Leveraging the power of molecular communities

Dr Diana Mitrea, Associate Director for Scientific Communications at Dewpoint Therapeutics, shares insight on developing therapeutics targeting biomolecular condensates to address neurological disorders.

Over six decades of target-based drug discovery, extensive effort has been focused on identifying target proteins whose structure and function is disrupted in disease and creating small molecules that repair the defect. This has proved successful in instances where the selected target is the main driver of the disease pathophysiology, and the target happens to present a binding pocket amenable for drug binding in a region of the protein that influences its function. For example, imatinib delivered the first disease-modifying therapy for chronic myelogenous leukaemia by targeting the active site of the disease-driving fusion oncoprotein BCR-ABL; direct acting antivirals can cure >95% of patients infected with Hepatis C virus by selective inhibition of viral proteins. However, the rate of success of this strategy decreases significantly with the increase in complexity of the disease-causing dysregulation, as is the case for many neurodegenerative diseases.

Biomolecular condensates

The biological processes of the cell are compartmentalised in membrane-bound and membrane-less organelles. The latter, also known as biomolecular condensates, are dynamic communities of biomolecules which assemble via phase separation – akin to the separation of oil from vinegar. They respond rapidly to environmental changes and cellular signals to compartmentalise and regulate a wide diversity of biological processes across all life forms.

Condensates integrate the functions of all community members within their microenvironment. Dysfunction in condensates is often responsible for complex causes of disease, that include both loss of function and toxic gain of function of one or more critical biomolecules. This makes them an attractive therapeutic target for diseases and targets that can't be addressed with conventionally developed drugs.

Developing strategies for targeting condensates requires a shift in perspective; condensate targets differ fundamentally from conventional single biomolecule targets because they are effectively a community of molecules, characterised by the structural features of each individual member, as well as collective properties of the community – termed emergent properties. These include local pH, viscosity, porosity, hydrophobicity, and surface tension (Figure 1). Importantly, the emergent properties can be leveraged for the development of a targeted, effective, and safe condensate-modulating drug (c-mods).

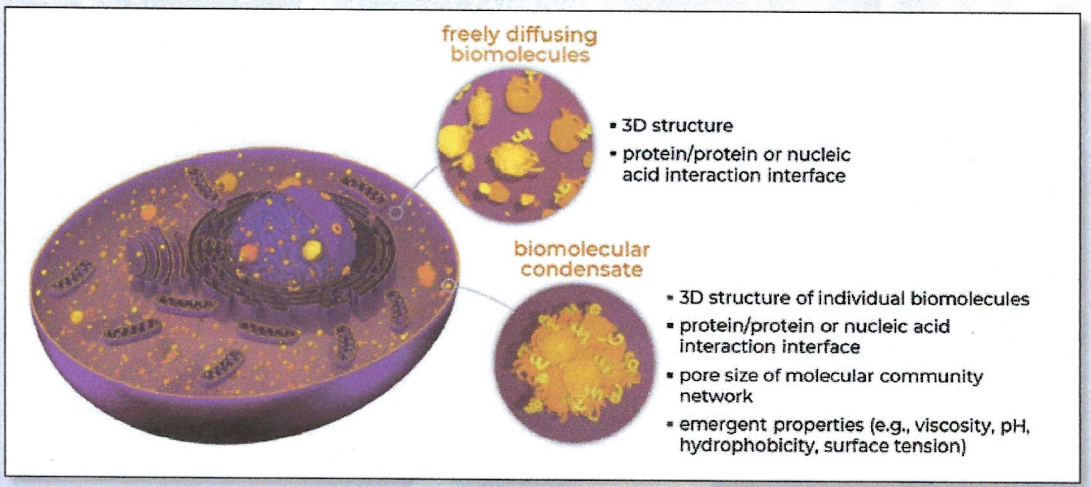

Figure 1 Features that can be leveraged to design and optimise drugs targeting individual biomolecules vs condensates.

Condensatoapthies

A condensatopathy is a condensate aberration that drives disease pathophysiology. An encyclopaedia of condensatopathies by Dewpoint Therapeutics shows their varied disease implications, including neurological disorders, cancer, drug resistance, heart and bone disease, and viral infections. The high unmet need in neurological disease therapeutics is fuelling increased interest and a steady stream of basic and clinical research efforts. These efforts demonstrate that at the root of many neurodevelopmental and rare diseases lies dysfunction integrated in condensates (Figure 2).

Take amyotrophic lateral sclerosis (ALS), an incurable neurodevelopmental disease: over 40 mutations in a variety of genes have been associated with ALS, each contributing to a small % of patients. Another fraction of patients does not present with any disease associated genetic mutations. Despite this highly variable genetic background, >97% of the patients share a common defect – aberrant cytoplasmic condensates that contain the splicing protein TDP-43 (Figure 3).

