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Predictive Network-Medicine Modeling Prioritizes Combinatory Drugs for Amyotrophic Lateral Sclerosis

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Short Report

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Abstract

Heterogeneity in amyotrophic lateral sclerosis (ALS) pathology has limited the success of monotherapy approaches in clinical trials. We implemented a network-medicine framework integrating ALS signatures with Connectivity Map to identify drug combinations that reverse disease-associated profiles while excluding those that phenocopied ALS. We identified dual targeting of HDAC and ATR kinase pathways may offer a mechanistically convergent therapeutic strategy, demonstrating the potential of network-medicine to prioritize combinatorial therapies for ALS.

Introduction

Despite decades of research, effective therapeutic options for ALS are limited.[1] The failure of numerous clinical trials reflects, in part, the profound clinical and molecular heterogeneity of ALS, which obscures therapeutic effects in unstratified trial populations. For example, ALS patients exhibit varying rates of progression and distinct underlying molecular mechanisms[2,3], and therapies that show promise in a subset of patients may fail to reach statistical significance when assessed across a poorly stratified cohort. This underscores the need for therapeutic strategies that target multiple disease pathways rather than a single pathway.

To overcome this challenge, we propose a systematic strategy to identify and prioritize repurposable drugs that show efficacy across all ALS phenotypes, regardless of clinical or molecular subclassification. The traditional drug development is lengthy and costly—often requiring more than a decade from target discovery to approval—drug repurposing offers a practical and efficient alternative. By focusing on compounds with established safety profiles and existing pharmacological data, repurposing enables a substantially accelerated route to clinical translation.

Network medicine depicts biological entities as nodes connected by functional interactions.[4] By embedding disease signatures and drug targets into this network framework, it becomes possible to systematically evaluate the therapeutic relevance of drugs and uncover rational combinations that may act synergistically on complementary aspects of disease biology. When combined with omics-driven drug repurposing[5], such methods can uncover therapeutically relevant drug combinations with known safety profiles, targeting multiple disease pathways and accelerating translation to clinical testing. We hypothesized that a two-stage selection, requiring strong reversal of ALS signatures while excluding compounds that phenocopy disease, would improve specificity and safety of repurposing predictions. We further applied the Syndrum algorithm[6] to predict synergistic drug combinations targeting complementary mechanisms.

The aims of this study are: (1) to develop a comprehensive framework for identifying drugs that robustly reverse ALS molecular signatures across phenotypic subtypes, and (2) to prioritize combinatorial therapies with mechanistic convergence and translational feasibility.

Results

To capture the heterogeneity inherent in ALS, we obtained the protein expression profile of eight distinct ALS phenotypes, ranging from high-to-low severity and fast-to-slow progression measured by the ALS Functional Rating Scale–Revised (ALSFRS-R) and the Cognitive and Behavioral Screen (CBS). (Fig. 1A) Each of the eight proteomic signatures were individually queried against the Connectivity Map (CMap) database, to quantify their perturbation correlations between various small molecule drugs with ALS. Small molecule drugs that share negative connectivity with the ALS signatures suggest its therapeutic potential to reverse the ALS phenotype, whereas small molecule drugs that exhibit positive connectivities with the ALS signatures potentially phenocopy or exacerbate the ALS phenotype.

Of the 1,878 drugs that were queried from the CMap database, twelve drugs consistently reversed ALS molecular signatures across motor and cognitive phenotypes while lacking phenocopy effects. (Fig. 1B and Supplementary Table 1) These included FDA-approved agents (vorinostat, everolimus), and investigational HDAC inhibitors (entinostat, mocetinostat), kinase inhibitors (BMS-777607, VE-821, ZM-447439, GSK-1070916), and metabolic or efflux modulators (U-18666A, zosuquidar). Dasatinib, an FDA-approved Abl kinase inhibitor, strictly reversed both the fast and slow progressing phenotypes. Concurrently, we identified five compounds that attained positive connectivities across the four ALS severity signatures. These five compounds are: FDA-approved methyltransferase inhibitor azacitidine, NMDA receptor antagonist gavestinel in Phase III clinical trials, cell-cycle kinase inhibitor JNJ-7706621, GSK-3α/β inhibitor CHIR-99021, and the methyltransferase inhibitor UNC-0638 which are preclinical investigational drugs.

