Innovations

Coming full circle
Vertex Pharmaceuticals, Inc.

Chemistry & Biology October 1998, 5:R267–R268

Establishing Vertex Pharmaceuticals Inc. (Cambridge, Massachusetts) was a bold move. Vertex was not based on the biotech model of injectable proteins and biology, but on the big drug company model of chemistry.

Vertex wanted to make the chemistry rational using structural information, a combination that only Agouron Pharmaceuticals Inc. (La Jolla, California) had relied on thus far. Both Agouron and Vertex have had their first successes with HIV protease inhibitors: Agouron's Viracept (nelfinavir) is on the market, and Vertex's Agenerase (amprenavir) has passed phase III trials (Figure 1).

But, as described in the book 'The Billion Dollar Molecule,' Vertex was founded with the promise that it would rationally design a blockbuster molecule — a nontoxic version of the immunosuppressant FK506. The book was primarily a tale of how Vertex, like almost any start-up, lurched from one crisis to the next and somehow survived. In the end the FK506 project was a failure, and the book was also an exhaustive chronicle of that failure.

Vertex rescued itself with other drug targets, including HIV protease. But in a strange twist, two of the many projects lined up behind amprenavir are based on molecules from the original FK506 program. The early toil was worth it after all.

Chasing the billion dollar molecule

Rejection of an organ transplant means a new transplant or death, so transplant recipients are willing to put up with the side-effects of immunosuppressive drugs. But for those with autoimmune diseases such as rheumatoid arthritis and psoriasis, the level of acceptable side-effects is far lower. The patient population and possible drug revenues in this market are, however, far higher. This is what drove Vertex to improve on FK506, which is now marketed by Fujisawa Pharmaceuticals (Japan) as the transplant drug Prograf (tacrolimus).

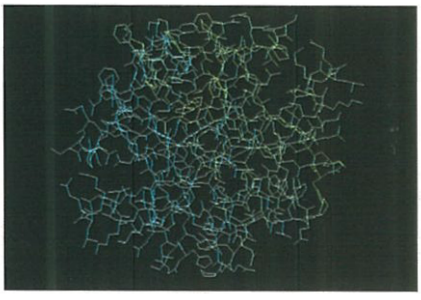
With hindsight FK506 appears to be a curious choice for a company hoping to exploit protein structure. Discovery of FK506's proposed target — the binding protein FKBP12 — was not announced until after Vertex was founded in 1989. A crystal structure of FKBP12 would not be forthcoming until mid-1991. Until then, Vertex's chemists were stuck doing traditional medicinal chemistry.

What Vertex did have was a simple assay, as FKBP12 was found to help protein folding by rotating particular bonds between amino acids. Unfortunately there was no proof that this rotamase activity, or even FKBP12, was relevant to the clinical effects of FK506.

As Vertex, Merck, and Stuart Schreiber of Harvard University made more FK506 derivatives, this lack of proof became a more urgent concern. Schreiber's FK506 derivative 506BD, for example, bound to FKBP12 and inhibited its rotamase activity, but had no immunosuppressant activity. Schreiber's result came out just three months after Vertex had convinced Chugai Pharmaceuticals (Japan) to commit $30 million to the project.

Vertex and Merck kept going. "If you start from the presumption that rotamase is the whole story then as soon as you find a 506BD that sends up a red flag and you would drop the project," says Mark Murcko, vice president and senior research fellow at Vertex. "But there was the therapeutic importance of the area, and there was already a drug [tacrolimus] that looked like it could be marketed. The red flag of 506BD simply meant the problem was harder than we thought."

Figure 1



Amprenavir (in yellow) bound to the active site of HIV protease.

The new problem was to inhibit the real target of FK506, which in early 1991 was unknown. Again the structure-based design team was stalled. And then the second blow came — the target for immunosuppression by the FK506–FKBP12 complex was the phosphatase calcineurin. As calcineurin is abundant in the brain, the neurological side-effects of FK506 were probably a result of the drug binding its true target, not a result of some unwanted interaction that could be designed away.

New targets

Luckily Vertex had secured its initial public offering the month before Schreiber announced the calcineurin results, so the company was financially on its way. And by now it had a back-up project, which was based on the HIV protease structure that Vertex's Manuel Navia had solved when he was at Merck. The concept for amprenavir began with chemist Roger Tung, who brought ideas for several new compound classes to the molecular modelers. They settled on the N,N-di-substituted sulfonamides.

A new drug application for amprenavir will be filed towards the end of 1998. Vertex will be hoping for an entry like that of Agouron's Viracept: first-year sales (in 1997) of close to $300 million. But amprenavir faces an increasingly crowded market, with four HIV protease inhibitors already approved.

