

Can In Silico Modeling Speed Development?

Although Unproven, Some Advocates Believe the Technology Can Save Both Time and Money

K. John Morrow Jr., Ph.D.

The current approach to drug development has not served the industry well. Fewer drugs are being approved, even as R&D investments spiral upward. The long-accepted protocol of in vitro testing of compounds followed by in vivo animal studies appears to be less satisfactory than in the past at predicting efficacy or toxicity. The changing picture is, no doubt, a reflection of the complex nature of drug targets.

An alternative to the traditional drug development protocol, however, has been maturing. The rise of computer technology, combined with a more sophisticated attack on targeted diseases, could eliminate unsatisfactory candidates early in the game, providing the possibility of vast savings and a new round of innovative therapeutics. Yet, numerous challenges remain, as was discussed at CHI's "BioIT World" meeting held recently.

"The systems biology approach allows us to map out the pathology of a complex condition and discover drugs that will treat the underlying disease," said Jordi Naval, Ph.D., research scientist at Anaxomics Biotech (www.anaxomics.com). Using algorithms that address multifaceted relationships within metabolic pathways, it is now possible to hone in on previously unrecognized drug targets.

The company uses existing databases to create cellular roadmaps to build discovery platforms. These databases employ text mining to define interactions within metabolic routes and signaling pathways. According to Dr. Naval, "we start with the seed interactome, that is, the set of already identified proteins that participate in the disease, and use this as the framework for constructing our model."

Dr. Naval and his colleagues take advantage of a large coterie of databases, including Entrez Gene and Uniprot, to look for possible targets. Building on the seed interactome they seek an expanded understanding of the target pathway. Promising leads are then validated in the laboratory using standard technologies for studying gene expression, such as the yeast two hybrid system, site-directed mutagenesis, and cellular and animal models. This expanded knowledge allows an exploration of perturbations of the system, which the company has pursued for the tumor necrosis factor pathway.

Combating Antibody Autoimmunity

Qingyu Cao, Ph.D., head of business development at AlgoNomics (www.algonomics.com), presented her company's approach to dealing with immunogenicity of therapeutic proteins. As all therapeutic proteins demonstrate some level of immunogenicity, this is a common problem.

AlgoNomics' Epibase® uses structural bioinformatics to identify potential epitopes on a protein based on the structural characteristics of HLA molecules and their

binding property to epitopes. The whole sequence of target protein is scanned using the Epibase algorithm, and the binding affinity between epitope and HLA is computed based on the interaction energies, taking into account side-chain flexibilities. A risk profile of the particular protein molecule is thus provided. This method becomes increasingly more accurate as the amount of experimental data grows, correlating in silico modeling with results from the company's T-cell activation assay.

While the in silico approach cannot replace thorough animal and clinical studies, it allows for a more rapid and accurate assessment of the immunogenicity risk and can provide substantial cost savings by elimination of blind alleys, Dr. Cao reported.

Dr. Cao's contentions were buttressed by clinical data from a study on patients with rheumatoid arthritis who were receiving Adalimumab directed against TNF- α . The study, performed in collaboration with Sanquin and Genmab, identified a number of potential troublemaker epitopes.

Of 109 patients enrolled in the study, 19 showed antidrug antibodies and 17 of these possessed HLAs that were targeted by unruly epitopes. These HLAs are also highly associated with RA.

While high-throughput screening is now



widely employed in the search for new pharmaceutically active molecules, there is little information available on the factors that affect the ability of positive hits to move to later stages of drug development. Novartis Pharma (www.novartis.com) has extensive experience in this area, according to Andreas Bender, Ph.D., post doctoral fellow at the Novartis Institutes for BioMedical Research.

Dr. Bender's approach is to combine in silico target prediction, which gives mechanistic information about compound action, with cellular, image-based readouts profiling multiple parameters, yielding holistic information but lacking the mechanistic parameter. "We combine both processes to have a better of idea of the true character of a compound, in order to measure both the effect of a compound as well as to develop an hypothesis of its function," he stated.

Dr. Bender and his associates compared the

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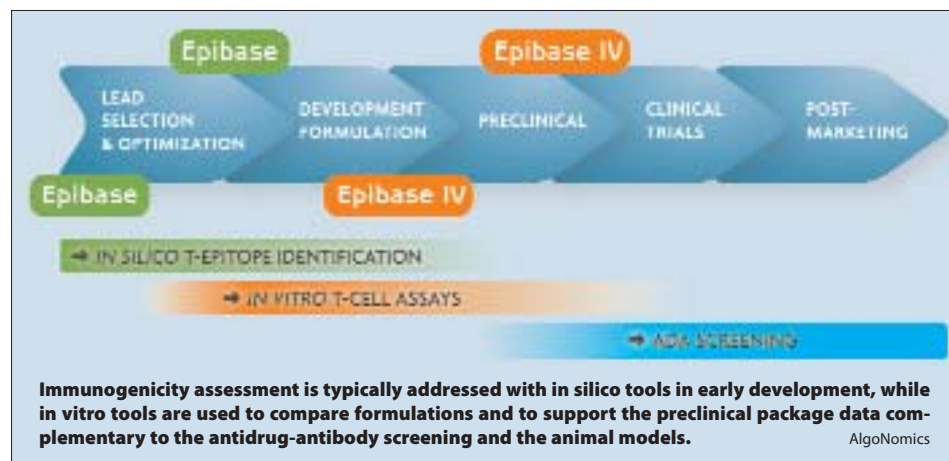
results of thousands of high-throughput screening runs using 15 different readout technologies and 18 target classes including protein kinases and GPCRs. The group collected data on which aspects of the screening process were empirically correlated with downstream success, defined as the fraction of HTS campaigns that advance into the later stages of drug discovery.

