CETSA®: a target engagement assay to improve drug discovery efficiency

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A drug must engage its intended target to achieve the therapeutic effect. Proof of drug-target engagement in physiologically-relevant contexts is a key pillar of successful therapeutic target validation. Several key analyses of the causes of clinical trial failures were recently published. In as many as half of the programs that failed due to ‘lack of efficacy’, a lack of demonstration of the intended target was identified. To better understand why some drugs, despite being potent inhibitors in biochemical assays, lose *in vitro* or *in vivo* activity, it is necessary to determine to what extent drugs engage their primary targets.

However, conclusively measuring target engagement in situ is challenging. This point is critical for topical drugs due to barrier function of the skin: “The reason for being” of the Epidermis. There are few technologies that enable the accurate measurement of drug-target occupancy in physiological contexts. In the present project we aimed to meet this challenge by developing a label-free, biophysical assay: the cellular thermal shift assay (CETSA®), which facilitates the direct assessment of target engagement in cells and tissues at various stages of drug development.

The CETSA® method builds on the concept of thermal stabilization of target proteins upon ligand binding in cells and tissue samples. By quantifying the melting temperature and shift induced by the ligand we can quantify the potency of target engagement. This potency determination then allows filtering of drug candidates by their ability to engage the target in its physiologically relevant form. For detecting thermodynamic stability using CETSA®, drug- or vehicle-treated cell lysates/intact cells were heated to different temperatures and the target proteins were detected by western blotting.

Our data demonstrates that CETSA® can assess drug target engagement in intact cells. These initial successes prompt us to develop alternative strategy to replace the low-throughput, manually intensive Western blot readout, with a quantitative, higher throughput assay. This would provide sufficient capacity to utilize CETSA® as a primary hit qualification strategy. We are also confident that this assay will prove to be a valuable tool to allow the direct confirmation of cellular target engagement in an ex-vivo human skin model, supporting clinical application.

CETSA® is likely to be a relevant tool applicable in many stages of drug development from High-throughput screening assays to clinical trials.