

Companies Team Up to Target Protein Kinase Inhibitor

The search for an effective protein tyrosine kinase (PTK) inhibitor that can target and impede specific causes of cancer-related cell proliferation has precipitated a joint inventorship patent between Signase, Inc.

(Houston, TX) and Tripos, Inc. (St. Louis, MO). The patent for a thienopyrimidine-based inhibitor of the Src family covers claims for composition of matter, methods of synthesis, and methods of use.

PTKs are enzymes that drive the cellular signaling process and promote the body's immunological defense against pathogens. When the cellular signaling process amplifies and PTKs proliferate, diseases such as cancer can occur. "The goal of PTK inhibitors in oncology is to inhibit the

overactive PTK in the cancer cells and moderate the cellular proliferation rate," said Tom Thrash, senior scientist at Signase. In normal cells, PTK activity is fairly low and remains in a regulated state. In a primary tumor cell, the PTK activity is "upregulated," and in a metastatic tumor cell, the activity is upregulated even further. If the PTK activity is controlled, the growth of tumor cells can at the least be slowed and at best, stopped. The key becomes the selective inhibition of PTK, which is what the two companies are striving to accomplish.

"Specificity is a complicated issue," said Thrash. "The cellular signaling cascade is redundant with multiple PTKs driving the process. Thus, inhibition of one branch of the pathway may not be enough to stop the signaling process,

and drugs that inhibit multiple PTKs, or a cocktail of several specific PTK inhibitors, may be most beneficial." However, harmful side effects are less likely to result from more-specific drugs because the drugs will not affect other targets. "We believe that the location of Src in the cellular signaling cascade represents the highest potential of a specific inhibitor producing the best cellular response," said Thrash.

In the drug development process, the drug is first screened against an individual target, in this case the Src family enzymes. Once a drug

is identified, the search continues for a cell line that expresses the target to see whether the compound is active in cells. If the compound is active in both assays and meets other criteria, it is tested in animal models. If there is no activity in animal models, several possibilities must be considered, among them that the drug is being metabolized into an inactive metabolite, the route of administration does not allow biouptake, or the drug is binding to the plasma proteins in blood and therefore is not bioavailable for its intended purpose.

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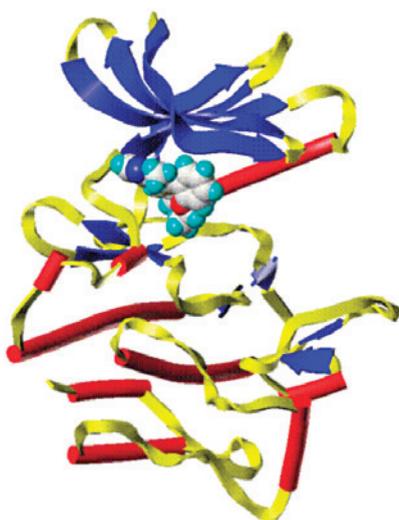


Figure 1: Highly potent c-Src inhibitor docked into the active site of c-Src. The docking conformation was generated by FlexX, available from Tripos, Inc. The c-Src coordinates are from the PDB file 2SRC (W. Xu et al., *Molecular Cell* 3, 629 [1999]). The image was created using Tripos's Sybyl software.

Abbott Ramps Up Biotech Manufacturing to Meet Humira Demand

Abbott Labs is increasing manufacturing capacity at its Bioresearch Center in Worcester, MA, to keep pace with demand for its new rheumatoid arthritis treatment. Given the tradename Humira, the new biologic drug hit the market in January.

According to Alejandro Aruffo, PhD, president of Abbott Bioresearch Center and Abbott Labs divisional vice-president, market acceptance of Humira has been "very good." Humira is supplied in a ready-to-use formulation for subcutaneous injection in a prefilled,



single-use syringe customized for arthritic hands.

Says Aruffo, Humira's early success demonstrates the value in developing not just a treatment but also a means of delivery targeted to patients. "From the beginning, we set a goal of a highly effective and patient-friendly compound," says Aruffo. "We considered not just the product but also the dosing and how it's used. The syringe was part of the design all along."

The patient-friendly syringe received an Arthritis Foundation ease-of-use commendation for its large plastic wings, large stopper and indented thumb rest grip (see photo), which make it easier for

Monoclonal antibodies take on rheumatoid arthritis

As targeted disease fighters, therapeutic monoclonal antibodies are revolutionizing the treatment of many illnesses, including rheumatoid arthritis. These agents work by recognizing and neutralizing antigens in the human body.

Rheumatoid arthritis is a chronic inflammatory disorder that damages joints by eroding bones and cartilage. The joints most commonly affected are those of the fingers and toes, hands, feet, and wrists. Although there is no known cure, sufferers seek treatments that alleviate the pain and inflammation and slow disease progression.

Before Humira, treatment for rheumatoid arthritis included nonsteroidal anti-inflammatory drugs (NSAIDs), which reduce swelling and relieve pain by reducing joint inflammation. NSAIDs include such prescription medications as Cox-2 inhibitors and such over-the-counter remedies as aspirin and ibuprofen. Although they help relieve pain and swelling, NSAIDs do not slow the progression of the disease and its resulting damage.

arthritis hands to hold and use than traditional syringes. Dosing is every other week. Treatment choices before Humira were either infusion or a product that first had to be reconstituted and then administered by injection.

