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Intracellular and Erythrocyte Partitioning of Venetoclax and its Metabolites: A Multi-Compartmental Pharmacokinetic Model Derived from Paired Patient Samples using High-Resolution Mass Spectrometry

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INTRODUCTION & AIM

Background: Venetoclax is a pivotal, highly selective BCL-2 inhibitor that has revolutionized the treatment of hematological malignancies.
The Problem: Standard Therapeutic Drug Monitoring (TDM) relies exclusively on plasma pharmacokinetics (PK). However, plasma concentrations often fail to accurately predict clinical outcomes, minimal residual disease (MRD), or drug resistance.
The Rationale: Because BCL-2 is an intracellular target, the true driving force of efficacy resides inside the cells. Venetoclax is highly lipophilic, suggesting it may significantly partition into blood cellular fractions, such as target Peripheral Blood Mononuclear Cells (PBMCs) and Red Blood Cells (RBCs).
Aims of the Study:
To simultaneously quantify Venetoclax and its two major metabolites across three matrices (Plasma, PBMCs, and RBCs) within the same patient cohort.

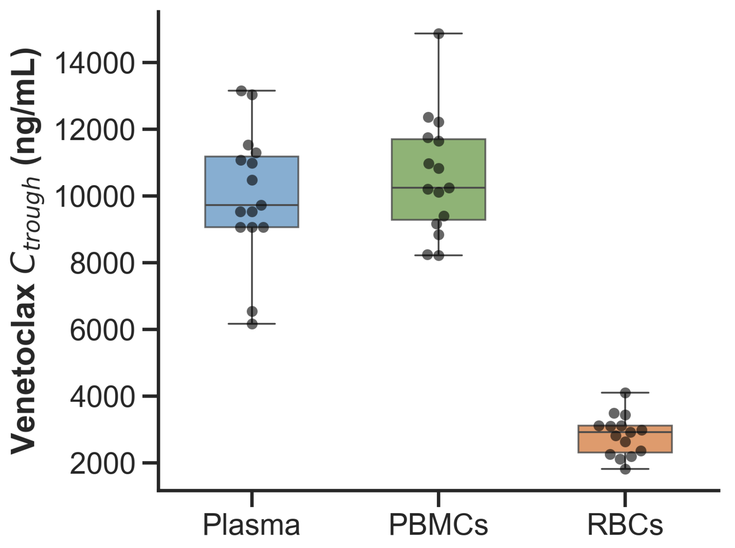
METHOD

Patient Cohort & Sampling: Peripheral blood samples were collected from 15 patients undergoing continuous Venetoclax therapy. All samples were taken at a single time point to capture steady-state trough levels (*concentrazione di valle*).
Sample Processing: Blood was immediately processed using optimized density gradient centrifugation to cleanly isolate Plasma, RBCs, and PBMCs, minimizing transmembrane drug leakage during handling.
High-Resolution Mass Spectrometry: Simultaneous quantification of the parent drug and its metabolites was performed using a ultra-high-performance liquid chromatography system (Shimadzu Nexera X3) coupled to a high-resolution QTOF mass spectrometer (Sciex ZenoTOF 7600), ensuring extreme sensitivity in cellular lysates.
Compartmental PK Modeling (MATLAB):
Since clinical data consist of single-point steady-state trough concentrations ($C_{ss,\ trough}$), a multi-compartment mass balance model was defined. At steady state, the net transfer rate between plasma and cells is zero ($\frac{dC}{dt} = 0$). Therefore, the model calculates the cellular accumulation ratio (K_p), which represents the dynamic equilibrium between influx (k_{in}) and efflux (k_{out}) rate constants:
$$\frac{C_{cellular}}{C_{plasma}} = \frac{k_{in}}{k_{out}} = K_p$$

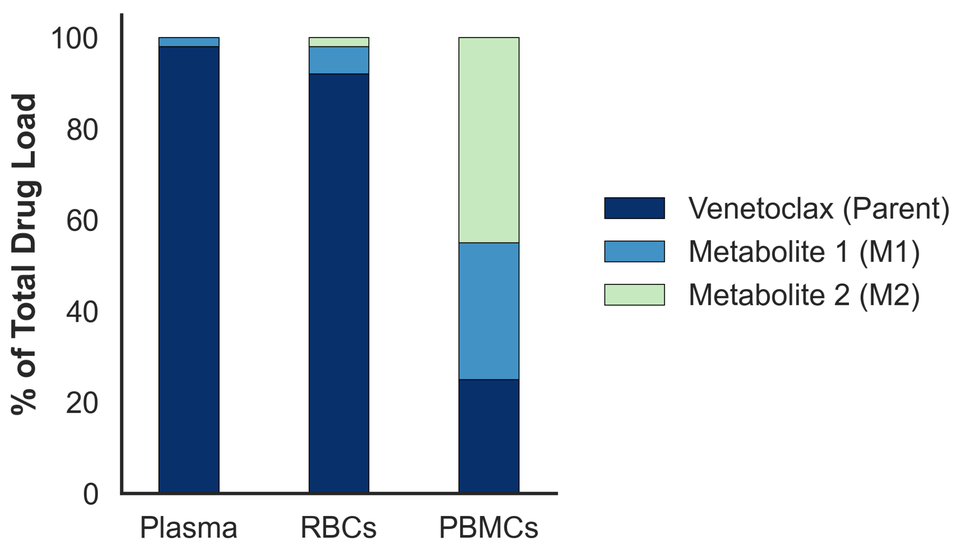
RESULTS & DISCUSSION

Analytical Performance: The UPLC-QTOF method demonstrated exceptional selectivity and sensitivity, successfully detecting and quantifying Venetoclax and its two main metabolites even at low intracellular volumes.
Plasma Baseline: The mean steady-state trough concentration ($C_{through}$) in the plasma fraction across the patient cohort was approximately **10,000 ng/mL**.
Target Cell Accumulation (PBMCs):
PBMC analysis revealed a significant intracellular accumulation of **+15%** compared to plasma, resulting in a mean target-cell concentration of **11,500 ng/mL**.
This confirms that Venetoclax effectively concentrates within its therapeutic site of action ($K_{p,\ PBMC}$ = 1.15).
Erythrocyte Sequestration (RBCs):
RBC concentrations were **-70%** relative to plasma, yielding a mean concentration of **3,000 ng/mL** ($K_{p,\ RBC}$ = 0.30).
Discussion: Although the concentration per unit volume is lower than in plasma, RBCs represent the largest cellular volume in whole blood. Consequently, a 3,000 ng/mL concentration implies that RBCs act as a massive absolute drug reservoir, significantly impacting total circulating Venetoclax mass and buffering its systemic availability.

A Steady-State Venetoclax Exposure



B Metabolite Distribution



C Key Kinetic Parameters

Parameter	Mean	%CV
k_{in_PBMC} (h^{-1})	0.045	18%
k_{out_RBC} (h^{-1})	0.100	19%
$C_{trough\_PBMC}/C_{trough\_Plasma}$	1.15	25%
$C_{trough\_RBC}/C_{trough\_Plasma}$	0.30	30%

CONCLUSIONS

This study successfully maps and models the real-world intracellular distribution of Venetoclax simultaneously in plasma, RBCs, and target PBMCs within a clinical cohort.
The data prove that plasma-only TDM severely oversimplifies Venetoclax pharmacokinetics, failing to account for the +15% over-concentration in target cells and the massive drug reservoir effect of RBCs.
Integrating mass-spectrometry cellular assays with MATLAB steady-state compartmental modeling offers a more precise, personalized oncology tool to understand drug resistance and optimize dosing regimens.

REFERENCES

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