Microfluidics-based screening platform identifies a novel therapeutic approach to targeting EML4-ALK driven cancers

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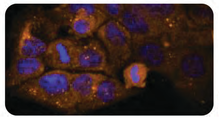
Abstract 1657

Introduction

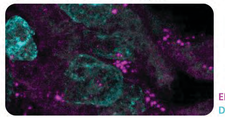
Opportunity
Address the **30-50% of patients** on ALKi who develop **acquired resistance** due to mutation(s) in kinase domain

Mechanism
EML4-ALK fusion protein forms **reaction hub condensate that drives constitutive oncogenic signalling** (*i.e.*, RAS-MAPK, PI3K & others) [1,2,3]

H2228 EML4-ALK⁺ NSCLC cell line



EML4-ALK⁺ NSCLC PDX tumor sample



PhaseScan: Microfluidics-based screening for modulators of phase separation

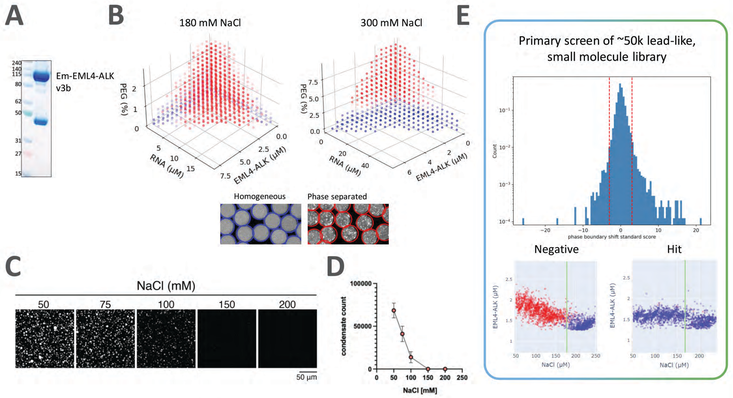
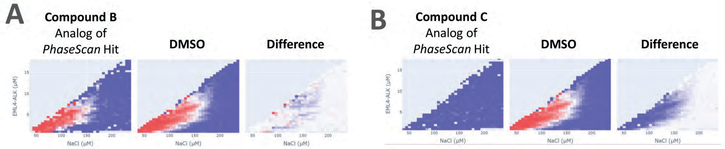
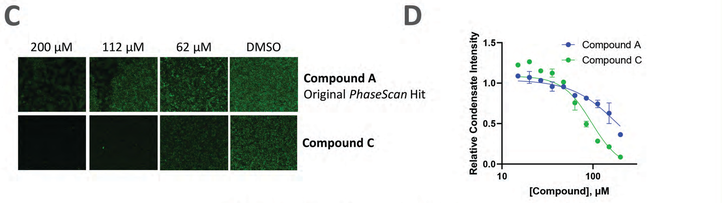


Figure 1. A. Phase diagrams demonstrating the effect of salt concentration on phase separation of EML4-ALK *in vitro*. Red, phase separated; Blue, homogenous. **B.** PhaseScan microfluidics droplet images of EML4-ALK condensates. **C.** Images of EML4-ALK condensates induced with decreasing concentration of salt. **D.** Quantification of data from (C). **E.** Summary of high-throughput *PhaseScan* screen of small-molecule library for modulators of EML4-ALK condensates, driven by low-salt. Example phase diagrams of compounds that have no effect (Negative) or compounds that prevent EML4-ALK condensate formation (Hit) are shown below.

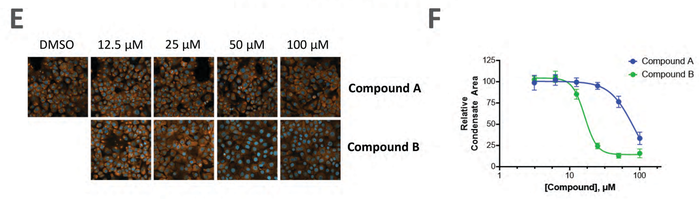
Pharmacologic dissolution of EML4-ALK condensates inhibits EML4-ALK signaling