Effects of an ALS c-mod

Dewpoint is leveraging this shared condensate defect to develop c-mods for ALS. In its approach, Dewpoint treats the aberrant condensate, and therefore the entire community of biomolecules enclosed within – as the drug target. This serves as a central node of dysfunction in ALS and contributes to multiple downstream misregulated processes, such as TDP-43 loss of splicing function, and gain of toxic function attributed to other proteins that wrongfully join the aberrantly formed biomolecular community. Dewpoint is developing brain-penetrant small molecule c-mods that repair the TDP-43 condensatopathy. The c-mods demonstrate effects on disease pathophysiology metrics (Figure 4); they systemically repair the TDP-43 specific transcriptional and splicing programming, restore neuronal health markers in cultured patient-derived motor neurons, and correct clinically approved ALS-biomarkers in animal models. Importantly, when tested against the current standard of care and other anti-ALS drugs under development, only the c-mod corrected the full complexity of the TDP-43

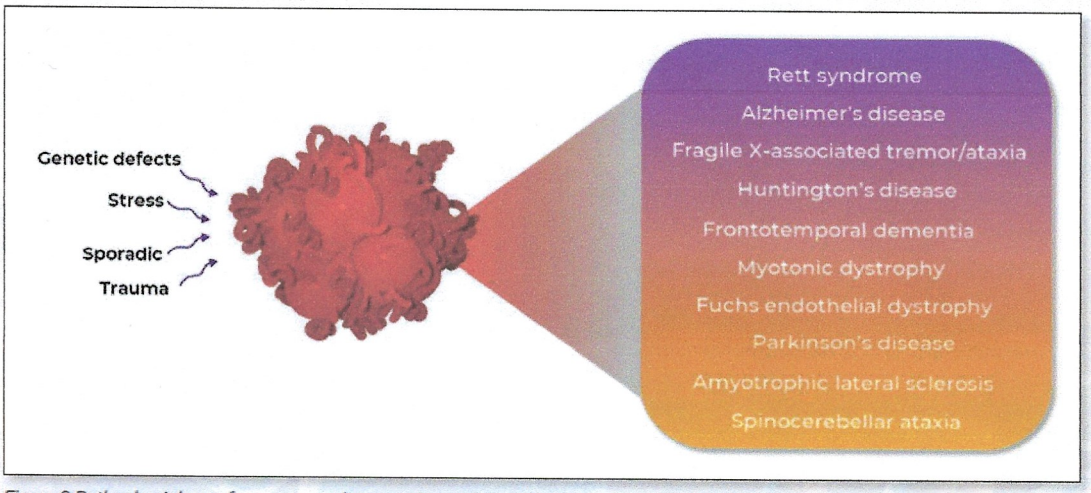

Figure 2 Pathophysiology of many neurodegenerative and rare diseases converges into aberrant condensates.

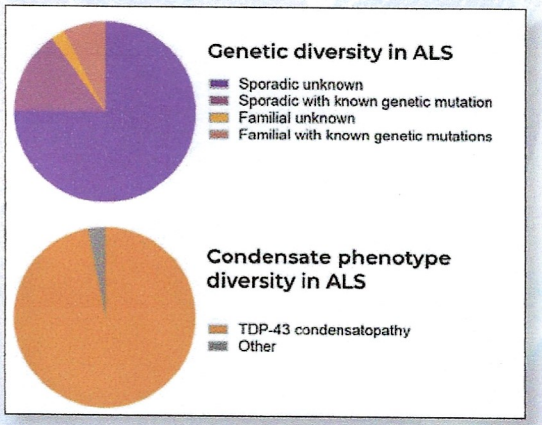

Figure 3 Despite high genetic diversity, >97% of ALS patients share an aberrant cytoplasmic TDP-43 condensate.

transcriptional profile. The c-mod's corrective effects are broad acting, across a panel of over a dozen patient-derived motor neuron cultures, of diverse genetic backgrounds. Collectively, these pre-clinical data show promising progress towards a true ALS disease-modifying treatment.

How is the drug being created?

Dewpoint uses an internal, AI-powered end-to-end platform to identify novel condensate targets, validate the condensate hypothesis, discover and optimise drugs (Figure 5). The platform is disease agnostic and is currently applied across Dewpoint's portfolio, comprised of wholly owned and partnered programmes, spanning neurological, oncology, cardiopulmonary and metabolic diseases.

