**OVERCOMING KINASE INHIBITOR RESISTANCE: FROM PROTEIN STRUCTURE TO CONTROL OF CELL FATES**

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Major problem encountered using small molecule cancer therapeutics in clinic is that even in susceptible cancers, these drugs rarely give durable responses, almost inevitably being hampered by signaling reactivation and development of resistance. Studying the causes of this resistance has revealed severe limitations in our understanding of the network properties and molecular mechanisms that control drug responses. We show that contrary to a common opinion, feedback loops by themselves cannot restore or overshoot steady state signaling1.

De novo synthesized negative feedback regulators can lead to a transient overshoot but still cannot fully restore output signaling. These findings can rationalize recent scientific and clinical disappointments that were based on the hypothesis that negative feedback loops can fully explain drug resistance. We demonstrate that there are two major means of complete, steady state revival of signaling, enabled by (1) the network topology or (2) molecular mechanisms rendering the primary drug target active again. Network topology analysis shows that at least two, activating and inhibitory, connection routes from a primary drug target to the output, must exist for complete reactivation or overshoot of steady-state output activity that existed before the inhibition1.

Irrespective of the network topology, drug-induced overexpression of the primary drug target or drug-induced increase in its dimerization or oligomerization can restore the pathway output activity. The formation of kinase homo- or heterodimers is a major course of resistance. In this constellation one protomer is drug-bound and allosterically activates the other, drug-free protomer thereby conferring resistance. The emergence of different drug affinities between protomers in a dimer has been enigmatic, but can be explained by thermodynamics2.

A striking example is so-called paradoxical activation of the extracellular regulated kinase (ERK) pathway by RAF inhibitors, which is caused by RAF homo- or heterodimerization. Exciting and counterintuitive discoveries of ways to overcome resistance were made using next generation computational modelling, which combines aspects of protein structure, posttranslational modifications, thermodynamics, network architecture, mutation data and dynamic reaction3.

As a specific example, we show that a treatment with Type I1⁄2 and Type II RAF inhibitors can counterbalance ERK pathway reactivation and concomitant drug resistance. Based on these computational results, Phase II Trial of Vemurafenib and Sorafenib in Pancreatic Cancer has started in the US (<https://clinicaltrials.gov/ct2/show/NCT05068752>). Understanding cell state transitions and purposefully controlling them is a longstanding challenge in biology.

Here, we present cell State Transition Assessment and Regulation (cSTAR), an approach to map cell states, model transitions between them, and predict targeted interventions that can convert cell fate decisions. cSTAR uses omics data as input, classifies cell states, and develops a workflow that transforms the input data into mechanistic models that identify a core signaling network that controls cell fate transitions by influencing global signaling networks. By integrating signaling events and phenotypic data cSTAR models how cells move in Waddington’s landscape and make decisions about which cell fate to adopt. Importantly, cSTAR can devise interventions to quantitatively and precisely control the movement of cells in Waddington’s landscape. Testing cSTAR in a cellular model of differentiation and proliferation shows a high correlation between model predictions and experimental data. Applying cSTAR to different types of perturbation and omics datasets demonstrates its flexibility and scalability and provides new insights. The ability of cSTAR to identify targeted perturbations that

interconvert cell fates will allow designer approaches for manipulating cellular development pathways and mechanistically underpinned therapeutic interventions4.

**References:**

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