Molecular assessment of Condensates





Cellular Condensate Assays



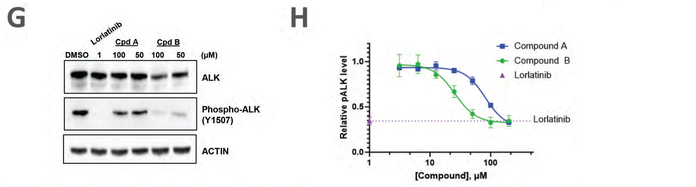


Figure 2. A. *PhaseScan* phase diagrams demonstrating the prevention of phase separation by Compound B and Compound C. Compounds B-C are analogs of a a hit from the primary *PhaseScan* screen with increased potency as compared to the original hit. Red, phase separated; Blue, homogenous. **B.** Images showing dose-dependent effects of Compound A and Compound C on EML4-ALK *in vitro* phase separation **C.** Quantification of data from (B). **D.** Quantification of data from (C). **E.** Images showing dose-dependent effects of Compound A and Compound B on EML4-ALK cellular condensates. SNU2535, crizotinib-resistant, NSCLC cells were treated for 6 hours with inhibitors and condensates were measured by immunofluorescence (IF). **F.** Quantification of data from (E). **G.** Western blot showing suppression of oncogenic ALK autophosphorylation after a 6 hour treatment of SNU2535 cells with Compounds A or B. **H.** Phospho-ALK levels were assessed by ELISA after a 6 hour incubation with compounds.

Transition Bio EML4-ALK inhibitor functions through a novel MoA

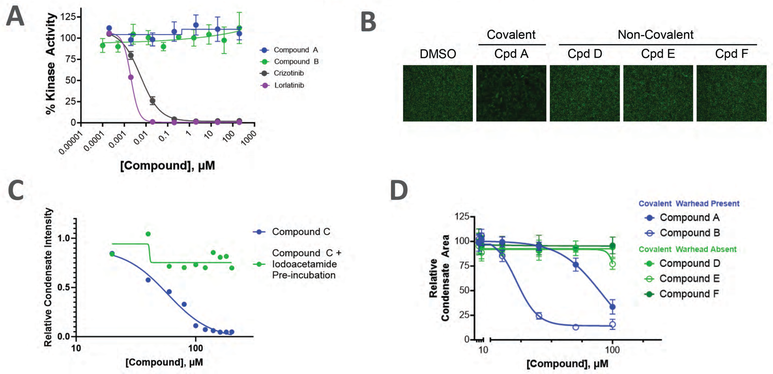


Figure 3. A. Compounds A and B do not directly inhibit the kinase activity of EML4-ALK. **B.** Images showing effects of compounds on EML4-ALK *in vitro* phase separation. Compound A, containing a covalent warhead, impacts phase separation, whereas there is no impact on phase separation by Compounds D-F, non-covalent compounds (from the same series with the covalent warhead removed) **C.** Pre-incubation of EML4-ALK with iodoacetamide prevents the effects of Compound C on EML4-ALK phase separation *in vitro*. **D.** Quantification of the effects of covalent and non-covalent compounds on EML4-ALK cellular condensates. Compounds A and B (covalent warhead present) disrupt EML4-ALK condensates in cells, whereas Compounds D-F (covalent warhead removed) have no impact on EML4-ALK cellular condensates.

Summary

- EML4-ALK fusion protein drives oncogenic signaling through biomolecular condensates
- *PhaseScan* technology provides a novel approach to screening for modulators of liquid-liquid phase separation, thus enabling identification of unique EML4-ALK inhibitors with differentiated MoA
- Transition Bio's EML4-ALK compounds disrupt biomolecular condensates *in vitro* and in cells, and suppress downstream ALK signaling
- Transition Bio's EML4-ALK inhibitors work *via* a novel covalent mechanism, unique from approved ALK kinase inhibitors, and provide a potential new path to treating TKI-resistant tumors

References

[1]Tulpule, A., *et al.* Kinase-mediated RAS signaling via membraneless cytoplasmic protein granules. *Cell.* 2021. 184, 2649-2664.
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[3]Sampson, J., *et al.* Phase-separated foci of EML4-ALK facilitate signalling and depend upon an active kinase conformation. *EMBO rep.* 2021. 22, e53693.
[4]Arter, W.E., *et al.* Biomolecular condensate phase diagrams with a combinatorial microdroplet platform. *Nature Communications*. 2022. 13, 7845.