Mounting evidence demonstrates that condensate structure and function is dependent on its composition and local environment, and can easily be altered by changes in protein levels, stress, cell cycle stage, signalling factors, etc. This also means that engineered systems, either *in cellulo* or *in vitro* are likely to push the system away from its disease-relevant state. After deliberating alternate approaches, Dewpoint chose to embrace the cellular complexity to prioritise biological relevance. As described in the ALS c-mod example, this approach paid off, as it led to translatable success across complex and clinically relevant model systems.

Dewpoint's workhorse discovery assay is high content imaging high throughput screening (HTS), which searches for phenotypic changes relative to the target condensate. This approach combines the advantages of phenotypic assays which cast a wide net, often without prior knowledge or bias on

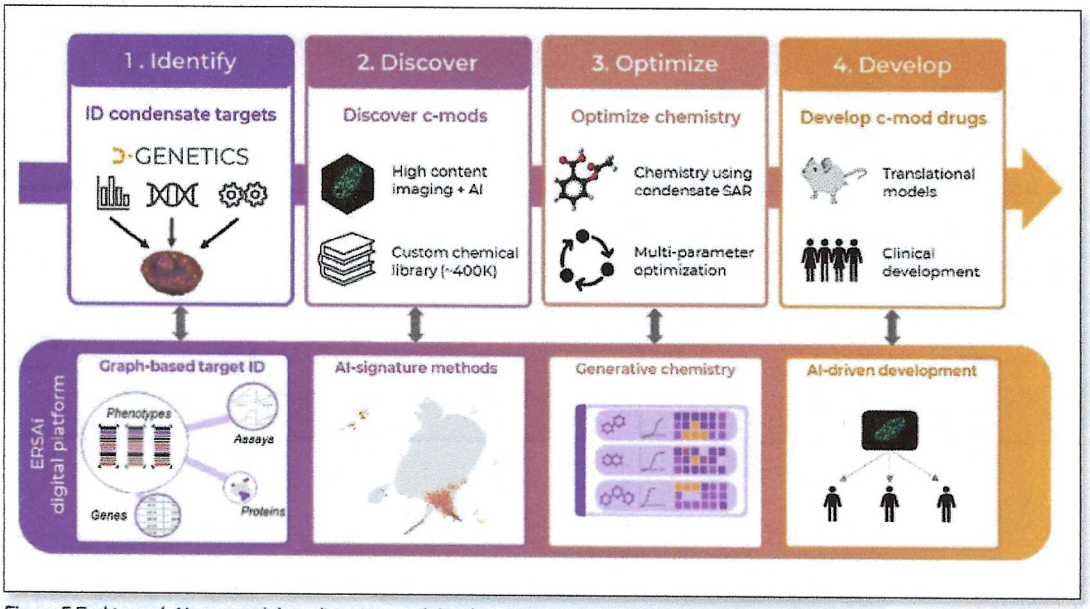

Figure 5 End-to-end, AI-powered drug discovery and development pipeline at Dewpoint Therapeutics.

