THE CHALLENGE

Our client, a global integrated pharmaceutical company, had completed Phase I studies with an investigative therapeutic, Drug A, for a cancer indication (Figure 1). In the Phase I study, a small number of patients experienced a specific serious adverse event (AE). This AE was correlated with the patients’ recent use of a second and unrelated therapeutic, Drug B. While Drug B was known to be associated with this type of AE, the affected patients had discontinued Drug B for some time prior to initiating Drug A. The client faced the possibility that initiation of Phase II trials could be delayed by regulatory authorities if the AE was not explained and steps were not taken to mitigate it. The client needed to test among three hypotheses: (1) the AE was caused by the client’s investigative drug, Drug A; (2) the AE was caused by prior courses of Drug B; or (3) low residual levels of Drug B in patients could synergize with Drug A to induce the AE.

Due to the small number of affected patients, there was limited clinical data on which to base a hypothesis, without which a risk evaluation and mitigation plan was not possible. Laboratory studies in animal models were not appropriate because both Drugs A and B were specific to humans. Studies in cell lines might not yield physiologically relevant results because of the clinical complexity of the AE. Human primary cells were a more physiologically relevant experimental system to test hypotheses that might then inform clinically relevant explanations of the AE. The client wanted to gain as much information as possible to bridge the gap from in vitro to clinical insights about possible drug interactions.

Figure 1. Developmental stage of our client’s drug
BIOSEEK’S APPROACH

The client met with BioSeek to learn more about BioMAP® human primary cell systems that mimic physiological microenvironments for a variety of disease states, including inflammation, wound healing, angiogenesis, proliferation, fibrosis, as well as monocyte, T-, and B-cell immune responses. The more than 30 BioMAP® systems yield quantitative, reproducible, and biologically relevant readouts of more than 400 well-documented protein, lipid and metabolite markers, as well as parameters of cell morphology and viability. The pharmacologic relevance of BioMAP® systems is validated with more than 3,000 known compounds. Drug responses in BioMAP® systems enable BioSeek to identify mechanisms of action, off-target effects, potential side effects, biomarkers, and drug efficacy in man—information that could be missed by in vitro assays using cell lines or simple primary cell systems.

As there were no mechanistic hypotheses available for the AE, BioSeek recommended applying 13 BioMAP® systems that mimic physiological microenvironments for a variety of disease states, including inflammation, wound healing, angiogenesis, proliferation, and monocyte, T-, and B-cell immune responses to scan for responses that could potentially be involved in the AE observed in the client’s clinical studies (Figure 2). For example, three of the 13 BioMAP® systems consist of early passage primary vascular endothelial cells co-cultured with PBMCs (lymphocytes, monocytes, natural killer cells, etc.) that are either resting or stimulated with LPS to model toll-receptor mediated monocyte activation, or with Superantigens to model T-cell receptor-mediated activation. These systems capture signaling cascades that recapitulate responses observed clinically in inflamed tissue, for example increased PGE2 (associated with pain and swelling), increased E-Selectin, VCAM and IL-8 (associated with leukocyte recruitment), increased TNFα etc. These and other markers are quantified in the presence of a test compound over a range of pharmacologically relevant concentrations.

BioSeek conducted three studies with the set of 13 BioMAP® systems: (1) Drug A only, (2) Drug B only, and (3) a combination of Drugs A and B. All protein markers were quantified and analyzed using BioSeek’s fully automated high-throughput ELISA platform, and the generated data fed into algorithms that map functional similarities to compounds in BioSeek’s annotated database of more than 3,000 known pharmacologically active compounds previously characterized in BioMAP® systems.

Figure 2. Three of the 13 BioMAP® systems applied to investigate our client’s hypothesis of drug interactions

Co-Cultures of Endothelial Cells and Peripheral Blood Mononuclear Cells

- **Stimulus:**
  - Resting
  - LPS
  - Superantigen

- **Measured responses:**
  - Activation of pro-inflammatory responses
  - Toll-receptor mediated responses
  - T-cell receptor mediated responses
**Figure 3.** The combination of Drug A and Drug B induces unexpected activities in the BioMAP® system consisting of a co-culture of resting endothelial cells and peripheral blood mononuclear cells. (A) BioMAP® profiles are shown for nine BioMAP® systems, each a different combination of human primary cells and activating factors. The horizontal axis lists the protein biomarkers measured for each BioMAP® system. The vertical axis shows the change in biomarker expression (log ratio) in response to the drugs. The blue, red, and dark gray lines show the BioMAP® profiles of Drug A, Drug B, or the combination of Drug A and Drug B, respectively. The light gray horizontal band shows the range outside of which a change in expression is statistically significant. (B) The “HPNo” BioMAP® profile is enlarged and shown as a column chart.
RESULTS

BioSeek's analysis showed that Drugs A and B alone induced BioMAP® profiles expected from each drug's known mechanism. The profile of Drug A was consistent with its mechanism, but showed no marker changes suggestive of AE. The profile of Drug B showed a limited number of low-magnitude marker changes that were indicative of weak pro-inflammatory activity, but again no marker changes were suggestive of AE. However, the combination of Drug A and Drug B induced reproducible and statistically significant activities in one out of the 13 BioMAP® systems that were not observed for each drug alone. In the BioMAP® system comprised of vascular endothelial cells co-cultured with PBMCs, both VCAM and TNFα protein levels were induced to levels several fold higher than when either Drug A or B was present (Figure 3). Both these proteins are pro-inflammatory, functioning in leukocyte recruitment.

Scientists at BioSeek consulted with the client to provide their insights on these findings. BioSeek explained that the client's drug, Drug A, showed no significant marker changes expected for pro-inflammatory activity. Hence, BioMAP® yielded no evidence that Drug A by itself would have caused the clinical AE. On the other hand, Drug B showed limited evidence of such activity, and from clinical experience Drug B was known to be associated with risk for the AE. However, the combination of Drugs A and B yielded marker changes for inflammation that were several fold greater than for Drug B alone, in addition to changes not observed for Drug B alone. BioSeek scientists suggested that these responses indicated that Drugs A and B were synergistically pro-inflammatory.

IMPACT FOR THE CLIENT

The client now had evidence from in vitro studies that their drug, Drug A, did not induce responses associated with the clinical AE. It also had evidence to explain the AE: that in the affected patients, low-residual levels of Drug B and therapeutic levels of Drug A caused a magnified inflammatory response to Drug B that led to the AE. In planning for further clinical studies, the client could use this insight to mitigate risk for the serious AE. Their protocol would be revised for those patients who had previously been administered Drug B so that Drug A would not be given until after an increased period of delay to allow further clearance of Drug B. BioMAP® results also provided the client with information on markers that could be used in the clinic to monitor risk for the AE.

BioMAP® studies provided novel insights on synergy of the client's drug with a second drug in an experimental system relevant to human physiology. BioMAP® in vitro models informed clinical observations, arriving at richer insights to mitigate risk in continued development of the client's investigative drug.

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