Murcko is confident. "There's no question that the world needs better protease inhibitors," he says. Whereas existing drugs have complicated dosing regimens and often show cross resistance, amprenavir is taken twice daily, with or without food, and shows few significant drug interactions. *In vitro*, the major amprenavir resistance mutation does not confer resistance to other protease inhibitors.

Using structure, any way you can

Amprenavir originated with the musings of a medicinal chemist, not with a computer constructing a molecule that was the best fit for an active site. Vertex, it seems, is not purely a structure company.

"The most important thing has been and always will be creativity," says Murcko. "Structure helps people think through their ideas. It's not so much about coming up with brand new ideas but a new form of information that can be integrated into a project."

Not that Vertex hasn't tried the pure approach. "In the early 1990s we were interested in exploring the limits of *de novo* design," says Murcko. Vertex researchers came up with computer programs that built possible drugs in an active site from scratch. "We could be very successful at generating novel ideas, but many of the compounds were too difficult to synthesize. The effort to make one compound was often equal to the effort to make hundreds of analogs of simpler compounds."

The alternative is to create a virtual library of chemicals, and narrow it down to a manageable size using various filters. First, a program discards chemicals that don't look like drugs (because they have toxic functional groups, are too large, too lipophilic, or too difficult to synthesize). Reagents or products can be clustered based on structural similarity, and one of each group selected as representative for testing purposes. The three-dimensional conformations of the remaining compounds can then be compared to the structure of known inhibitors, and only those compounds with certain key structural features (such as two hydrogen-bond donors separated by 10 Å) are selected.

The chemicals are now ready for docking into the active site of the target. Those compounds that can be docked must then be scored for binding, taking into account the effect of entropy changes, water displacement, lipophilic contacts, hydrogen bonds, and ionic contacts. The winners are then synthesized and tested in an assay.

All the *in silico* filtering is needed because the final steps are computer-intensive. "The slow step is generating three-dimensional conformations for the ligand you want to dock," says Murcko. And to get through a reasonable number of compounds, scoring functions that are already error-prone must take short cuts.

Numerous groups have taken a stab at creating scoring functions. "We have implemented all of the public ones, and approximately six of our own," says Murcko. "It works best with consensus scoring — you run all of [the scoring functions] and then pull out compounds that do well with the large majority of functions."

The final result is an idea, not a specific compound. "If you had a perfect scoring function you could do a *de novo* design and hand [an idea for] a single chemical to the chemist," says Murcko. "But that's not what we have. When we have a good concept, we're best off making a small series of compounds around the idea."

The amount of structural information used in different project varies widely. "We are big fans of high-throughput screening as well," says Murcko. But for a project designed to stop inflammation by targeting interleukin-1 converting enzyme (ICE), structure led the way. "The ICE lead was a designed compound from a crystal structure," says Murcko. "It's a clean example of structure-based drug design. In a matter of weeks after the structure we had the ideas that led to [the clinical candidate] VX-740."

The return of FK506

As the FK506 project was dying, a remarkably similar project was rising in its place. Like FK506, mycophenolic acid (MPA) is a toxic natural product with immunosuppressant activity. But it differs from FK506 in being a simple uncompetitive inhibitor, in this case for the rate-limiting enzyme for *de novo* guanosine biosynthesis. There are no FKBP12 complications here. Vertex solved the structure of the MPA–enzyme complex, and used it to design VX-497, a novel inhibitor unrelated to nucleosides, which is entering phase II trials. MPA is toxic because it gets glucuronidated and therefore concentrates in the bile, but VX-497 should not be glucuronidated.

FK506 itself is back on the scene in two guises. Like many large lipophilic molecules, FK506 binds the multi-drug resistance channels that are responsible for pumping cancer drugs out of the cell. A compound from the FK506 program that blocks two types of channels is in phase II cancer trials.

A move into the nervous system started with Solomon Snyder (Johns Hopkins University, Baltimore, Maryland), who found that FKBP12 was highly expressed in the nervous system, and Bruce Gold (Oregon Health Sciences University, Portland, Oregon), who used Vertex compounds from the FK506 program to speed nerve regeneration. Snyder is collaborating on similar work with Guilford Pharmaceuticals Inc. (Baltimore, Maryland).

This neurophilin project, as Vertex calls it, is truly reminiscent of the original FK506 quest. Once again, the therapeutic mechanism is unknown, and the drug development process is largely non-structure-based. But with a role for calcineurin in nerve regeneration ruled out, Vertex is hoping that the return of FK506 will prove to be triumphant.

William A. Wells, Biotext Ltd
1095 Market Street #516, San Francisco, CA 94103-1628, USA; wells@biotext.com.