APPLICATION NOTE

2D OncoSignature™ Long-Term Assay: Extending the reach of cell panel screens.

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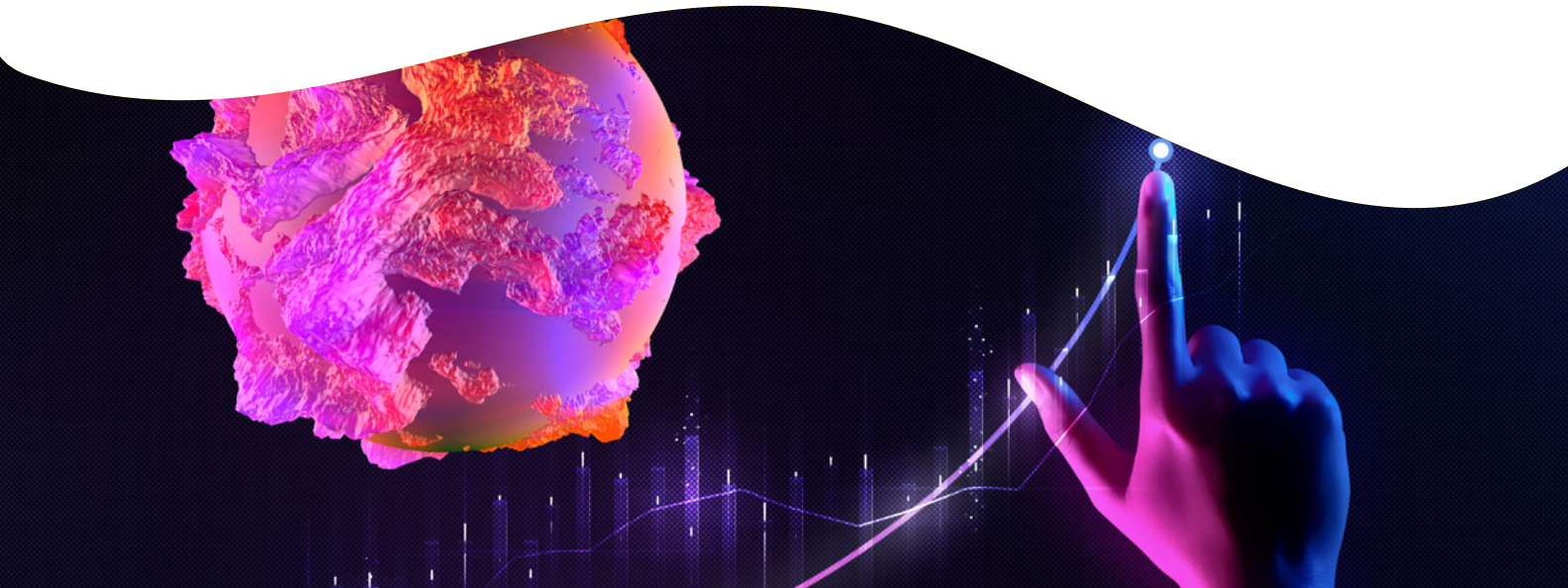
Introduction

Cell panel screens are an essential part of the drug discovery toolbox for identifying sensitive tumour types and the molecular biomarkers underpinning response. Epigenetics has a significant yet mostly mysterious role in the development of cancer, but recent findings are starting to unlock significant opportunities for identifying new therapeutic targets and treatment strategies.[1,2] However, fully resolving the response profiles of slower-acting therapeutics, such as those targeting epigenetic pathways remains challenging with conventional short term 384-well assay formats with 3–6-day treatment windows (Figure 1). Traditionally, treatments requiring longer than six days have therefore been evaluated in colony-forming assays that have limited throughput precluding their routine use for large cell panel screens. To address this bottleneck there is a real need for longer-term assays that can be run in formats more amenable to automation-based workflows. Here we describe the development of our 2D OncoSignature™ Long-Term Assay (LTA) drug discovery service which enables the profiling of slower acting or epigenetic drugs in 384-well plates for 10 days across a diverse, clinically relevant panel of 248 cell lines.