Next, we adopted a network-based approach to search for pairwise combinations of CMap drug compounds that may deliver therapeutic benefits towards ALS. Drug-pair combinatorial therapeutic potential was evaluated by applying the Syndrum (CITE Iida) framework and quantified using a predictive score (PS), which ranges from 0–4, where a score of 4 indicates that the drug combination has maximal predicted therapeutic synergy. In total, we evaluated the therapeutic potential of 1,413,721 drug-pair combinations (derived from 1,682 individual drugs), against each of the eight ALS signatures. From among the twelve strictly-reversing drugs identified from the first stage of this study, two drug combinations — Mocetinostat-VE-821 and Entinostat-VE-821 — attained a strong PS against three of the ALS severity signatures. (Table 1)

Table 1
Combinatorial therapeutic prediction score (PS) of Mocetinostat-VE-821, Entinostat-VE-821 and the highest PS attained, against eight ALS signatures. ALS-FRS-R: amyotrophic lateral sclerosis functional rating scale-revised, ALS-CBS: amyotrophic lateral sclerosis cognitive and behavioural screen

	PS with ALS signature (vs control)	Mocetinostat-VE-821	Entinostat-VE-821	Highest PS attained
ALS severity	ALSFRS-R high	3.09	3.09	3.15
	ALS-CBS high	3.15	3.14	3.19
	ALSFRS-R low	3.09	3.10	3.15
	ALS-CBS low	3.06	3.07	3.16
ALS progression	ALSFRS-R fast	3.00	3.02	3.17
	ALS-CBS fast	0.53	0.57	3.14
	ALSFRS-R slow	3.04	3.04	3.15
	ALS-CBS slow	2.83	2.81	3.26

Discussion

This study provides a stringent, network-based computational framework to prioritize single and combinatorial repurposing candidates for ALS. By coupling reversal screening with phenocopy exclusion, we improved the specificity of drug predictions and flagged potentially harmful compounds. The subsequent Syndrum[6] analysis identified HDAC–ATR-axis combinations (Mocetinostat–VE-821 and Entinostat–VE-821) with consistent synergy across heterogeneous ALS phenotypes. Mocetinostat / Entinostat are histone deacetylase (HDAC) inhibitors currently in clinical trials, with potential to modulate aberrant gene expression and protein homeostasis[7,8], while VE-821, a preclinical tool compound, is an ATR kinase inhibitor that may influence DNA damage response pathways.[9] The identification of an ATR kinase inhibitor as a synergistic partner to the HDAC inhibitor points to a therapeutically relevant axis. DNA damage and impaired genomic maintenance are central features of ALS pathophysiology, with elevated DNA damage biomarkers such as γH2AX observed in patient-derived neurons and postmortem tissue.[10–12] Mechanistically, this HDAC-ATR dual targeting paradigm resonates with the design logic of AMX0035, an FDA-approved ALS therapy that combines sodium phenylbutyrate (an HDAC inhibitor) with tauroursodeoxycholic acid (TUDCA), a bile acid known to modulate the unfolded protein response and cellular stress pathways, including components of the DNA damage response.[13,14]

We acknowledge several limitations: First, the predictions generated were derived through computational and network-based methodologies. Therapeutic efficacy and safety of the identified compounds remain to be validated through rigorous preclinical and clinical investigations. Second, the CMap database is largely composed of cancer-derived cell lines, and may not accurately reflect the drug perturbation

effects in neuronal or glial tissues. The development or inclusion of more neurologically-relevant perturbation datasets such as from primary tissue or from iPSC-derived motor neurons, astrocytes, and microglia would improve the specificity and translational potential of future drug repurposing efforts for ALS. Thirdly, CMap drug library remains biased towards oncology drugs, potentially excluding neuroprotective agents or metabolic modulators of interest for neurodegeneration.

Our next steps will involve validating top drug and combination effects in ALS iPSC-derived neurons, quantifying γH2AX, CHOP, and acetyl-histone biomarkers, integrating multi-omics readouts, and leveraging these data to design adaptive platform trials for ALS. By linking *in silico* prioritization with *in vitro* functional validation and *in vivo* biomarker development, this study lays the groundwork for a rational, mechanism-based combinatorial therapy pipeline in ALS that may accelerate the translation of repurposed drugs into clinically actionable interventions.

Methods

ALS Proteomic Signatures

Eight ALS differential protein expression profiles were obtained from the study by Huber et al.[15], generated from induced pluripotent stem cell (iPSC)-derived motor neurons obtained from ALS patients and healthy controls. Samples were stratified using the ALS Functional Rating Scale–Revised (ALSFRS-R) and the ALS Cognitive and Behavioral Screen (ALS-CBS). To examine disease severity, protein expression profiles were compared between ALS patients with high or low ALSFRS-R and ALS-CBS baseline scores, each independently contrasted against healthy control samples. To study disease progression, protein expression profiles of fast and slow progressors were generated by comparing ALS patient samples stratified by ALSFRS-R and ALS-CBS progression slopes against controls. This approach resulted in eight comparative proteomic datasets (Fig. 1A).

