Targeted Protein Degradation Profiling by the Cellular Thermal Shift Assay (CETSA®)

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Targeted protein degradation is an emerging therapeutic modality and there is a growing appreciation for PROTACs as chemical probes for target identification and validation. Pelago Bioscience´s patented CETSA® technology is a well-established label-free technology that can be used to assess protein-ligand interactions and subsequent effects in the native cellular environment. In this study we show how CETSA® in combination with other technologies can be applied to profile protein degraders, linking target binding to the desired degradation effect along with addressing selectivity.

We have confirmed binding of three different degraders; BSJ-04-132 (targeting CDK4 and CDK6), BSJ-03-204 (targeting CDK4/6), and ARV-825 (targeting BRD4) to both the respective protein of interest (POI) and the E3 ligase Cereblon (CRBN). This was accomplished using the targeted CETSA® Navigate HT platform based on AlphaLISA® detection, enabling robust and sensitive confirmation of target engagement (TE). The PROTAC-mediated POI degradation was monitored in parallell using the same Alpha*LISA®* SureFire® Ultra™ assay. This allowed quantification of concentration dependent degradation.

Using the unbiased proteome-wide format CETSA® Explore, we showed binding of BSJ-04-132 to CDK4 and CRBN as well as potential off-targets in cell lysate. The correct MoA for the degrader could be confirmed in intact cells, with a stabilization of CRBN whereas CDK4 and CDK6 was not detected. In addition, the primary substrate for the degraded proteins was destabilized.