The Novartis high-content screening platform gives 36-dimensional readouts in three channels. HeLa cells are grown for 24 hours, stained with a laundry list of fluorescent dyes, then analyzed by automated microscopy following a factor analysis to

as inhibitors. The company has targeted renin because, as Suresh Singh, Ph.D., director of computational drug design, explained, the renin-angiotensin system is essential for regulating vascular function.

The company's drug discovery platform, Contour™, was designed to create thousands of novel molecular structures through visualizing the most relevant potential inhibitors. The program starts with a defined active site on a target to which an anchor fragment is fitted; other fragments are added stepwise until a molecular weight limit is reached. The in silico molecule is given a final score and saved as one of thousands of different candidate structures that are assembled using high-speed computers.

According to Dr. Singh, Vitae scientists have used the top scoring conformations to build two series of potent and selective



inhibitors. In less than 18 months they were able to construct candidates with patentable design, high potency, and bioavailability, all of which demonstrated blood pressure lowering ability in a number of different species. The company is moving its renin inhibitors toward clinical evaluation and also pursuing a number of other disease targets.

These targets include 11 β HSD-1, an enzyme critical in the production of cortisol that has been implicated in diabetes and cardiovascular disease. Yet another program targets the Aurora A and Aurora B serine/threonine protein kinases, which are overexpressed in many human tumors.

Vitae is also developing orally active inhibitors of BACE1 (β -site APP cleaving enzyme 1) for the treatment of Alzheimer's disease and low molecular weight inhibitors of the enzyme HCV NS3 protease for HCV treatment.

Epix Closes In on the Clinic

"The PREDICT Methodology represents an innovation in small molecule selection," according to Sharon Shacham, Ph.D., svp for drug development at Epix Pharmaceuticals (www.epixpharma.com). In her presentation, Dr. Shacham described how the company's platform has resulted in four novel drug candidates in less than four years.

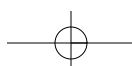
Focusing on a rational drug design technology, Epix has developed a suite of modeling and optimization algorithms leading to rapid lead discovery and optimization. The discovery program is aimed at GPCRs,

reduce dimensionality of the readout. This approach yields a multidimensional readout that details the assembled complexity of the signaling network. "We can build statistical ligand-target relationships to predict the targets of molecules, based on their molecular structure," Dr. Bender said.

When Dr. Bender and his colleagues analyzed the results of these large-scale screens of 6,500 compounds they found that "similar effects (phenotypes) can be obtained from dissimilar molecular structures. On the other hand, similar structures can cause dissimilar effects. Different information is obtained from both sources and employing both types of knowledge is crucial."

Drug Design

Vitae Pharmaceuticals (www.vitaepharma.com) is one of a number of companies assessing low-molecular weight compounds





which rarely have well-described 3-D protein structures given the extreme difficulty of isolation, purification, and crystallization of hydrophobic molecules.

To overcome this daunting roadblock, the company's Predict technology was designed to encompass the in silico screening of a library of more than four million-prospective compounds. Epix seeks to target proteins with clear biological validation that are on the market or in development, with the goal of improving on existing selectivity, half-life, and pharmacology.

Validation

Applicable to all GPCRs, each new model undergoes a validation process to establish the internal consistency of structure. This encompasses agreement with available site-directed mutagenesis data and binding pocket location data including correct interactions with key residues in the binding pocket.

Another component of the platform is a reconstruction protocol in which success in an enrichment experiment proves that the docking procedure is able to pick out known ligands that have been embedded in a large and random 10,000 member drug-like compound library. The validity of this approach is proven by the fact that more than 20 candidates have successfully passed through this validation process, according to Dr. Shacham.

The next phase of the program involves the in silico screening process in which the candidate receptors are confronted with the virtual compound library, and in which the Predict program looks for virtual hits using the criteria of $K_i < 10$ mM, a validated dose response, and a clear intellectual property search.

Subsequently, the qualifying candidates are put through an optimization program featuring extensive use of computational tools including 3-D structures and predictive ADME to navigate the multiple possible optimization pathways. These prioritize choices to synthesize or not to synthesize. "This is an efficient process, robust in theory, and agnostic to the receptor class," Dr. Shacham said. "It is hypothesis driven so there must be a specific reason for each synthesized compound; those that don't fit the model binding site or bind to an off-target structure will not be synthesized."

Speeding Up the Process

So what role does high-content screening play when it comes to drug discovery? In theory, technology that pole vaults over in vivo and in vitro testing of vast numbers of compounds should go a long way toward optimizing the discovery process, saving time and money. However, in silico results are not definitive, since they do not cover a plethora of factors that influence the fate of a project. These include biophysical properties of the compound, strategic and safety issues, and, frequently, IP considerations.

Bender argued that "high-content imaging fares rather well when it comes to the success of HTS campaigns, and potentially, by integrating in silico tools such as ligand-

target prediction, those campaigns may fare even better in the future."

Delphic prophecies predicting breakthroughs in drug development using in silico modeling technologies abound.

A recent Deloitte report, *The Changing Face of R&D in the Future Pharmaceutical Landscape*, bemoans the current state of the pharmaceutical industry and predicts that companies will exploit "virtual R&D processes with significant outsourcing to

maximize flexibility and manage development risk."

While in silico modeling holds much promise, it still remains an unproven technology, as there are no drugs that have traveled the epic voyage from lead compound optimization to the marketplace. A number of anti-HIV drugs have been developed through programs that include in silico modeling, however.

As pharma companies are understand-

ably reticent to reveal the details of their discovery programs, it is not surprising that the precise role of in silico modeling in drug development is somewhat opaque. Moreover, the very long gestation period required in today's world of drug development means that new strategies may take years to see their effects realized. With many small and large players in the field, the next few years should see the vindication of this approach. **GEN**

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