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ADME A CASE STUDY

HAO CHEN, Vice President of Research and Development at **Caliper Life Sciences**, explains how companies can push their high throughput absorption, distribution, metabolism and excretion (HT ADME) technologies forward

MATT TROUTMAN The next generation of high throughput ADME assays will likely require measurements beyond analysis of the compound itself. For instance, to gauge a propensity of a compound to elicit enzyme induction, resultant changes to enzyme mRNA is typically determined. **What new technologies or approaches are on the horizon for quantification of compound disposition and drug-drug interaction of the nature described above that can be performed at high capacity with a short turnaround for the sake of extensive profiling and SAR-generation in early discovery?** And the second question is about key tenants of success in HT ADME profiling, which includes providing discovery teams with sufficient data to form decisions, ensuring the data has sufficient quality and granularity, and within the right timescale, typically tied to a design cycle that a medicinal chemist might work on. **Could you discuss any new approaches or technologies that will significantly enhance these elements and that may also affect the efficiency by which data is generated?**

HAO CHEN Both questions pose rather complex and intricate challenges that we face nearly every day. The question is: What do we need to do to generate sufficient data that is timely enough to be useful and relevant to guide the

decision-making processes?

The demand for increasing capacity, for example numbers of compounds against a particular enzyme, and providing sufficient data granularity, that is the number of enzymes, within a compressed timeframe are logistic challenges we face every day. We have capacity issues, data granularity issues, and you also have the turnaround to support the medicinal chemists who lead generation.

We here at Caliper Discovery Alliances & Services (CDAS), which is a CRO business unit of Caliper Life Sciences, have realized such demand, especially from the angle of supporting medicinal chemistry activities, for elite optimization process for different clients across the country. Our initiative is to increase data granularity, as well as increasing our screening capacities without added expense. With that, you're increasing your production data matrixes. We also need to meet the challenges of time constraints.

To increase data granularity, for example, we have assembled a very large panel of CYP450 assays that encompasses nearly all known human, rat, and rodent isoforms. This way, in one shot, we can screen many different enzymes per compound. The first way to increase screening capacity without expanding resources is a real challenge. Our approach is to change the experimenters' mindset about how to organize experiments. One of the attempts we're carrying out is to arrange logistics, reagents and cells for example, in such formats that we can conduct multiple experiments in parallel. Sometimes we pre-arrange or half-assemble these reagents, like the cells, enzymes and buffers, so that these

experimental components may be assembled quickly according to particular groups' or clients' or medicinal chemists' needs with the assistance of appropriate automation. CDAS is very much engaged in fast discovery and that lead optimization programs require a one-week turnaround, for example. These fast turnaround time frames will provide support to the medicinal chemists. Panels are customized for each clients' and end users' need. We see that multiplexing experiments will ultimately increase our screening capacity, providing high-density and high-quality information in a very short time frame.

Producing high screening capacity, high-density screening services with highly refined granular information and short turnaround can be readily implemented. This is because we're blessed with many commercially available enzymes, or primary cells, from different providers. Reagents for testing a panel of compounds against a panel of enzymes can be easily assembled from multiple sources, such as mRNA array, protein expression profile analyses using dot-blot, flow-based beads, and instruments or reagents for multiplexed enzyme inhibition assays, which are all readily accessible. The tools are actually out there. For us, we need a new way of thinking about how to set up our experiments so that the logistics and arrangements can be readily made to increase production capacity, data granularity, as well as throughput.

MT Increasingly, ADME data is being produced on a large scale for multiple endpoints, in some cases from various sources (internally and at CROs), and consumed in a short time cycle, the whole notion of throughput typically being about a week. HT ADME data is being increasingly used to inform design decisions aimed at achieving the right PK profile rather than just profiling for information. Complimentary to this primary value in profiling and also decision making, the data is typically utilized to create *in silico* models for these endpoints that are used to predict likely properties, and this often informs design decisions. Data management plays a critical role in all of these processes, and in some cases directly affects how these activities are performed. **What new data management solutions are on the horizon that will enable this process?**

HC This is a real challenge, especially when we

run multiplexing assays, which present challenges for data flow and management. However, many e-notebook providers, like ActivityBase or Symyx, are designed to continuously capture datasets so long as the proper templates are built. Disassembling multiplexed datasets — multiple compounds versus multiple end-point readouts — and disseminating them to multiple users correctly has been our strength. In fact, we've been providing such data to a multitude of end users for the past 15 to 20 years. What we find useful as a data provider is to understand the need of the end users. In other words: Who uses the data and what intended uses do they have in mind?

