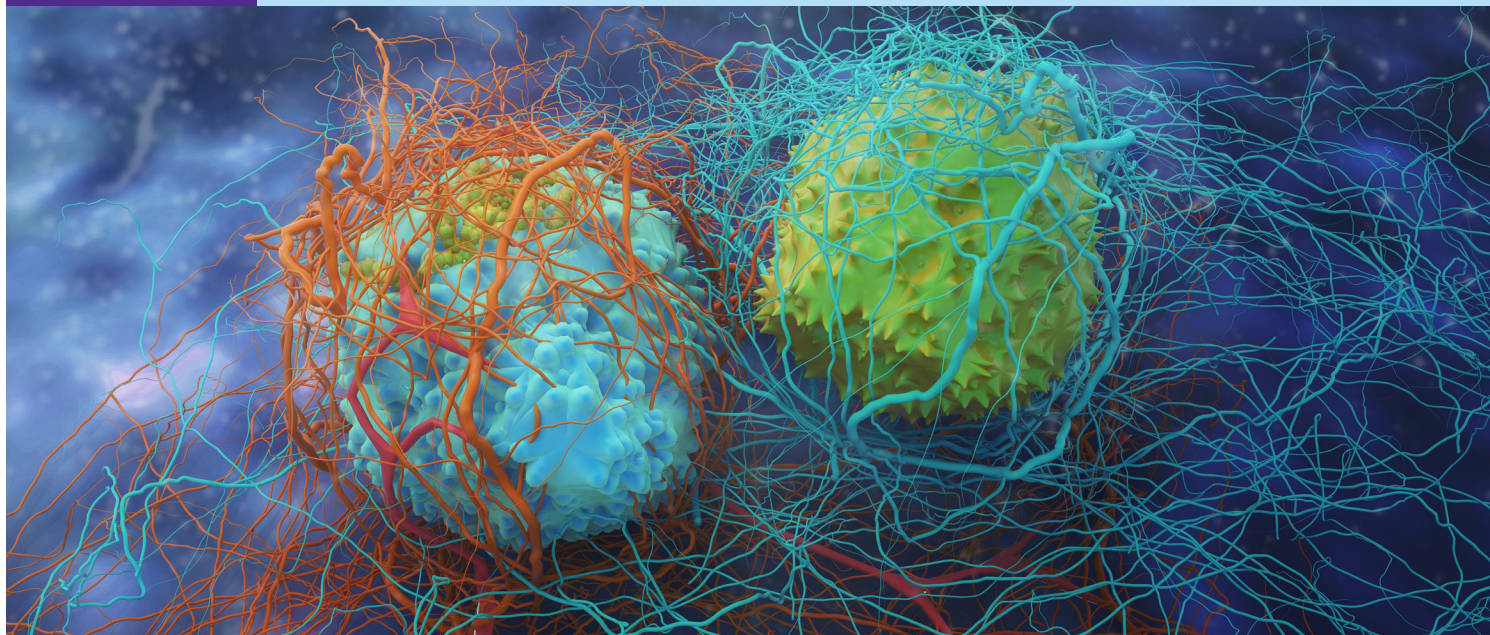


Data integration and Advanced Analytics: The Frederick National Laboratory for Cancer Research



Supporting Precision Medicine Workflows Through Data Integration and Advanced Analytics

The delivery of effective therapeutics to market requires new approaches to maximize the utility of existing drugs and progress promising pre-clinical data to clinical evaluation. Development of tools to support these efforts is driven by a growing trend toward precision medicine, with researchers increasingly demanding streamlined integration of in-house data with animal models, clinical trials, and publicly available information to expedite improved therapeutic outcomes.

Data integration can be challenging, since assay results are often reported in different formats, scattered throughout various data handling packages, or generated across multiple sites. To gain the greatest possible value from new or legacy data, researchers require a solution that supports the complete precision medicine

workflow. With the capacity to quickly and easily aggregate data from various sources, PerkinElmer Signals™ Translational empowers researchers to perform self-service querying and analytics to accelerate the realization of precision medicine.

Researchers at the Frederick National Laboratory for Cancer Research (FNL), sponsored by the National Cancer Institute (NCI), used PerkinElmer Signals Translational to interrogate complex data from a study designed to evaluate the therapeutic potential of combining FDA-approved oncology drugs. By harmonizing biomarker data from a phospho-histone H3 immunofluorescence assay (pHH3 IFA) and multiplex apoptosis immunoassays performed on the Luminex® platform, novel insights were revealed to complement previous discoveries and instigate further investigation.

