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, MD Candidate[1,2]
[1]Department of Clinical Medicine and Surgery, School of Medicine and Surgery, Università degli Studi di Napoli Federico II, Naples, Italy
[2]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy
Corresponding Author: Luca Celebrano, MD Candidate (`l.celebrano@studenti.unina.it`), ORCID: `0009-0007-0931-3555`

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Open Code & Data Repository: `https://github.com/Lukecele/inflammatory-onco-physics`
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Supplementary Note 1: First-Principle Derivation of Radial Drug Transport (K_0 Bessel Solution)

In solid tumor cords, convective interstitial fluid transport is severely compromised by elevated interstitial fluid pressure ($IFP \approx 20$–35 mmHg) and the collapse of intra-tumoral functional lymphatics, rendering the transmural pressure gradient negligible ($\Delta P \approx 0$). Under these conditions, the Péclet number:

$$Pe = \frac{v_{\text{fluid}} L}{D_{\text{eff}}} \ll 0.1$$

confirming that interstitial mass transport operates in a pure diffusion-dominant regime (Baxter & Jain, *Microvasc Res* 1989). Around an idealized cylindrical microvessel of radius $r_v \approx 10$ μm, steady-state radial transport with first-order cellular uptake and metabolic clearance k is governed by:

$$D_{\text{eff}} \left(\frac{d^2C}{dr^2} + \frac{1}{r}\frac{dC}{dr} \right) - kC = 0$$

Dividing by the effective interstitial diffusion coefficient D_{eff} and defining the characteristic tissue penetration length $\lambda = \sqrt{D_{\text{eff}}/k}$:

$$\frac{d^2C}{dr^2} + \frac{1}{r}\frac{dC}{dr} - \frac{1}{\lambda^2}C = 0$$

Substituting the dimensionless radial variable $\xi = r/\lambda$ transforms this into the modified Bessel differential equation of order zero:

$$\xi^2 \frac{d^2C}{d\xi^2} + \xi \frac{dC}{d\xi} - \xi^2 C = 0$$

The general analytical solution is:

$$C(\xi) = AI_0(\xi) + BK_0(\xi)$$

Where I_0 and K_0 are modified cylindrical Bessel functions of order zero of the first and second kind, respectively.

Physical Boundary Conditions:

1. **Distal boundary condition ($r \to \infty$):** In the deep cord/necrotic boundary, drug concentration must asymptotically approach zero: $\lim_{\xi\to\infty} C(\xi) = 0$. Since $I_0(\xi) \sim \frac{e^\xi}{\sqrt{2\pi\xi}} \to \infty$ as $\xi \to \infty$, coefficient A must vanish identically ($A = 0$).
2. **Capillary wall boundary condition ($r = r_v$):** At the outer microvascular endothelial boundary ($r = r_v, \xi_v = r_v/\lambda$), concentration matches the free unbound intravascular trough concentration C_0:

$$C(\xi_v) = BK_0(r_v/\lambda) = C_0 \implies B = \frac{C_0}{K_0(r_v/\lambda)}$$

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

$$C(r) = C_0 \frac{K_0(r/\lambda)}{K_0(r_v/\lambda)}$$

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

- Molecular Weight (MW): 499.6 g/mol.
- Hydrodynamic Stokes Radius: $r_H = 0.58$ nm.
- Free diffusivity in water (37°C): $D_0 = 3.78 \times 10^{-6}$ cm^2/s.
- Effective interstitial tissue diffusivity in desmoplastic stroma: $D_{\text{eff}} = 59.29$ μm^2/s.
- Elimination rate constant: $k = 1.51 \times 10^{-4}$ s^{-1}.
- Characteristic tissue penetration length: $\lambda = \sqrt{D_{\text{eff}}/k} = 627.6$ μm.
- Intravascular free unbound concentration (80 mg daily oral dosing, free fraction $f_u = 0.053$, $C_{\text{trough}} = 540$ nM): $C_0 = 28.62$ nM.
- Cellular target inhibitory concentration: $\text{IC}_{50} = 10.0$ nM (therapeutic threshold $\theta = \text{IC}_{50}/C_0 = 0.349$).
- Critical threshold crossing radius: $r_{\text{crit}} = \lambda \cdot K_0^{-1}(\theta \cdot K_0(r_v/\lambda)) \approx 166$ μm (165.89 μm; sensitivity envelope: 140–180 μm).
- Critical viability oxygenation boundary: $r_{\text{ox}} = 200.0$ μm (corresponding to cellular hypoxia threshold $p\text{O}_2 \geq$ 1–2 mmHg).
- **Zonal Cord Compartmentalization & Volume Fractions:**
  - *Proximal Perivascular Zone ($r_v \leq r \leq 166$ μm, i.e., $10 \leq r \leq 166$ μm from the vascular lumen):* Therapeutic concentration ($C \geq$ 10–14 nM); active suppression of sensitive clones (N_S).
  - *Distal Cord Sanctuary / Marginal Transitional Niche ($166 < r \leq 200$ μm):* Viable tissue ($p\text{O}_2 \geq$ 1–2 mmHg), but sub-therapeutic drug level (2–8 nM); nominal width $\Delta r \approx 34$ μm, harboring uneradicated persisters in cytostatic G_0/G_1 arrest.
  - *Exact Geometric Volumetric Fraction:* In cylindrical coordinates, accounting for the microvascular lumen ($r_v = 10$ μm), the proportion of the viable cord volume occupied by the distal sanctuary is:

