Germ Cell	133	Overall Survival	0.36 (0.04 - 3.21)	0.379	Correct Negative Control
TCGA-SARC	Soft-Tissue Sarcoma	255	Overall Survival	1.18 (0.79 - 1.76)	0.423	Correct Negative Control
TOTAL	**Multi-Center Master**	3,443	**All Endpoints**	—	—	REMARK-Compliant Total (10 Cohorts)

Supplementary Note 5: REMARK Guidelines Full Compliance Checklist & Ethical Declarations

1. Hypothesis & Study Rationale: Explicitly prespecified: evaluation of whether coupled histological architecture, interstitial transport, and inflammatory markers provide independent prognostic stratification in retrospective surgical cohorts.
2. Patient Selection & Cohorts: Strict complete-case analyses (across 10 cohorts). For raw blood chemistry (), incomplete records () were excluded a priori. No imputation was performed.
3. Data Modalities & Assay Methods Mapping:

Data Modality	Cohorts	Epithelial Polarity Domain (P_{hist})	Desmoplasia Domain (M_{mech})	Inflammatory Soil Domain (Φ_{soil})
Modality 1: Raw Blood & Surgical Path	PLOS ONE ($N = 1,056$)	Histological tumor differentiation grade	Pathologic invasiveness / local spread	Pre-op CBC NLR, serum CRP, and Albumin (mGPS)
Modality 2: Bulk Transcriptomics	Solid: TCGA ($N = 996$), GEO GSE31210 ($N = 226$), Controls ($N = 561$)	Polarity/cilia module z-score (*TJP2*, *CDH1*, *PKHD1*, *IFT88*)	Stromal matrix module z-score (*COL1A1*, *ACTA2*, *LOXL2*)	Myeloid cytokine module z-score (*IL1B*, *IL6*, *CXCL8*, *TNF*)
Modality 3: Digital Histology & Clinical	MSKCC 2020 ($N = 604$)	Lepidic/acinar vs solid/micropapillary architecture	Peritumoral desmoplastic fibrosis	Smoking pack-years & inflammatory covariates

4. Study Design: Retrospective translational multi-cohort study analyzing de-identified open-access public repositories.
5. Statistical Analysis: Kaplan-Meier survival curves, log-rank tests, multivariable Cox proportional hazards with Schoenfeld residual testing, Aalen-Johansen competing risks cumulative incidence functions, 500-fold bootstrap cross-validation, and time-dependent receiver operating characteristic (ROC) curves.

6. Negative Controls & Falsification: Proof-by-contradiction negative controls evaluated in liquid tumors (TCGA-LAML), germline tumors (TCGA-TGCT), and sarcomas (TCGA-SARC), confirming model failure where biophysical assumptions are absent.
7. Ethical Approval & IRB Exemption: Secondary analysis of fully de-identified, publicly accessible datasets. In accordance with national guidelines and institutional policies of the Computational Oncology Laboratory, Independent Research, this study is exempt from formal Institutional Review Board (IRB) review.
8. Regulatory Notice: All computational scores and calculator prototypes are designated strictly for Research Use Only (RUO).