The NCI-ALMANAC is a Comprehensive Screening Resource to Detect Oncology Drug Pairs

NCI researchers recently published data demonstrating the therapeutic potential of combining small molecule FDA-approved oncology drugs with distinct mechanisms of action. A primary aim of this study was to address the inherent heterogeneity of tumors by using selected drug combinations to overcome single-agent resistance. Following an *in vitro* screen of 104 pairwise drug combinations in the NCI-60 panel of human tumor cell lines, data were uploaded to a searchable database known as the NCI-ALMANAC (A Large Matrix of Anti-Neoplastic Agent Combinations)¹.

A subset of drug combinations was subsequently identified for *in vivo* evaluation using human tumor xenograft models. Selection was based on the ability of pairwise combinations to achieve enhanced growth inhibition or cytotoxicity profiles compared with either agent independently. Strikingly, the combination of paclitaxel with nilotinib resulted in complete tumor regression in an MDA-MB-468 triple-negative breast cancer xenograft model, with no tumor regrowth observed for over 80 days following the end of therapy, leading researchers to interrogate the mechanisms by which the drug combination was exerting its effects.¹ Data illustrating the greater-than-additive *in vivo* efficacy of paclitaxel with nilotinib as compared to the single agents, reported as median tumor volume in an MDA-MB-468 xenograft model, is shown in Figure 1.

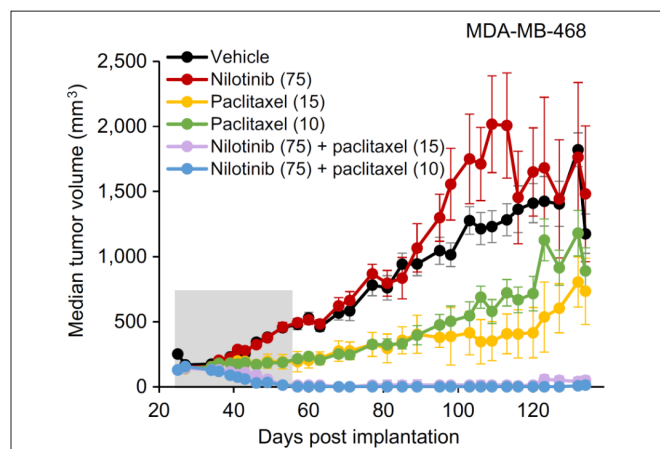


Figure 1. The combination of paclitaxel and nilotinib demonstrated greater-than-additive *in vivo* efficacy compared to the single agents in an MDA-MB-468 xenograft model.¹

Investigation of the paclitaxel-nilotinib drug combination included a panel of multiplex apoptosis assays performed on the Luminex[®] platform. While no increase was observed in levels of the pro-apoptotic marker cleaved caspase-3, elevated expression of the anti-apoptotic marker Mcl-1 was seen in both the paclitaxel and combination groups compared to the control. These results suggested tumor growth to be suppressed by a caspase-3 independent mechanism of cell death and, in combination with *in vitro* and *in vivo* efficacy data, provided sufficient evidence to support the initiation of a phase I clinical trial of the drug pair.

Streamlined Data Integration is Essential to Gain Actionable Insights

As datasets become larger and increasingly complex, it is necessary to address certain challenging aspects of the data life cycle to achieve clinical utility. In addition to the initial generation and collection of experimental results, effective data leveraging requires that researchers have the capacity to answer questions such as:

- How do we store and manage data with limited computational resources?
- How can we share and collaborate with our colleagues in a secure environment?
- How can we improve access to, and aggregation of, our on-going and legacy datasets?
- How can we facilitate data exploration and analysis so that end-users with limited bioinformatics experience are able to engage with the datasets to test their hypotheses and extract actionable insights?

To address these challenges and interrogate the data generated during investigation of the paclitaxel-nilotinib drug combination, FNL researchers initiated a pilot study to evaluate PerkinElmer Signals Translational as an alternative to existing methods of data storage, aggregation and analysis. By simultaneously leveraging multiple sources of data from the ALMANAC study instead of using Microsoft Excel and Prism to perform traditional, two-dimensional analysis – a method which is frequently prone to human error – FNL researchers expected to extract further actionable insights.

