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# Automated Data Processing and Annotation for Untargeted Plasma Metabolomics in TKI-Associated ADR Research

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## 1 Abstract

- **Background**: Tyrosine kinase inhibitors (TKIs) are widely used in cancer therapy but frequently induce adverse drug reactions (ADRs) that limit treatment efficacy and adherence (1)
- **Objective**: Establishment of an untargeted serum metabolomics workflow to identify predictive biomarkers of ADR frequency and severity during TKI therapy
- **Analytical workflow**: protein-precipitation sample preparation optimized for metabolite recovery; UHPLC-HRMS with SWATH/DIA
- **Data processing**: R-based pipeline for automated data preprocessing, feature detection, annotation and analysis
- **Application/Impact**: Method will be applied to longitudinal serum samples from RCC patients, enabling early ADR risk stratification and supporting personalized TKI therapy

## 2 Experimental Setup

- **Samples**: Human plasma aliquots
- **Instrumentation**: UHPLC-QTOF, HILIC/CSH C18 column
- **Acquisition**: Data-independent acquisition (DIA/SWATH)
- **Data format**: .mzML (converted via MSConvert)
- **Software environment**: R 4.5.1

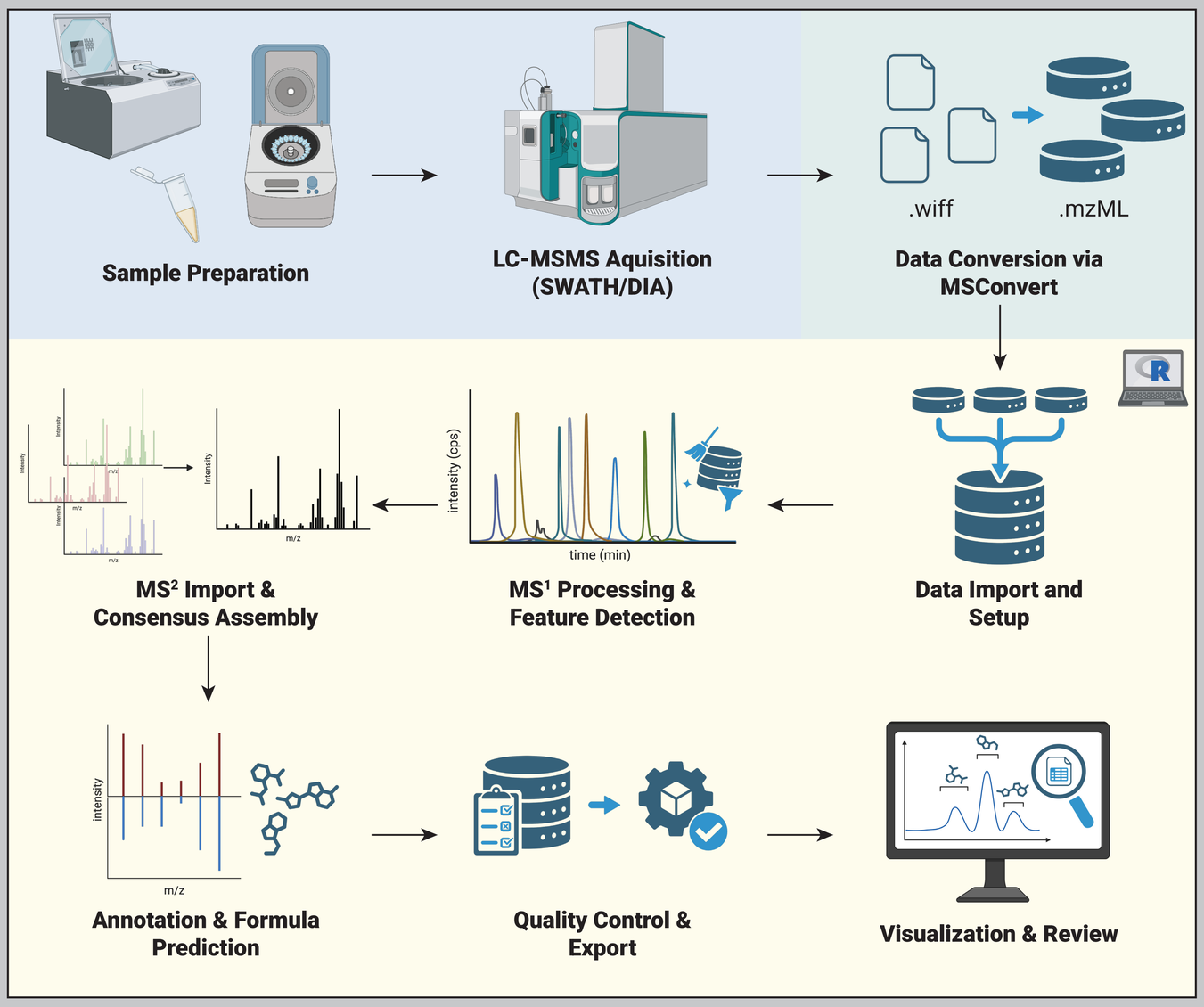


*Figure 1:* **Overview of the plasma metabolomics workflow, highlighting the automated data processing and annotation pipeline performed in R.** (Figure created with illustrations from https://BioRender.com)

## 3 Data Processing and Annotation Pipeline in R

1. **Data Import and Setup**: All converted mzML files are automatically detected, categorized by ionization polarity and chromatographic mode (HILIC/CSH C18) → ***avoids manual data sorting, and contains metadata structure***
2. **MS¹ Preprocessing and Feature Detection**: Feature detection with *XCMS* using the *CentWave* algorithm; RT-alignment using the *obiwarp* method; grouping of features across samples using a density-based approach → ***batch-consistent XCMS processing with integrated logging and standardized RDS***