$$\frac{V_{\text{sanctuary}}}{V_{\text{viable}}} = \frac{r_{\text{ox}}^2 - r_{\text{crit}}^2}{r_{\text{ox}}^2 - r_v^2} = \frac{200.00^2 - 165.89^2}{200.00^2 - 10.00^2} = \frac{12,480.51}{39,900.00} = \mathbf{31.28}\%$$

  - *Anoxic Necrotic Core ($r > 200$ μm):* Severe ischemia ($p\text{O}_2 < 1$ mmHg).

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: ~ 0.5 kDa.
  - Hydrodynamic radius: $r_H \approx 0.58$ nm(< 1 nm).

  - Diffuses into tissue based on small-molecule mobility.
- **Circulating Tumor DNA (Mononucleosomal fragment):**
  - Molecular weight: > 200 kDa (~ 166 bp wrapped around histone octamer).
  - Hydrodynamic diameter: ≈ 11 nm ($r_H \approx 5.5$ nm).
  - Stokes-Einstein free diffusion coefficient:

$$D_{\text{Stokes}} = \frac{k_B T}{6\pi\eta r_H}$$

    In water at 37°C, $D_{\text{nucleosome}} \approx 2.5 \times 10^{-7}$ cm^2/s, which is over an order of magnitude smaller than D_{drug}.

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 10–50 nm. For an 11 nm nanoparticle, steric hindrance and hydrodynamic drag reduce effective interstitial diffusivity according to the Ogston-Renkin relationship:

$$D_{\text{eff, ctDNA}} = D_{\text{Stokes}} \cdot (1 - \lambda_{\text{pore}})^2 \cdot F(\lambda_{\text{pore}})$$

Where $\lambda_{\text{pore}} = r_{\text{particle}}/r_{\text{pore}}$. This physical formulation is strictly valid for $\lambda_{\text{pore}} < 1$ (where $(1 - \lambda_{\text{pore}})^2$ is the cylindrical steric partition coefficient and $F(\lambda_{\text{pore}})$ is the Faxén hydrodynamic drag factor).

In hyper-crosslinked collagen regions where pore diameters approach ≤ 11 nm ($\lambda_{\text{pore}} \geq 1$), nucleosomes undergo **complete steric filtration** ($D_{\text{eff, ctDNA}} \to 0$ and convective velocity $v_{\text{fluid}} \approx 0$). In the absence of convective flow ($\Delta P \approx 0$), 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 (< 70 bp); * 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 ($\gamma_{\text{shed}} \to 0$), explaining false-negative plasma ctDNA assays even with ultra-sensitive detection limits (LOD $\leq 0.01\%$).

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-1β, TNF-α, IL-6, TGF-β):

$$\mathcal{E}_{\text{imm}}(t) = \mathcal{E}_0 \exp\left(-\gamma \int_0^t \Phi(x, \tau) d\tau\right)$$

Where: * $\mathcal{E}_0$ is baseline cytolytic capacity of tumor-infiltrating CD8+ cytotoxic T lymphocytes and NK cells. * $\Phi(x, \tau)$ is local and systemic inflammatory tension at time τ. * γ is the epigenetic exhaustion susceptibility parameter (0.08–0.15 month^{-1}).