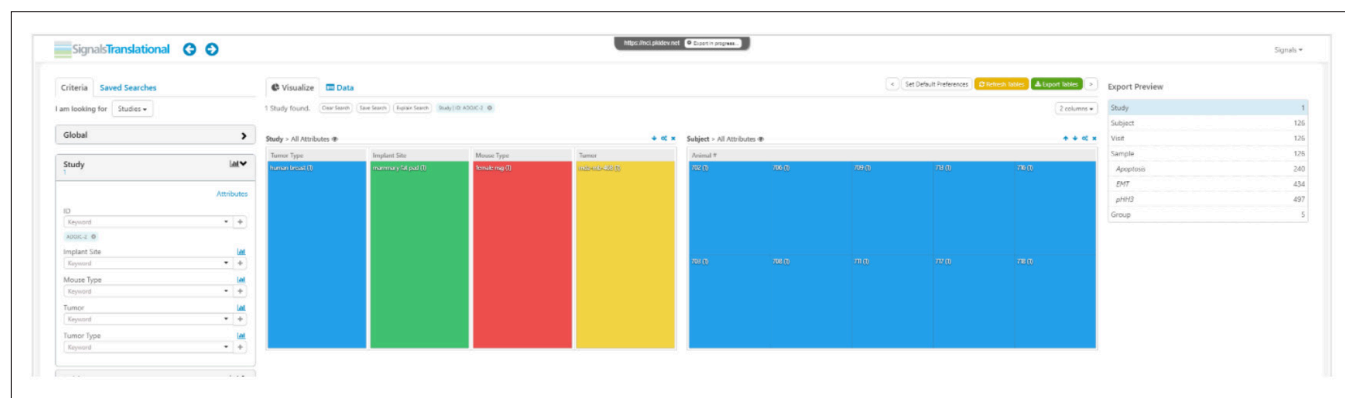


Figure 2. PerkinElmer Signals Translational allows intuitive harmonization and aggregation of assay results from multiple sources into a single platform for seamless data management and self-service analytics. The dashboard represented here makes for easy data searching and the Export Preview dialogue (top right corner) shows the number of entities and measurement types ready to be exported for the study that has been selected.

Harmonization Adds Value to Existing Data

Within the PerkinElmer Signals Translational pilot study, FNL researchers integrated biomarker data from an MDA-MB-468 xenograft model treated with paclitaxel and nilotinib. This included pHH3 IFA data from xenograft sections and data from multiplex apoptosis immunoassays performed on the Luminex® platform. These assays are highly complex and deliver distinct readouts - pHH3 IFA uses Definiens software to measure the percentage of nuclei that stain positive for pHH3 (Figure 3A), while Luminex results are reported as protein concentration in ng/mg of total protein (Figure 3B).

The multiplicity of these datasets highlights the need for data aggregation to better inform discovery. Using PerkinElmer Signals Translational, data from all platforms were uploaded

in conjunction with *in vivo* efficacy data and coupled with detailed information regarding the study design (e.g. mouse number, treatment group, dose, timepoint).

By harmonizing data using PerkinElmer Signals Translational, FNL researchers could easily conduct searches as simplistic as 'bring me all the samples that have measurements for pHH3,' or more complex searches such as 'inform me which samples have data for pHH3 and active caspase-3 for a specific time point.' After querying the data in this manner, additional visual analytics could be performed, allowing fast, simple and interactive dashboarding to refine the data for extraction of actionable insights.