With that in mind, we will arrange the format of the multiplexed assays allowing the data migration to a preset template with the output formatted for different end users. Not just data, but including the visual aid. Communication and understanding between the data provider and the data end user is a key component; once such understanding is achieved, then data handling templates, migration schedules, automatic review criteria and conflict resolution policies may be established using different scripts provided by the software tool set.

Continuing capturing the data over extended time was consistent policy of data curation. Conflict resolution, accumulation, migration and integration are essential for constructing high quality *in silico* training sets for CAD programs. Although the entire process may be driven on an automatic schedule without human interference, it's really necessary to be examined by experienced scientists in light of capturing data.

In fact, this has reminded me that one of the most important issues relating to ADME is to identify the undesired activities, eliminating those compounds that exhibit one. These activities could be 3A4 inductions or rendering some kind of transporter activities. Eliminate these early in the drug discovery process. For some time already, we've been experimenting on constructing a high-density, full-rank database with our screening platforms and commercially available compounds that were previously shown to be biologically active. Using this dataset as a computational training set, we have worked with many different clients and end users in de novo design and library selection. The negative data has proven to be particularly useful in selecting compounds devoid of certain activities, whether side effects or ADME concerns.

MT HT ADME and ADME in general, is an applied discipline that borrows heavily from biology, physics and analytical chemistry. In many

cases, some of the breakthroughs that we've seen that have been very impactful in the past have been the application of technology that was not originally designed specifically for ADME, or HT ADME. **Do you envision any technologies not currently employed in HT ADME profiling that may lead to positive enhancements in this area? Things in the technological realm, software or biology assay solutions?**

HC From the service end, we see two emerging trends. One is in the *in vitro* area. The other one is in the *in vivo* area. The *in vitro* trend is to characterize using the platform normally developed by the proteomics. And the *in vivo* trend is to use emerging techniques to characterize and capture real time pharmacodynamic properties.

Some of the *in vitro* induction assays have been with recombinant cells and reporter genes, for example 3A4 induction or primary cells and primary mRNA expression profiles. These assays are convenient, but have limitations. Our initiative is to characterize the induction protein directly from relevant cells. Not just a single induction protein, but multiple proteins. Our instrument initiative is to design using automated high throughput or medium throughput western techniques, but this initiative, including making the instruments as well as the reagent kits, is still in the research phase, or prototypes. So, short of accessing the mature technique and instruments, we at CDAS are experimenting with primary cells, dot-blot and bead-based flow techniques for the expression profiling. Both techniques have shown promise in providing reasonable throughput and high quality data.

The second trend we see is mostly *in vivo*. A key part of a pharmacokinetic study traditionally is to characterize pharmacodynamic properties. Traditionally, this information has been derived based on kinetic information like C_{max} and T_{1/2}. However, as we come to understand more and more about the cell signal transduction mechanisms and events, the simple appearance and disappearance of the chemical entities from plasma or a specific tissue has little to do with the real effect of the drug on a particular cell or its respective signal transduction events, which is where the original drug was intended or designated to mediate. These imaging techniques may not be in the real high throughput realms, but we see *in vivo* imaging techniques and reagents — implanting recombinant cells with engineered light-producing reporters, allowing signal transduction pathways, using light-producing transgenic animals, such as NFκB, NOS, GFAP — are being increasingly explored in the realm of



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pharmacokinetic or pharmacodynamic characterizations. In fact, we have encountered examples in our study, where the drug action reaches far beyond what the normal realm of PK-derived PD may predict. Imaging technique in this case is not really a prediction, but a real time measurement of signal transduction events and that could produce more relevant pharmacokinetic data.