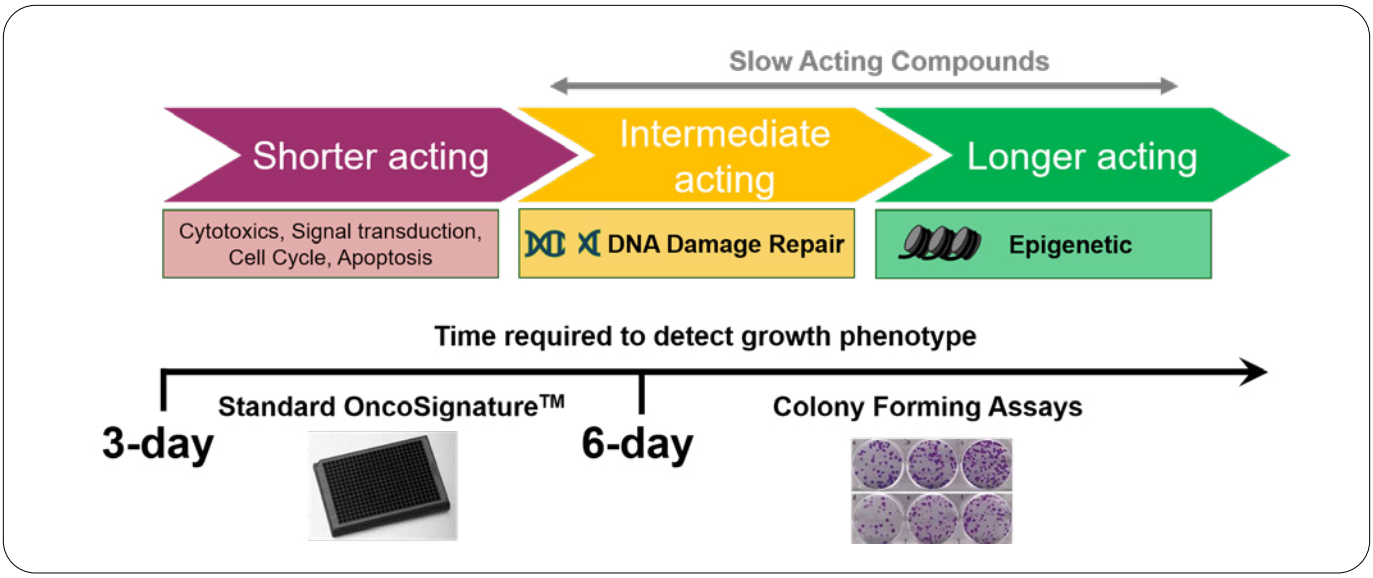


Figure 1: Classifications of therapeutics based on treatment durations. Therapeutics can be classified as 'shorter', 'intermediate' or 'longer' acting based on treatment duration required to see growth effects in a standard 3-6-day OncoSignature assay. Although both intermediate and longer-acting classes are often considered as 'slower-acting', only intermediate-acting therapeutics, such as some DDR inhibitors, can be observed in a standard OncoSignature assay using the longest timepoint (6d), with longer-acting therapeutics such as epigenetic inhibitors requiring longer assay durations and have traditionally been evaluated in low throughput colony-forming assays.

Methods

For the 10-day assay cells were analysed using growth conditions specifically optimised for this extended duration. For the 6-day assays cells were grown using our Standard 2D OncoSignature assay conditions. For both assays cells were seeded in vendor recommended medium in black Culturplate-384 plates (Revvity), equilibrated by gentle centrifugation (1200 rpm for 2 min) and placed in incubators (37°C, 5% CO2). Compounds were added after 24 h using a nine-point titration plus untreated control for the single agent assays or a 9x9 dose matrix for the combinations. After a further 6 (standard) or 10 days (Long-Term) incubation, cell viability was assessed using a standard luminescent viability assay that measures ATP levels. A baseline T0 measurement (at the time of drug addition) was taken to enable calculation of growth inhibition. Single agent responses were analysed by calculating the area under the dose-response curve (response area) and the maximum response, and combinations were analysed using synergy score which was calculated using the Loewe additivity model using Revvity's proprietary Chalice Analyzer software.[3]