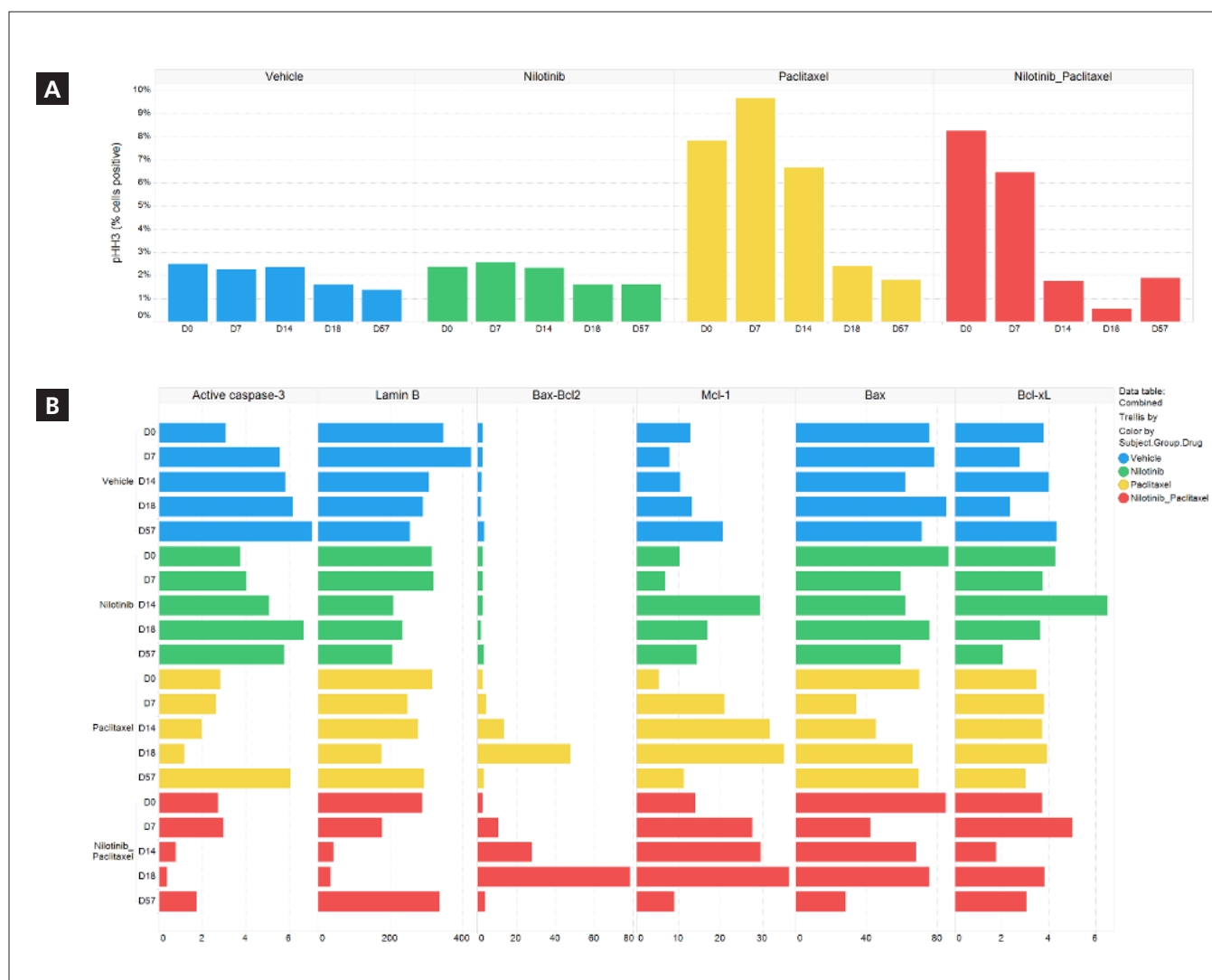


Figure 3. (A) pHH3 data is reported as percent cells positive for each of the four groups ((vehicle, nilotinib, paclitaxel, and nilotinib combined with paclitaxel whereas (B) Luminex® results from multiplex apoptosis immunoassays are reported as protein concentration in ng/mg of total protein.

As an example, FNL researchers used PerkinElmer Signals Translational to visualize the Luminex® apoptosis immunoassay data as percent change relative to vehicle over time following normalization for total protein, as seen in Figure 4. This showed levels of the pro-apoptotic marker cleaved caspase-3 to decrease in both the paclitaxel and combination groups, accompanied by a corresponding increase in the anti-apoptotic markers Bax-Bcl2 and Mcl-1.

In another instance, FNL researchers reported pHH3 data as a percent of cells stained positive for pHH3 (Figure 5). An increase in pHH3 was observed in the single agent paclitaxel and paclitaxel-nilotinib combinations at early timepoints, indicating that cells were entering mitosis. The difference between paclitaxel and combination treatment was likely due to the decrease in tumor cell number in the combination group and not a true difference due to treatment, since the analysis did not control for tumor content in the biopsies.

