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Multiplexed High Content Imaging assay optimization for the analysis of apoptosis/necrosis in breast cancer cells after treatment with Vorinostat analogues

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Background. Vorinostat is ahistone deacetylase inhibitor approved for the treatment of acute T-cell lymphoma The recent studies have shown a significant potential of Vorinostat in the mono– and polytherapy of breast cancer as well. Administration of Vorinostat causes cell cycle arrest and apoptosis in neoplastic cells both *in vivo* and *in vitro*. Several analogues of Vorinostat were synthesized at Enamine Ltd. to improve the pharmacokinetics, selectivity and efficacy of the parental molecule. In this study, we aimed to test the effect of Vorinostat and its analogues on the viability of breast adenocarcinoma MCF-7 cell line using multiplexed High Content Imaging assay. **Methods.** Automated confocal microscopy was used to study MCF-7 cell viability. After treatment with the compounds the cells were labelled with fluorescent dyes to detect activated caspase 3/7, nuclei, and assess membrane permeability. Moreover, the assay was multiplexed with a luminescent CellTiter-Gloreagent to evaluate mitochondrial health and ATP content. The experiment was performed in 384-well plates to ensure the high throughput format of the study. InCarta v.1.17 image analysis software was used to analyze the results. **Results.** MCF-7 cell seeding density and incubation time with compounds were optimized to identify the best conditions for High Content Imaging assay. It was revealed that a seeding density of 8000 cells per cm^2 over a 72-hour incubation period yielded the best balance of linear growth and suitable confluency for imaging in 384-well plates. Vorinostat and Staurosporin were used to test the assay performance.Both reference compounds exhibited cytotoxicity and chromatin decondensation, with Vorinostat inducing a significant specific increase in caspase 3/7 apoptotic activity in a dose-dependent manner. Image analysis allowed the successful identification of early-apoptotic, late-apoptotic/necrotic, and viable cells. The assay demonstrated potential for multiplexing with CellTiter-Glo to assess ATP production and compound cytotoxicity. IC50 values for the reference compounds aligned with the literature data. The optimized assay was used to compare the effect of Vorinostat analogues and parental molecule on the viability of the MCF-7 cell line. **Conclusions.** High Content imaging assay coupled with luminescent cytotoxicity assessment was successfully optimized to investigate the potential cytotoxic and apoptotic anticancer effects of Vorinostat analogues in the breast cancer model, laying a foundation for further exploration of their therapeutic potential. We identified optimal conditions for the cell growth and confluency, as well as concentrations for staining solution components and reference compounds.

K e y w o r d s: Vorinostat, breast cancer, cytotoxicity, confocal microscopy, assay optimization.