Figure 1. Overview of the project timeline and workflow across seven sequential steps. Step 01 corresponds to the retrospective phase: aggregation and preprocessing of existing datasets from ConLiGen and PGBD (see Sections 2.1-2.2). Steps 02-07 comprise the prospective phase. Step 02 initiates prospective recruitment of 100 lithium-naïve patients with bipolar disorder (BD) (Section 2.2). Step 03 involves multi-omics and clinical data collection (Section 2.3). Steps 04-06 describe model development: training a multilayer feed-forward neural network (MFNN) using prospective data (Step 04; Section 2.6), validation with a dedicated internal subset (Step 05; Sections 2.6-2.7), and final testing on a held-out dataset (Step 06; Section 2.7). Step 07 captures longitudinal follow-up at 6-month intervals for up to 36 months, supporting outcome refinement and model updating (Section 2.8). Collectively, these steps follow the two-phase structure detailed in Section 2.1: retrospective pre-training followed by prospective fine-tuning and validation. Figure created using Canva.com.

Step	Description	Target N	Key Outcome	Tools/Considerations
1. Retrospective Data Collection *(Phase I)*	Aggregate and preprocess existing lithium response datasets (e.g., ConLiGen, PGBD) to extract SNPs, available RNA-seq/methylation data and Alda scores. Format features for initial model input.	**retrospective datasets**	Foundation for pre-training and feature selection	Alda scale (≥7 = responder), data normalization, SMOTE, dimensionality reduction (LASSO, PCA)
2. Prospective Cohort Recruitment *(Phase II)*	Enroll 100 lithium-naïve BD patients via partner clinics. Screen for inclusion/exclusion and obtain consent for clinical and molecular data collection.	**100**	Consent-based, diverse cohort for multi-omic study	Batch control, standardized protocols, consistent sample timing, ethics/REB clearance
3. Multi-Omics Data Collection *(Phase II)*	Collect blood for RNA-seq and DNA methylation profiling. Store serum for future metabolomics. Gather standardized clinical data (e.g., YMRS, MADRS, CGI-BP).	**100 (multi-omics)**	High-resolution multi-modal input dataset	Biobanking, SOPs, QC, morning sampling to reduce circadian noise
4. Model Training *(Phase II)*	Train a multilayer feed-forward neural network (MFNN) using multi-omics and clinical data from 50 patients. Address class imbalance.	**50 (training subset)**	Trained model with internal cross-validation	SMOTE, class weighting, 10-fold CV, dropout, embedded feature selection (LASSO/PCA/autoencoders)
5. Validation *(Phase II)*	Optimize architecture and tune hyperparameters using a dedicated validation set. Assess feature importance.	**25 (validation subset)**	Refined model with interpretability checks	Early stopping, SHAP explanations, performance tuning
6. Testing *(Phase II)*	Evaluate final model performance on an unseen hold-out set.	**25 (test subset)**	Generalizability assessment; benchmark metrics	AUROC, accuracy, F1, precision/recall, calibration curve, confusion matrix
7. Longitudinal Follow-Up *(Phase II)*	Conduct 6-month follow-ups for up to 36 months. Track response durability, refine labels, and support model retraining.	**100 (full cohort)**	Outcome validation, label refinement, and model retraining	Imputation, survival analysis, sensitivity testing, model updating feedback loop

Table 1. Sequential project steps from retrospective dataset processing to real-world follow-up. Steps 1-7 align with the core workflow described in the methodology (Sections 2.1-2.8). Step 1 involves retrospective data aggregation from the International Consortium on Lithium Genetics (ConLiGen) and the Pharmacogenomics of Bipolar Disorder (PGBD) study for pre-training. Steps 2-6 represent the prospective pipeline: recruitment of 100 lithium-naïve patients with bipolar disorder (BD), multi-omics and clinical data collection, training of a multilayer feed-forward neural network (MFNN), model validation, and testing. Step 7 includes longitudinal follow-up to refine outcome labels and improve model performance. Training/validation/test subsets derived from prospective cohort of N=100.
[Abbreviations: BD - bipolar disorder; SNP - single-nucleotide polymorphism; RNA-seq - RNA sequencing; MFNN - multilayer feed-forward neural network; CGI-BP - Clinical Global Impressions Scale-Bipolar Version; YMRS - Young Mania Rating Scale; MADRS - Montgomery-Åsberg Depression Rating Scale; SHAP - SHapley Additive exPlanations; PCA - principal component analysis; LASSO - least absolute shrinkage and selection operator; SMOTE - Synthetic Minority Oversampling Technique; CV - cross-validation; SOP - standard operating procedure; QC - quality control; REB - Research Ethics Board.]