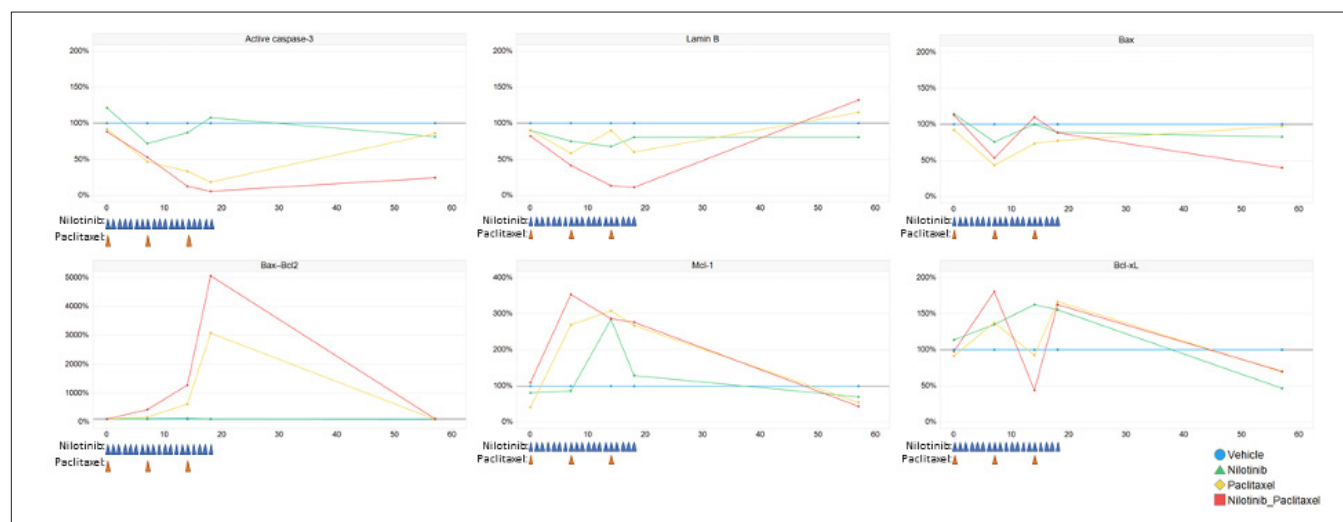


Figure 4. Luminex® apoptosis immunoassay data was normalized by total protein and reported as percent change relative to vehicle over time. A decrease was observed with the pro-apoptotic marker active-caspase 3 and an increase with the anti-apoptotic markers Bax-Bcl2 and Mcl-1 in both the paclitaxel and combination groups. Lamin B was observed to largely be reading out cell number, which is consistent with the large decrease in tumor cell number with the combination treatment due to cell kill. Bax and Bcl-xL were seen to be noisy and largely uninformative.