When cumulative inflammatory damage reduces $\mathcal{E}_{\text{imm}}(t)$ below the net persister expansion rate ($r_R - \mu_R$), the dormant equilibrium fixed point at $N_R = 0$ undergoes a transcritical bifurcation:

$$\left.\frac{dN_R}{dt}\right|_{N_R \to 0} = (r_R - \mu_R - \mathcal{E}_{\text{imm}}(t))N_R > 0$$

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 ($C < 14$ 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 $N = 604$)

Covariate	Hazard Ratio (HR)	95% Confidence Interval	Wald Statistic	P-Value
HTBI (Continuous, Decoupled)	**2.49**	**1.00 - 6.18**	**Wald** $z = 1.96$	**0.050**
— HTBI Binary (Median Split)	*1.89*	*1.07 - 3.35*	*Wald* $z = 2.18$	*0.029*
Pathologic Stage (Continuous)	2.53	2.00 - 3.19	Wald $z = 7.80$	< 0.0005
Patient Sex (Male vs Female)	0.91	0.59 - 1.40	Wald $z = -0.45$	0.654
***EGFR* Driver Mutation (Mut vs WT)**	0.91	0.57 - 1.46	Wald $z = -0.38$	0.704

Global Schoenfeld Residual Proportional Hazards Test: $\chi^2 = 3.82, p = 0.431$ *(HTBI term* $p = 0.813$*).*

Supplementary Table S2: Complete-Case Blood Chemistry Risk Stratification ($N = 1,056$)

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 (%)	**69.4%**	**56.7%**	**Log-Rank** $\chi^2 = 25.8$	**3.81 × 10^-7**
Adjusted Cox HR (Binary, Stage/Grade-adjusted)	**1.000 (Reference)**	**1.434 (1.138 - 1.807)**	**Wald** $z = 3.06$	**0.00224**
Adjusted Cox HR (Continuous, Stage-adjusted)	—	**2.202 (1.434 - 3.382)**	**Wald** $z = 3.53$	**0.00041**

Note: Mann-Whitney U tests for NLR and CRP reflect descriptive separation across the predefined stratification criteria (NLR < 2.5 *vs* ≥ 2.5*; mGPS* 0 *vs* ≥ 1*); 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 ($N = 3,461$)

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	2.49 (1.00 - 6.18) cont / 1.89 (1.07 - 3.35) bin	0.050 / 0.029	Significant (Independent)
PLOS ONE Complete	Surgical Mixed Solid	1,056	Overall Survival	1.43 (1.14 - 1.81) bin / 2.20 (1.43 - 3.38) cont	0.0022 / 0.0004	Significant (Independent)
TCGA-COADREAD	Colorectal Adenocarcinoma	592	Overall Survival	1.68 (1.18 - 2.39)	0.014	Validated Solid Epithelial
TCGA-PAAD	Pancreatic Ductal Adenoca	177	Overall Survival	1.74 (1.13 - 2.68)	2.6e-5	Validated Solid Epithelial
TCGA-ESCA	Esophageal Adenocarcinoma	181	Overall Survival	1.59 (0.99 - 2.54) [Adeno: 2.09]	0.053 [Adeno: 0.019]	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

Cohort Identifier	Cancer Type	Sample Size (N)	Primary Endpoint	Hazard Ratio (95% CI)	P-Value	Hypothesis Status
CHOL-OMICS	Inherited Cholangiopathy	18	Desmoplasia / Resistance	$BSIR =$ 3.6–22.1	—	High Concordance
TCGA-LAML	Acute Myeloid Leukemia	149	Overall Survival	1.28 (0.85 - 1.93)	0.246	Correct Negative Control
TCGA-TGCT	Testicular Germ Cell	133	Overall Survival	2.33 (0.23 - 23.09)	0.471	Correct Negative Control
TCGA-SARC	Soft-Tissue Sarcoma	253	Overall Survival	0.85 (0.57 - 1.26)	0.413	Correct Negative Control
TOTAL	**Multi-Center Master**	**3,461**	**All Endpoints**	—	—	**REMARK-Compliant Total**

Note: Of $N = 3,461$ *clinically indexed records across 11 cohorts,* 36 *TCGA samples lacked paired RNA-seq matrices, yielding* $N = 3,425$ *complete matched molecular and blood profiles analyzed (604 + 1,056 + 986 + 226 + 18 + 535 = 3,425).*

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 ($N = 3,461$ across 11 cohorts). For raw blood chemistry ($N = 1,056$), incomplete records ($N = 274$) were excluded a priori. No imputation was performed.
3. **Data Modalities & Assay Methods Mapping:**

Data Modality	Cohorts	Epithelial Polarity Domain ($\mathcal{P}_{\text{hist}}$)	Desmoplasia Domain ($\mathcal{M}_{\text{mech}}$)	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 Department of Clinical Medicine and Surgery, Università degli Studi di Napoli Federico II, 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)**.