Drug Perturbation Signature Matching and Prioritization

Each ALS signature was queried against the Library of Integrated Network-based Cellular Signatures (LINCS) Connectivity Map (CMap)[16], restricted to skeletal myoblast, neuronal and neuroblastoma cell lines which represent the neuromuscular system (SKB, NEU, NPC, SKNSH, SH-SY5Y). (Fig. 1A, B) The Zhang score quantified similarity between ALS and drug perturbation profiles.[17] This metric ranges from − 1 to + 1, where a score of + 1 denotes that a drug's perturbation signature perfectly mimics (phenocopies) the query profile, and a score of − 1 indicates a complete reversal of the signature.

Strictly reversing compounds were defined as drugs that appeared among the top 50 reversal candidates for both ALSFRS-R and ALS-CBS signatures, and were absent from all phenocopy lists. The top 50 drugs with the most positive Zhang scores were classified as phenocopy candidates.

Drug Combination Prediction Using the Syndrum Pipeline

The pairwise combinatorial therapeutic potential between 1,682 drugs was evaluated using the Syndrum pipeline as described by Iida et al..[6] This framework integrates protein-protein interaction (PPI) networks with transcriptomic perturbation data to assess the combinatorial efficacy of drug pairs against a given disease signature. Briefly, Syndrum constructs a functional drug module for each compound using a curated PPI network and the LINCS database of drug-induced gene expression profiles. These modules represent the set of proteins perturbed by each drug, mapped onto the broader interactome. For each combinatorial pair, a therapeutic prediction score (PS) was calculated by summing three key components: 1) The network proximity between the ALS disease module and each drug module individually, 2) the transcriptional association between the ALS signature and each drug's perturbation profile (i.e. Zhang score) and, 3) the network relationship of the disease module with both drug modules. The resulting prediction score ranges from 0–4, with a score of 4 indicating that the drug combination has maximal predicted therapeutic synergy, and they are predicted to reverse the ALS phenotype across multiple mechanistic axes.

Abbreviations

ALS	Amyotrophic Lateral Sclerosis
ATR	Ataxia Telangiectasia and Rad3-related protein
CBS	Cognitive and Behavioural Screen
CHOP	CCAAT/Enhancer Binding Protein (C/EBP) Homologous Protein
CMap	Connectivity Map
FDA	Food and Drug Administration
FRS-R	Functional Rating Scale - Revised
HDAC	Histone Deacetylase
iPSC	Induced Pluripotent Stem Cell
LINCS	Library of Integrated Network-based Cellular Signatures
NPC	Neural Progenitor Cell
PPI	Protein-Protein Interaction
PS	Predictive Score

Declarations

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure of Conflicts of Interest

None of the authors has any conflict of interest to disclose.

Code Availability

The underlying code for this study is not publicly available but may be made available to qualified researchers on reasonable request from the corresponding author.

Author Contribution

C.Y.: Conceptualization. N.T. and C.Y.: Formal Analysis, Methodology, Visualization, Writing – Original Draft Preparation. N.T., J.N. and C.Y.: Writing – Review & Editing. J.N. and C.Y.: Supervision.

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Data Availability

This study only used publicly available datasets for analysis. The ALS proteomics signatures analysed in this study are available from Supplementary Information from Huber et al.. 15 The Connectivity Map dataset of drug perturbation signatures was accessed at https://clue.io/data. 16 The pre-built PPI and code used for the Syndrum analysis are available from Iida et al.. 6