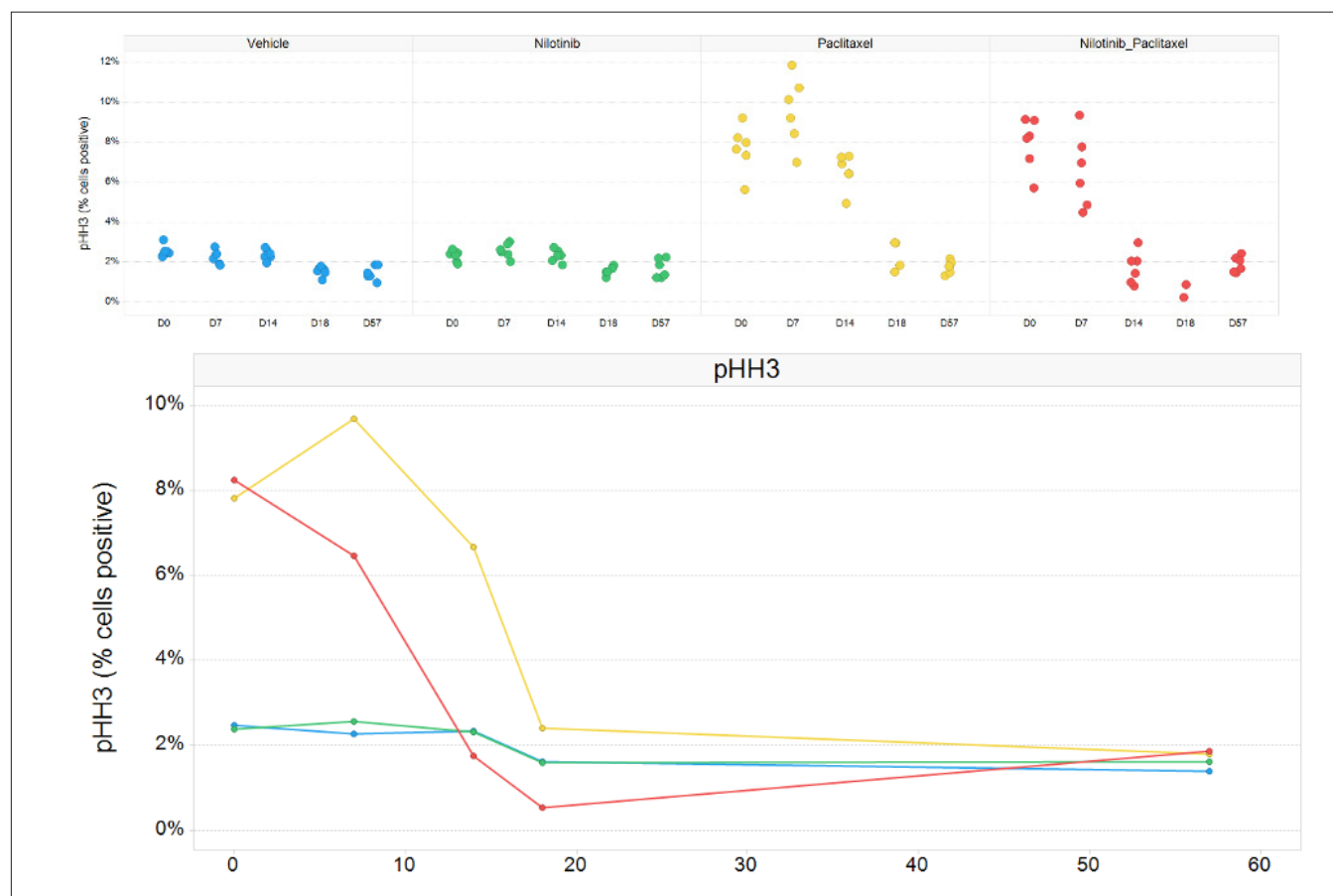


Figure 5. Reporting pHH3 data as percent of cells stained positive for pHH3 it was possible to observe an increase in pHH3 in the single agent paclitaxel (yellow) and paclitaxel-nilotinib combinations (red) at early timepoints. A similar effect was not seen in the vehicle or single agent nilotinib groups.

An Integrated Solution That Delivers Many Benefits

Through the provision of an intuitive and user-friendly environment, PerkinElmer Signals Translational empowered FNL researchers to better understand the relationship between different biomarkers in pre-clinical specimens. Multi-dimensional analysis allowed comparisons to be made between distinct assays within a single model, providing answers to complex questions that could not be addressed fully when examining assay results in isolation.

For example, by plotting changes in the expression of various apoptotic markers against changes in levels of pHH3 (Figure 6), researchers concluded that cell death is not proceeding through intrinsic apoptosis or mitotic catastrophe and is most likely to be necroptotic. This theory was supported by visualizing the data over time (Figure 7), with findings paving the way for future studies.