Results

Optimisation of growth Conditions

We optimised the conditions required to support cell growth in 384-well plates across an extended 10d cell culture timeframe for all 248 adherent cell lines in our OncoSignature™ cell line panel. Optimised conditions had to meet several acceptance criteria: 1) sufficient 10d assay signal in control untreated wells to indicate optimal cell growth; 2) no steep decline in 10d assay signal relative to 6d, which would be indicative of a reduction in viability with the extended duration; 3) minimize risk of over-confluence; 4) yield acceptable drug response data quality (Figure 2). Assay robustness was assessed at 10-days for each cell line using the Z'-factor[4] calculated from replicate positive and negative plate controls (Figure 3). Within the context of a phenotypic assay for the evaluation of dose-responses the majority of cell lines exhibited good (Z'>0.4) or acceptable (0.4 > Z' > 0.2) values (Figure 3).

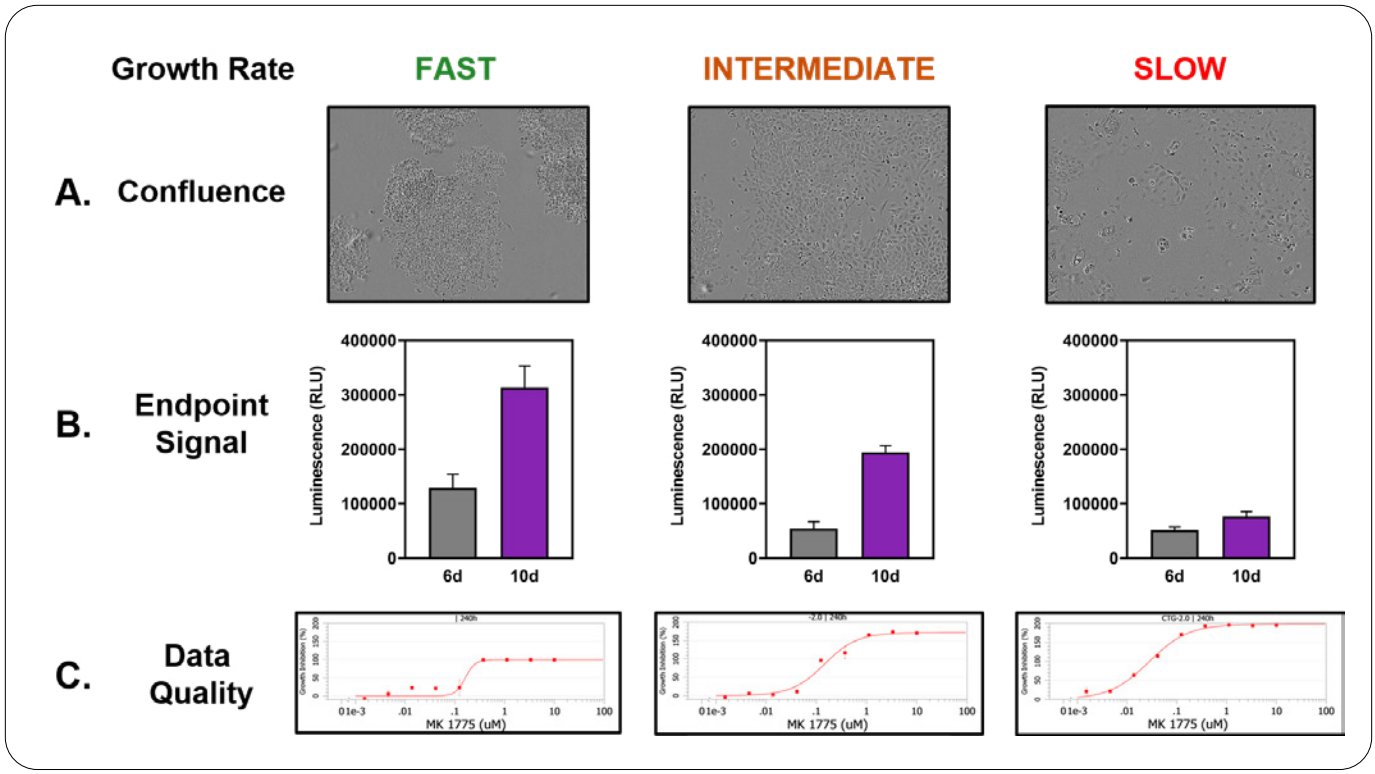


Figure 2: Optimisation of Long-term assay growth conditions. Three example cell lines representing fast, intermediate, and slow growth rates are shown. Conditions were optimised for all cell lines to meet several acceptance criteria: (A) minimize risk of over-confluence; (B) sufficient 10d viability assay endpoint signal in control untreated wells to indicate optimal cell growth; no steep decline in 10d assay signal relative to 6d, which would be indicative of a reduction in viability with the extended duration; (C) yield acceptable drug response data quality.

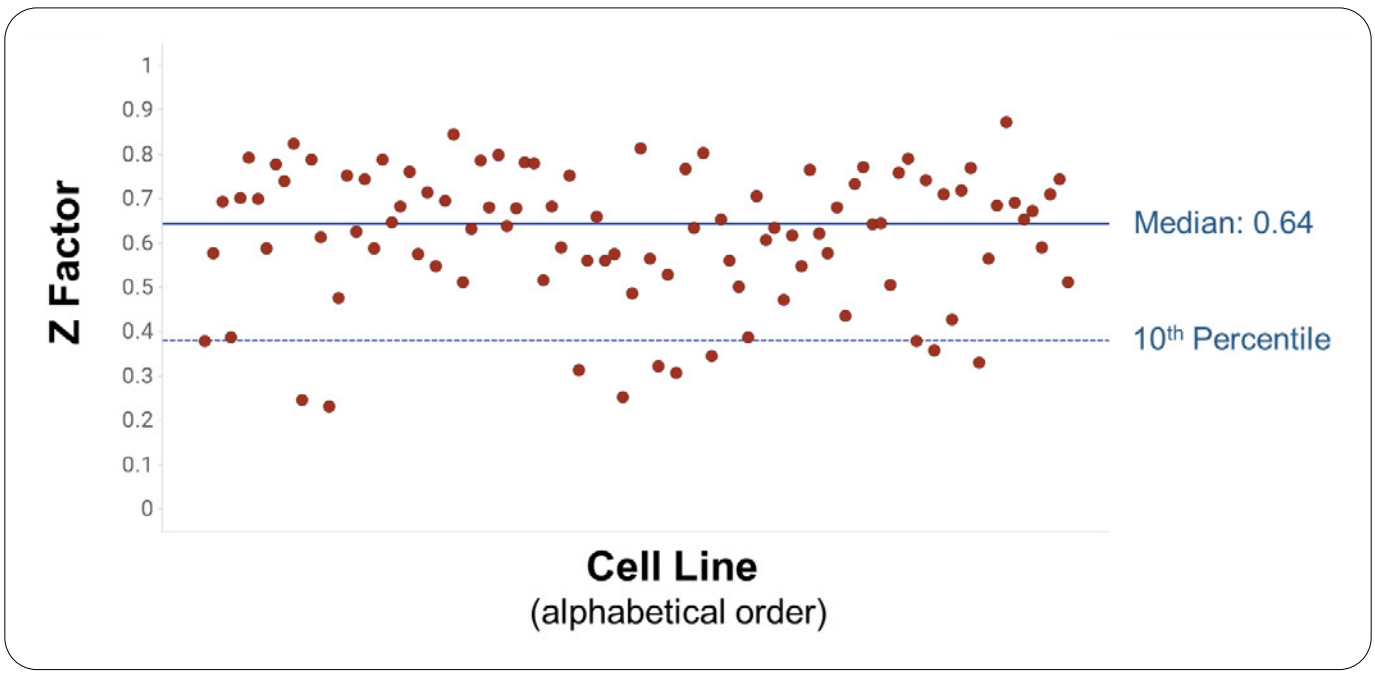


Figure 3: Long-Term Assay robustness. Z′ was calculated at the 10-day endpoint for each cell line using positive and negative controls. In the context of a phenotypic assay for the evaluation of dose-responses we consider a value of Z′>0.4 as good and 0.4 > Z′ > 0.2 as acceptable. Example data for 100 cell lines is shown.