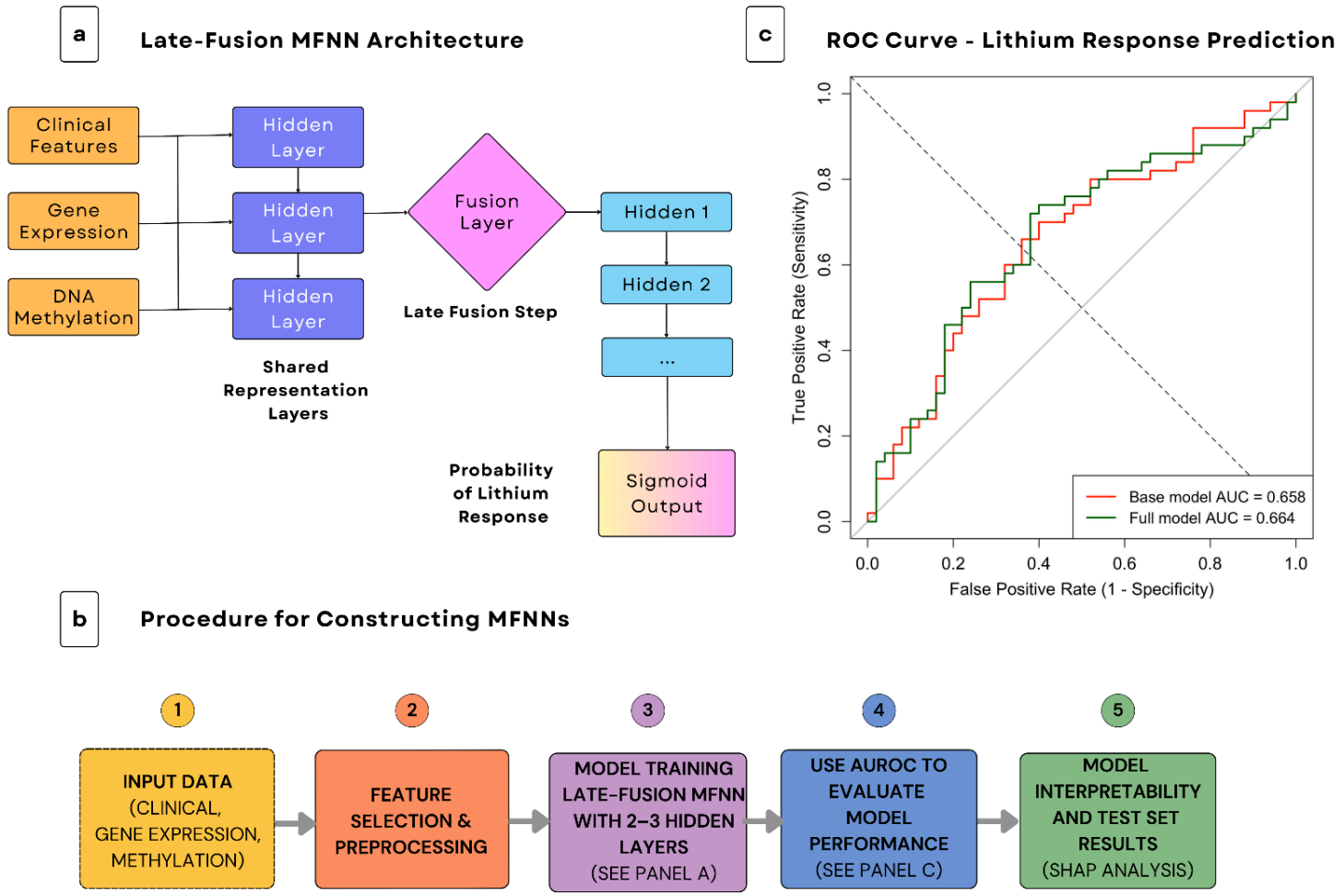

Figure 2. Overview of model development and evaluation workflow for predicting lithium response using a multilayer feed-forward neural network (MFNN). (a) Late-fusion MFNN architecture: clinical, transcriptomic, and methylation features are processed through modality-specific hidden layers before integration via a fusion layer, followed by additional hidden layers and a sigmoid output for binary classification. (b) End-to-end pipeline from input data preprocessing to model interpretation. (c) Example ROC curves comparing a base model (e.g., clinical + genetic data only) with a full model that includes multi-omics inputs. The full model demonstrates improved predictive performance (AUC = 0.664) over the base model (AUC = 0.658). This reflects a realistic effect size for deep learning models in early-stage pharmacogenomic studies, where sample sizes are limited and outcomes are complex. AUC was selected as the primary evaluation metric due to its threshold-independent assessment of classification performance. See Sections 2.5-2.7 for full methodological details. Figure created using Canva.com.