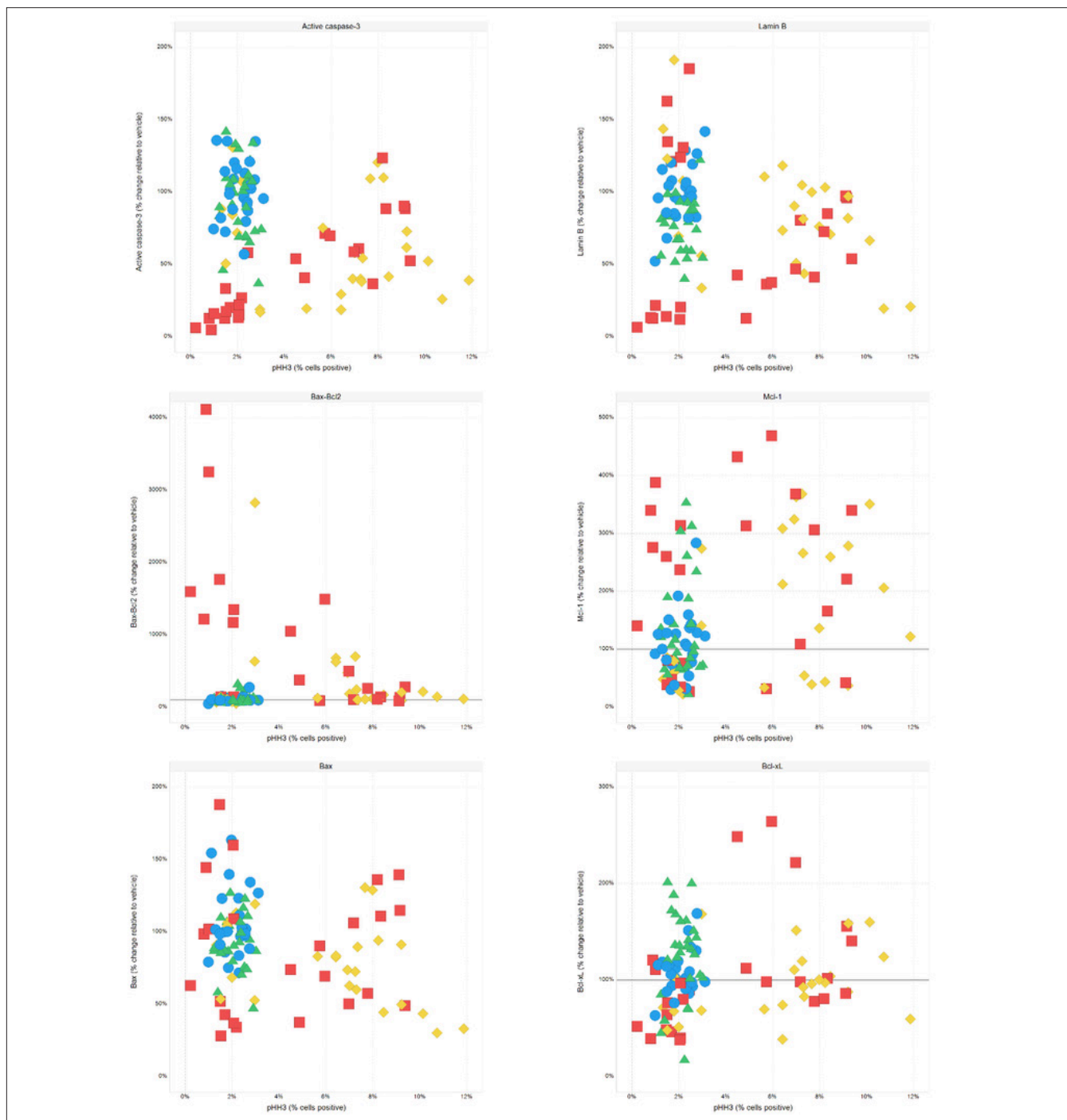


Figure 6. Comparison of apoptosis marker expression against changes in pHH3 levels provides insight to the mechanism of cell death, indicating that it is not proceeding through intrinsic apoptosis or mitotic catastrophe and is most likely to be necroptotic. The time points - Days 0, 7, 14, 18, and 57 are superimposed and the color and shape represents treatment: Vehicle=blue circle, Nilotinib=green triangle, Paclitaxel=yellow diamond, Combo=red square.