Slower-acting therapeutics show greater responses in the OncoSignature™ Long-Term Assay

Therapeutics can be classified as 'shorter', 'intermediate' or 'longer' acting according to length of time required to see responses to single agent treatment in a standard OncoSignature assays (Figure 1). Although both intermediate and longer-acting classes are usually considered as 'slower-acting', only the effects of intermediate-acting therapeutics, such as DDR inhibitors, can be observed in a standard OncoSignature assay using the longest timepoint (6d), with this treatment duration typically being too short to sufficiently reveal responses of longer-acting therapeutics such as epigenetic inhibitors. To evaluate the utility of the LTA for profiling slower-acting therapeutics as single agents we compared responses to the DDR inhibitor Olaparib (PARPi) and the epigenetic inhibitor Tazemetostat (EZH2i) between the 10-day LTA and standard 6-day assays across the 248-cell line panel (Figure 4). As expected, in the shorter 6-day assay Olaparib responses could be more readily observed compared to the Tazemetostat. However, both inhibitors showed greater responses in the 10-day LTA compared to the standard 6-day assay. In contrast, responses to a control faster acting therapeutic (WEE1i) were generally similar in both assays.

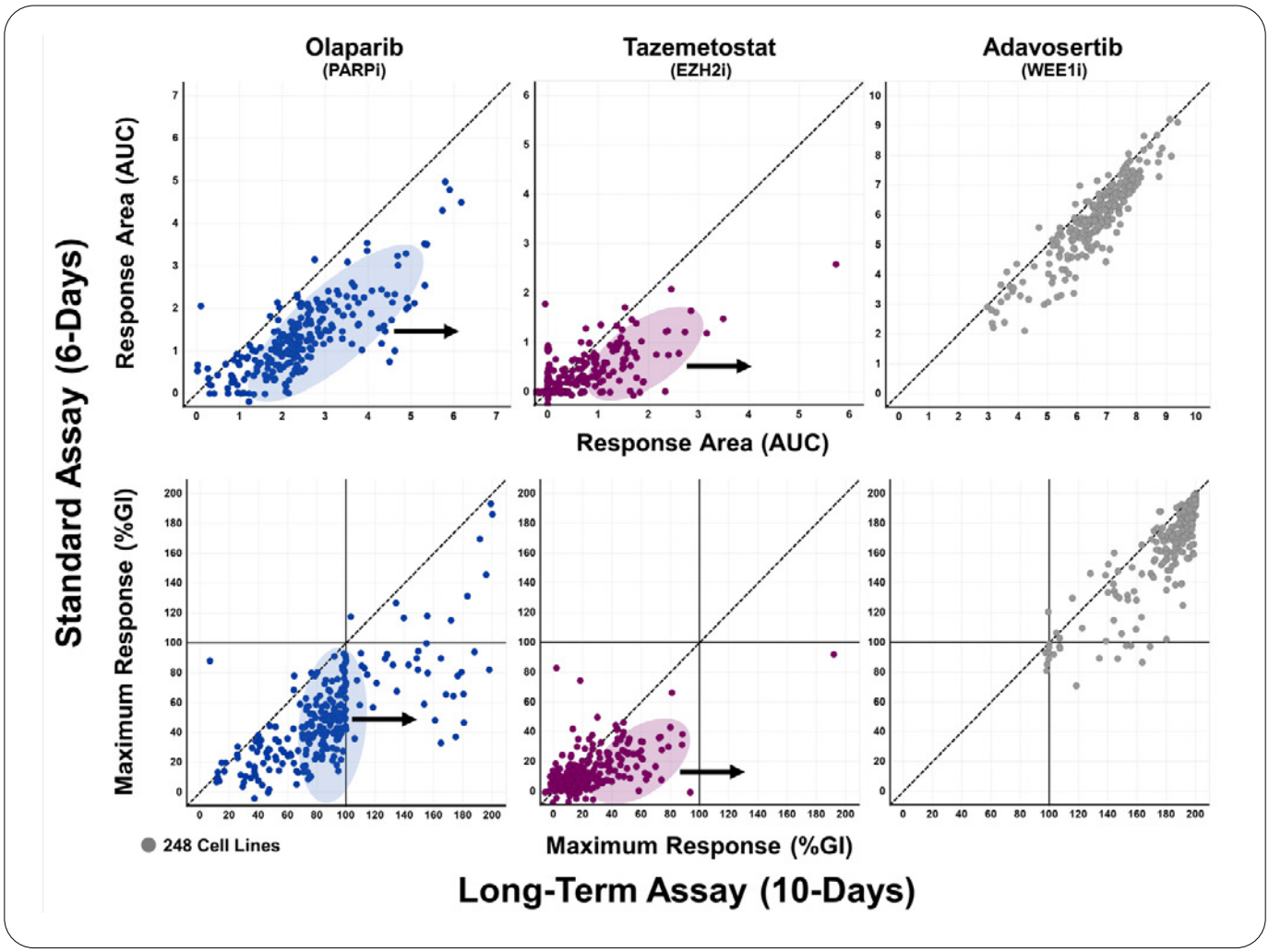


Figure 4: Slower-acting therapeutics show greater responses in the OncoSignature™ Long-Term Assay. Slower acting inhibitor classes (PARP and EZH2 inhibitors) showed greater responses in the 10-day LTA (shift to right along X axis, as indicated by arrows) compared to the standard 6-day assay in a panel of 248 cell lines. In contrast, responses to a faster acting therapeutic (WEE1i) were generally similar (points distributed along X=Y line indicating equal response at 6-days and 10-days).

OncoSignature™ Long-Term Assay for the analysis of combinations

To demonstrate the use of the LTA for the analysis of combinations we evaluated the responses of a small panel of 7 cell lines to the combination of Olaparib with the ATR inhibitor AZD6738, a known synergistic pairing undergoing evaluation in clinical trials.[5] This combination exhibited widespread synergy across the cell line panel (Figure 5). While the overall pattern of synergy was similar in both the assays, the magnitude of synergy was greater in the 10-day LTA.