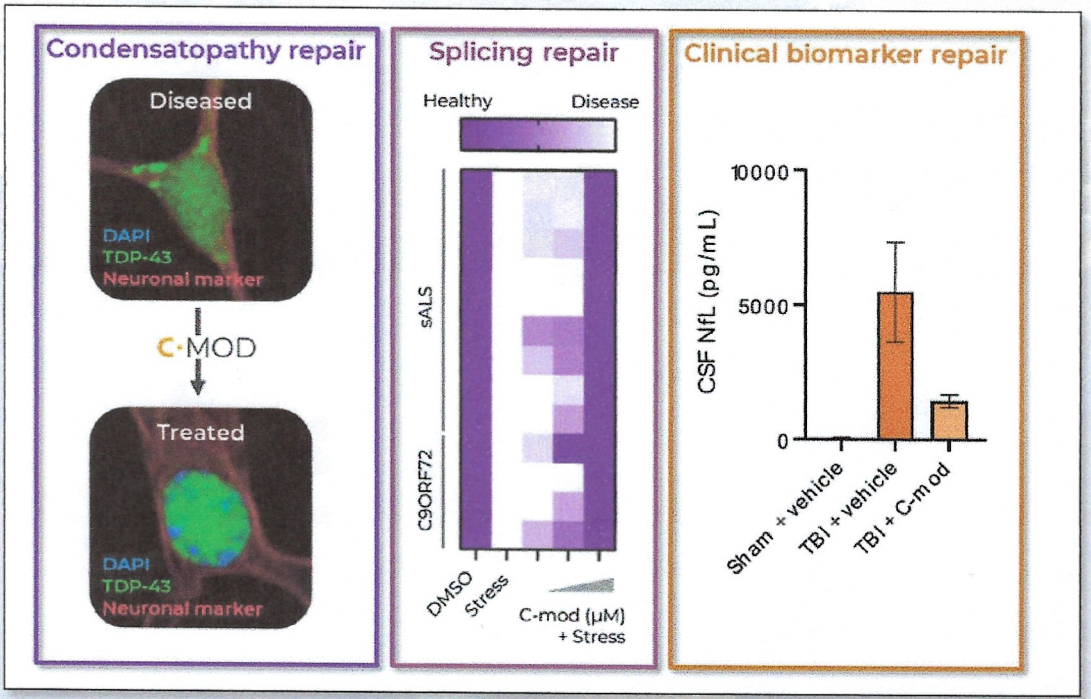

Figure 4 TDP-43 condensatopathy repair in cultured motor neurons (left) with a small molecule c-mod translates into broad-acting repair of TDP-43 splicing function in patient-derived motor neurons (middle) and repair of a clinically accepted biomarker in an ALS mouse model (right).

the pathways that need to be repaired to treat the disease, with those of targeted drug discovery by focusing on the integrating hub of the pathway(s) dysregulation responsible for the disease. The phenotypic perturbations within the condensate are then validated in downstream, functional assays in disease-relevant model systems to confirm the expected systemic and on-pathway effect of the hits.

The direct target can be one of the hundreds of members of the condensate community, or a regulatory factor that acts upstream of the condensate. With efforts to deconvolute the direct target which allows for conventional structure-based drug optimisation, Dewpoint advances its structure-activity relationship efforts based on a combination of condensate and functional assays, to ensure that the disease relevance and efficacy is maintained in the complex cellular setting.

What do we know of c-mods now?

Following Dewpoint's inception in 2018, and the subsequent founding of other biotech companies with the shared goal of developing medicine based on targeting condensates, such as Neriad Therapeutics, Transitions Bio, and ETERN Therapeutics, the industry started hunting for drugs by intentionally targeting condensates.

Recent studies are finding that FDA-approved drugs, developed through conventional methods, function – at least in part, through condensate mechanisms, and/or modulate condensates as part of their off-target effects. Dewpoint's c-mod encyclopaedia recorded 237 compounds experimentally demonstrated to modulate condensates; 63 of which are FDA approved. These c-mods come in multiple modalities – from small molecules to peptides, oligonucleotides, proteins and more. Systematic analysis of this evolving public dataset, further enriched with proprietary data from HTS campaigns and optimisation cycles generated by the biotech industry, will help define unwritten rules that pertain to determining selectivity and specificity towards a community of biomolecules. Together, these novel insights will drive refinement in strategy towards an accelerated path to successful optimisation of c-mods with superior clinical safety and efficacy.

Clinical trial explanation and limitations

To date, there are no drugs discovered or developed through a condensate-centric approach in the clinic. Dewpoint is on track to be the first that brings a drug discovered through a condensate lens into the clinic, scheduled for the H2 of 2025. As part of this journey, several safety and efficacy considerations will have to be revisited and potentially revised, primarily due to the complex nature of the target. For example, while safety studies for c-mods can be designed following well established, conventional approaches, defining selectivity and specificity towards a condensate (ie. a community of molecules) is uncharted territory. The current model that Dewpoint adopted is to define selectivity and specificity relative to the pathways integrated within the condensate as opposed to a single biomolecule. This model will continue to evolve with new data.

Implications for the future of treatment

Refocusing the notion of target from a single biomolecule to a community of biomolecules that function synergistically to perform a specific biological process provides previously unattainable access to effectively modulating the function of molecular targets previously considered 'undruggable'. These targets have earned their 'undruggable' designation due to lack of well-defined pockets where drugs can bind to effectively modulate their function. With c-mods, one gains the ability to modulate this function by altering the structure and environment of the compartment where the 'undruggable' target resides.

C-mods, by virtue of their mode of action of modulating a central node of dysfunction in disease, promise to exhibit disease-modifying effects and activity across broader, more diverse patient populations. Albeit early in the game, Dewpoint's results demonstrate encouraging translatability of outcomes between cell lines, patient-derived cells (eg. iPSCs) and animal models.

There is more work to be done, but the potential implications are substantial. For example, understanding the rules that drive a drug to preferentially accumulate in a specific condensate can be a game changer for optimsing target engagement and improving drug safety and efficacy.

Furthermore, understanding the condensate mechanisms by which the organism responds to stress has the potential to revolutionise how we prevent or combat acquired drug resistance to existing therapies.

Dr Diana Mitrea is the Associate Director for Scientific Communications at Dewpoint Therapeutics. She has a background in biochemistry and protein engineering, and over 14 years' experience in condensate biology and biophysics & structural biology of folded and intrinsically disordered proteins.