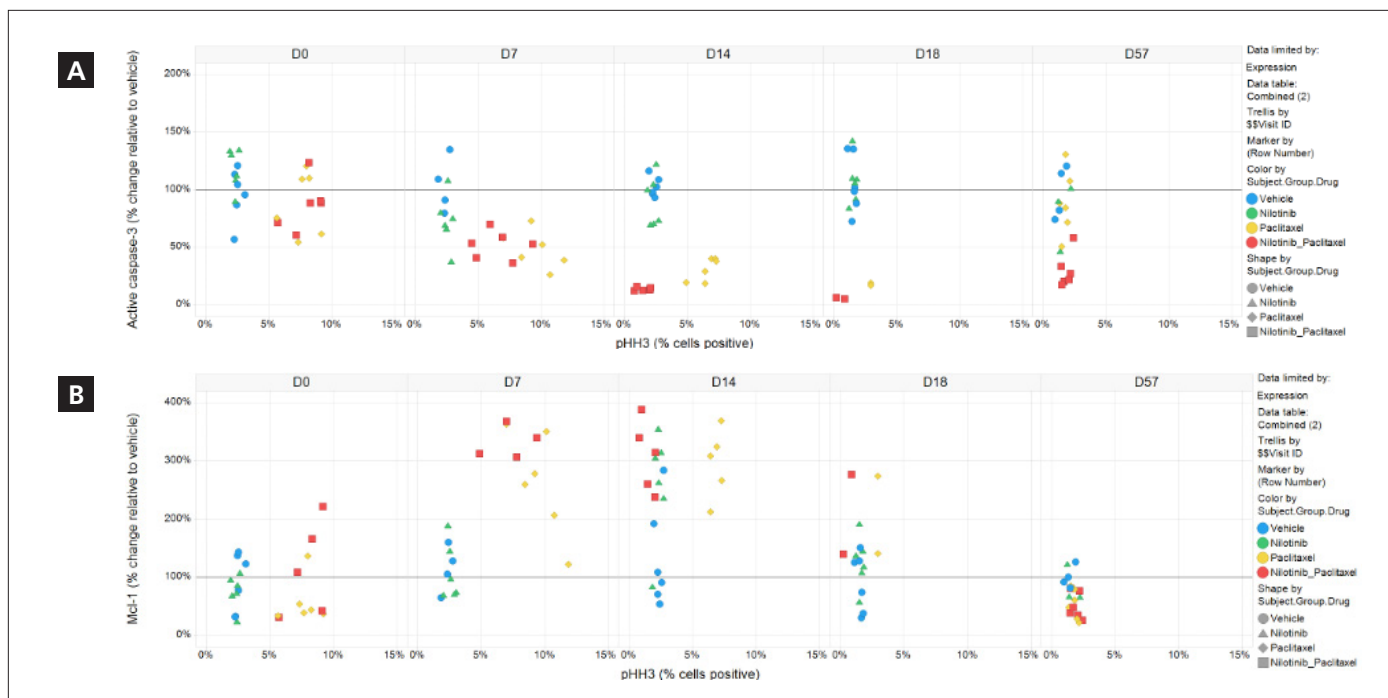


Figure 7. Splitting the data from Figure 6 by time point provides a time course of (A) pro-apoptotic marker active caspase-3 and (B) anti-apoptotic marker Mcl-1 versus pHH3. This supports the hypothesis that cell death is not due to intrinsic apoptosis or mitotic catastrophe.

Although the FNL project focused on multi-dimensional aspects of a single study, PerkinElmer Signals Translational is ideally suited to perform cross-study analyses since it serves as a unifying data source with the capacity to search, aggregate and perform analytics. FNL researchers plan to exploit this utility of PerkinElmer Signals Translational by performing similar data mining on assay results from clinical samples across multiple trials.

A further advantage of PerkinElmer Signals Translational is that by integrating search and analytics functions within a single solution, the need for additional upstream data preparation is eliminated, significantly reducing the burden on IT. Flexibility and scalability in response to ever-increasing data sizes are achieved by leveraging the cloud, an approach which also lowers costs and serves to increase the speed and efficiency of drug development.

In combination, these functionalities deliver an incredibly powerful self-service querying and analytics solution, empowering researchers to test and refine their hypotheses in an ad-hoc manner. The FNL case study highlights the utility of PerkinElmer Signals Translational to deliver novel insights, generating findings with significant potential to expedite the progression of promising pre-clinical data to clinical evaluation.

Reference

1. The National Cancer Institute ALMANAC: A Comprehensive Screening Resource for the Detection of Anticancer Drug Pairs with Enhanced Therapeutic Activity, Holbeck SL *et al*, Cancer Res. 2017 Jul 1;77(13):3564-3576.

Acknowledgements

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PerkinElmer Signals Translational is a cloud-based data analytics solution that allows intuitive aggregation of assay results from multiple sources into a single platform for seamless data management and self-service analytics. With visual analytics powered by TIBCO Spotfire®, PerkinElmer Signals Translational provides all the necessary tools to harmonize, manage, search, aggregate and analyze data consistently across large datasets for use in translational research in a highly scalable manner. This delivers novel insights that cannot be achieved when examining assay results in isolation, providing researchers with the capacity to more rapidly develop and test hypotheses, and expediting the discovery and development of new therapeutics.

Disclaimer: This document does not imply nor convey any endorsement of the aforementioned technology by either the Frederick National Laboratory for Cancer Research, currently operated by Leidos Biomedical Research, Inc. or the National Cancer Institute.

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