MT I think both of these are great illustrations of things that will really move the state of the science and discovery forward. Recently there’s been some interest to create assays that produce higher content information than what was previously available in the realm of HT ADME for the compounds themselves. An example of this would be early identification of major metabolites generated from *in vitro* studies that could be profiled to provide highly valuable information that discovery teams can use to make decisions and

to plan future efforts. At this point in time however, analysis of the volume of data and speed to perform such experiments is limiting. **Can you please discuss some technologies that might be forthcoming that are expected to enhance the level of content or information that can be generated for the compounds themselves? What about corresponding technology being developed to help manage this volume of data so that it could be routinely performed in HT ADME approach?**

HC There are a number of companies that provide different versions of HT metabolite identification, and this research is ongoing. The work is being done by Thermo, Waters, and Agilent, for example, but I don’t think they’re sufficiently matured to be implemented in a high or medium throughput ADME format.

As far as data generation, different e-notebooks can provide these capturing mechanisms, but it’s sufficient enough to say we have to provide

additional guidance and templates so that the data can be easily merged together. So in this case, the technologies are out there, but they’re not sufficiently matured to be used in a high throughput format to provide the metabolite identification yet.

MT That is definitely what we are seeing here at Pfizer. We are starting to get our feet wet with this. I think you really hit the nail on the head that technologically we can get a lot of this information, but then it becomes about what is the right information and how do we manage that data. I think there is going to have to be a lot of partnership between people who need to produce the data and people who built the tools that collect it in order to get us really where we need to be.

HC The communication is very important. Programmers will write any type of template you want, but you need to communicate very clearly so that they will be able to provide you with the proper tools in terms of metabolite identifications; you have to have a database that can be trained over time to quickly recognize these metabolites. The database has to be expendable.

MT Absolutely. It is going to be quite a challenge. **FP**



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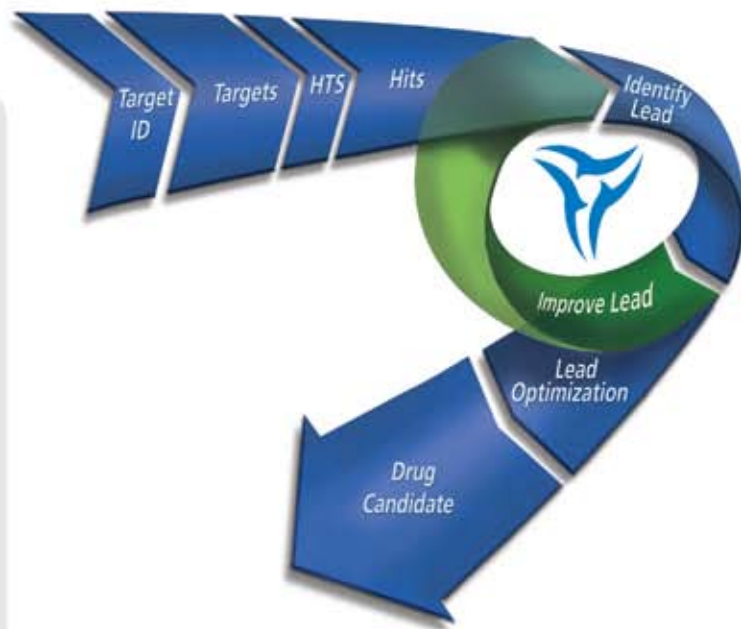
HAO CHEN, PH.D., Divisional Vice President, Research and Development, Caliper Discovery Alliances and Services. CDAS is a branch of Caliper Life Sciences, a discovery alliance and contract research organization. CDAS provides drug discovery and development services and consultation worldwide. Prior to Caliper Lifesciences, Mr. Chen was the vice president of research and development of NovaScreen Biosciences, the predecessor of CDAS. In this role, he led, organized and assisted numerous cooperative alliance and contract service projects with biotech and pharmaceutical companies as well as different NIH institutions.

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