3. **MS² Import and Consensus Assembly**: Import using *Spectra*; custom indexing step (m/z, RT) to enable fragment-to-feature mapping; normalization and filtering → ***aggregation of DIA fragments dynamically per feature across samples + normalization and standardization of peak intensities enable consistent downstream matching***
4. **Library Matching and Annotation**: Consensus spectra are compared against public spectral libraries (e.g. MoNA, HMDB, MassBank) in a first step using *MetaboAnnotation* → ***simultaneous multi-library matching, allowing in-house libraries + theoretical formula generation***
5. **Quality Control and Export**: QC metrics (dot-product distribution, explained intensity, hit rate) are computed to assess spectral match quality; export of the results in both .rds and .csv files → ***reproducible, object-based data layer; reloadable, script-accessible, and version-consistent computational states in the form of .rds objects***
6. **Interactive Visualization and Curation (Shiny App)**: Interactive interface that enables multi-sample EIC visualization per feature, mirror plots comparing query vs. library MS² spectra, and feature table filtering by mode, column and annotation score → ***dynamic exploration of annotation results directly linked to the pipeline's outputs***

## 4 Outlook

- Creation of an in-house metabolomics library
- Metabolomics analysis of longitudinally collected plasma samples from patients with renal cell carcinoma (RCC) treated with axitinib and cabozantinib
- Integration of the metabolomics data with drug plasma concentrations, individual patient data, clinical parameters, proteomics measurements, and ADR occurrence for a TDM approach

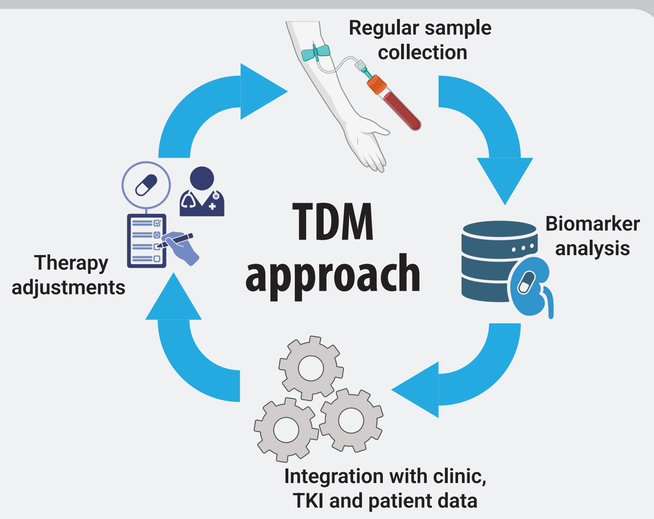


*Figure 2:* **Proposal for a Therapeutic Drug Monitoring Regimen, incorporating biomarker data.** (Figure created with illustrations from https://BioRender.com)

## 5 References

(1) Shyam Sunder S, Sharma UC, Pokharel S. Adverse effects of tyrosine kinase inhibitors in cancer therapy: pathophysiology, mechanisms and clinical management. Signal Transduct Target Ther. 2023;8(1):262.

**TALK TO THE AUTHOR**

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