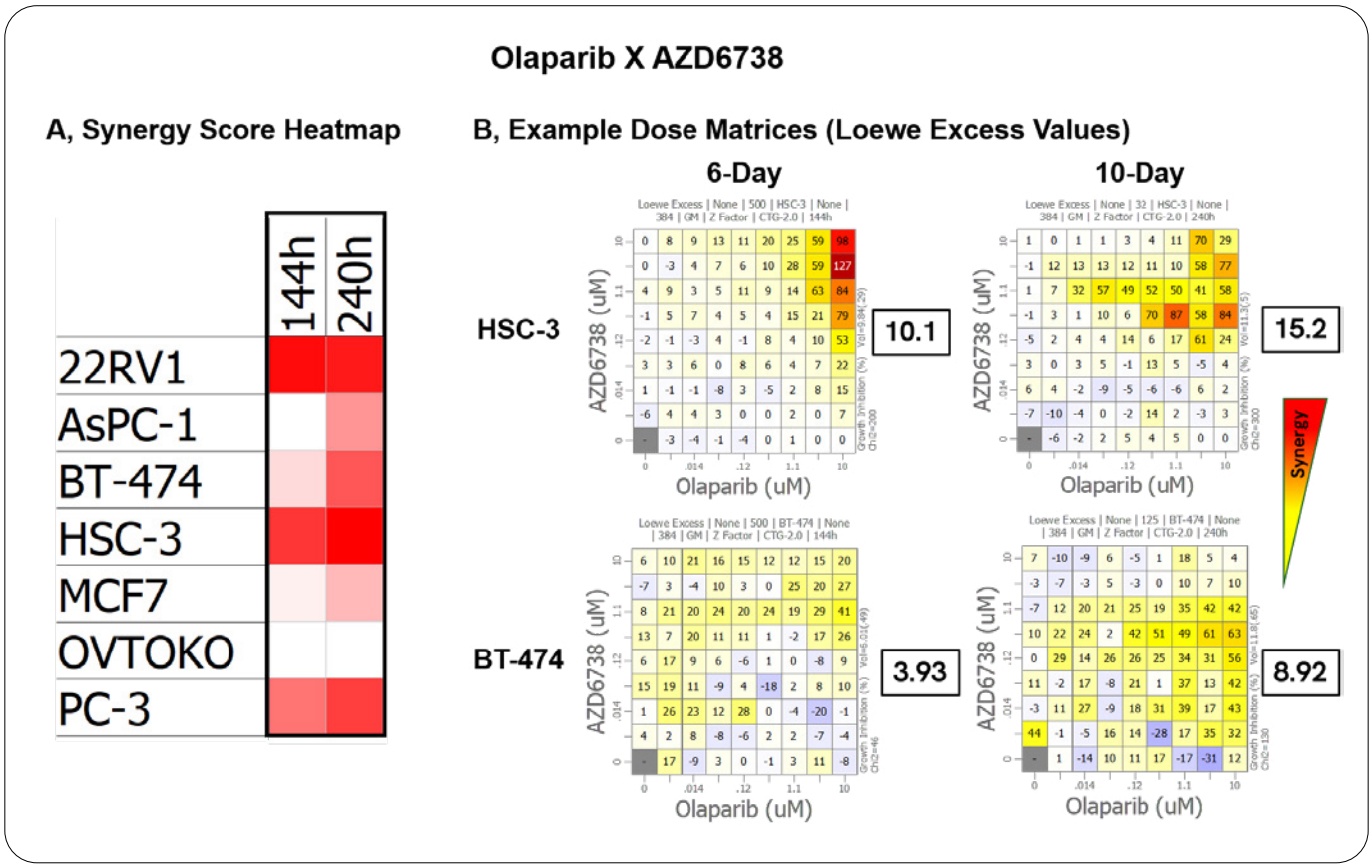


Figure 5: OncoSignature™ Long-Term Assay for the analysis of combinations. A 9x9 dose matrix of Olaparib (PARPi) combined with AZD6738 (ATRi) was evaluated across a panel of 7 cell lines in the 10d (240h) LTA compared to the standard 6-day (144h) assay. A) Heat-map representation of synergy scores calculated from each dose matrix using the Loewe additivity model. Darker red indicates greater synergy. B) Example dose matrices with the Loewe excess values shown. Loewe excess values are determined by subtracting the level of inhibition predicted as additive by the Loewe model from the actual observed inhibition. Therefore, positive excess values indicate synergy and negative values indicate antagonism. The synergy score for each matrix is shown on the right of each example. A minimum synergy score of 2.8 was considered the threshold for the combination to be considered synergistic and values over 10 are considered strong synergies.

Conclusion

The 2D OncoSignature™ Long-term Assay drug discovery screening service enables the profiling of compounds or biologics of interest over a prolonged assay duration of 10 days across the 248 adherent cell lines from the OncoSignature™ cell line panel. We demonstrate that the LTA assay enabled greater resolution of the activities of slower acting agents either as single agents or in combination. Importantly, this new service complements our existing standard 2D OncoSignature™ screening service by enabling the evaluation of longer-acting therapeutics such as epigenetic inhibitors that show little activity in the shorter-term assay in adherent cell lines. While intermediate-acting agents such as DDR inhibitors have been successfully profiled in standard 2D OncoSignature™ screens[6], the greater resolution of responses observed in the Long-term assay may also offer benefits for the evaluation of these classes of therapeutic.

References

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5. Wilson et al., *Cancer Res* 82(6): 1140-1152 (2022)
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