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Figures

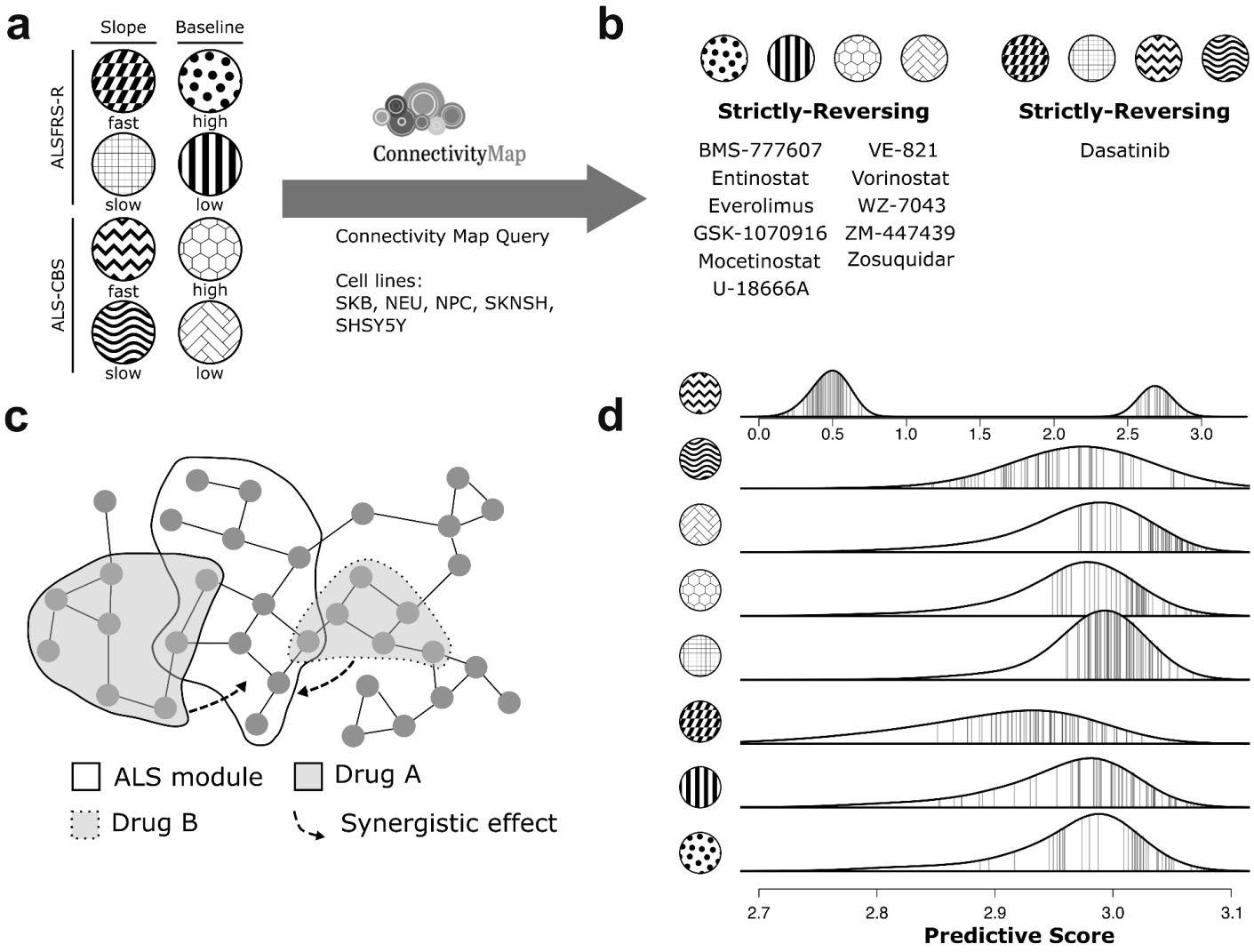


Figure 1

a) Eight differential protein expression signatures were derived from Huber et al.[15] across ALSFRS-R and ALS-CBS clinical scales. Slanted checkers: ALSFRS-R slope fast vs control, waffle pattern: ALSFRS-R slope slow vs control, polka dots: ALSFRS-R baseline high against control, vertical stripes: ALSFRS-R baseline low against control, zig-zag pattern: ALS-CBS slope fast vs control, wavy pattern: ALS-CBS slope slow vs control, hexagonal tiles: ALS-CBS baseline high against control, rectangular brick pattern: ALS-CBS baseline low against control. b) These signatures were queried against the Connectivity Map database of drug perturbation profiles (SKB, NEU-NPC, and SKNSH-SHSY5Y cell lines), identifying 11 and one strictly-reversing compounds across baseline and slope signatures, respectively. c) The network-based implementation[6] quantified a Predictive Score (PS) as a metric of drug combination synergy toward the ALS protein expression signatures. d) Distribution of PS for all possible drug combinations, separately for each ALS protein signature. Black vertical lines denote the PS for combinations of strictly reversing compounds identified in panel B. ALSFRS-R: ALS Functional Rating Scale-Revised, ALS-CBS: ALS Cognitive Behavioural Screen.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ALSDrugCombinationSupplementary.docx