Indications are that Humira may go on to become "a bona fide blockbuster," says Eric Bolesh, senior analyst at Cutting Edge Information, a business intelligence consultancy in Durham, NC. "And for these you don't skimp." He believes that Abbott will try to derive maximum revenues from Humira and has "shaken things up" internally to get the product launched.

Cutting Edge Information's chief operating officer Adam Bianchi agrees: Abbott has gone to great lengths to free up resources "to get manufacturing and operations up as fast as possible. The entire organization is pitching in."

Abbott recently announced a manufacturing expansion to meet future demand for Humira as well as other biologic products in its pipeline. "Manufacturing is going well—we are exceeding expectations," says Aruffo. As of February, Abbott is hiring at the Worcester facility, which provides biologics manufacturing services to the company and selected partners. Independent manufacturing suites are available for prod-

Corticosteroids are another treatment for rheumatoid arthritis. These are hormones taken orally or given by injection to reduce inflammation.

Humira, by contrast, is a monoclonal antibody that mimics antibodies that occur naturally in the human body. In the case of rheumatoid arthritis, monoclonal antibodies recognize and neutralize the protein associated with inflammation.

The earliest monoclonal antibodies, from about 25 years ago, were derived entirely from mouse (murine) cells. Chimeric and humanized monoclonal antibodies followed, with both types still produced in mice but the latter containing some 90% human components.

Human monoclonal antibodies are generated of human-derived components, but in test tubes rather than mice. FDA has approved 10 monoclonal antibodies for use in humans. More than 50 others are in clinical trials worldwide.

ucts based on both microbial and cell culture technologies. An operations staff of 200 provides manufacturing and support functions.

Humira is the result of a collaboration between Abbott and UK-based Cambridge Antibody Technology. As part of the collaboration, Abbott selected several target antigens and a joint Abbott/Cambridge research team then isolated and optimized Humira. Abbott owns the worldwide rights to Humira and books all revenues. Cambridge Antibody Technology receives a royalty fee based on sales.

According to Abbott, Humira is the first human monoclonal antibody approved for reducing the signs and symptoms, and inhibiting the progression of structural damage, in adults with moderate to severe rheumatoid arthritis, who have had insufficient response to one or more traditional antirheumatic drugs. In contrast to molecules made from mice (the norm for monoclonal antibodies), Humira is derived from human sequences (see sidebar).

Humira received FDA marketing approval in late 2002, a quick nine months after Abbott filed its application. One reason for the speedy approval may be the product's potential to lower

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Flexible Simulation Tool Aids Filter System Selection

Flexibility is an important parameter in all aspects of process development, but particularly when determining filtration system requirements. Recognizing this need, Pall Corporation (East Hills, NY), has developed the Palltronic filter manager. As described by Derek Pendlebury, Pall director of marketing, in addition to lessening the burden of the scale-up and scale-down process by computerizing traditional methods of predicting filter requirements, "the Palltronic system provides process developers with new levels of flexibility. It allows clients to test two different filters at any prefilter and final-filter combination, and all of the combinations can be tested under constant flow and constant pressure. That type of versatility is paramount in process development environments."

Depending on the production environment, the Palltronic system simulates production conditions at constant flow or constant pressure within the laboratory. This means users can determine the required size of a filtration system for full production without the need for several sizing trials.

Through the use of separate filter holders, the system has the ability to test two filters simultaneously, but independently, of one another. This capability allows users to analyze several different combinations of pre- and final filters to determine the optimal combination within the product environment.

Through a series of electronic prompts, users set test parameters such as product volume, viscosity, and filter type. Says Pendlebury, "Once the start button is pressed, the system records measurements at the rate of 10 readouts



per second." Test results are displayed on the screen in table or graph format. As many as 50 test results can be stored in RAM memory and made available for downloading. All electronic records are 21 CFR Part 11 compliant.

System security is organized around three personnel levels (operator, supervisor, and manager), which controls the type of access granted to specific users. User identification and password-protection ensures that only authorized users access the system and that unauthorized users cannot change the parameters of the program.

The Palltronic Filter Manager will be introduced during Interphex, 31 March–2 April 2003 in New York City.

Felicia Pride

PDA appoints new president



The Parenteral Drug Association (PDA) has appointed Neal G. Koller as president, succeeding Edmund M. Fry, who resigned from the position in September 2002 to join IVAX Pharmaceuticals. In making the announcement,

PDA board of directors chairman, Floyd Benjamin, stated, "I am extremely pleased that Neal Koller has accepted the chief staff position at PDA. His track record as a highly successful global manager in this industry will ensure that PDA maintains its strategic focus in this challenging environment."

Koller joins PDA with more than 22 years of experience in medical devices and biopharmaceuticals. Most recently he was president of WelCare Group in Rome, Italy. Before his post at WelCare, Koller was president and CEO of Dovetail Technologies, president and CEO of Sound Diagnostics, and had a long affiliation with Sherwood–Davis & Geck, subsidiary of Wyeth Pharmaceuticals.

Commenting on his appointment, Koller offered, "I am truly excited about the opportunity to lead PDA as president and look forward to building upon the strong foundation of the organization."

Originally formed in 1954, PDA currently boasts a global membership of more than 10,500 scientists involved in the development, manufacture, quality control, and regulation of pharmaceuticals.

Pharmaceutical and Biotech Sectors Drive Cleanroom Market Growth

Technology advances and market growth in the pharmaceutical and biotechnology fields are trickling down to the benefit of the contamination control market.

"Strength in pharmaceutical and biotechnology, flat panel, and food sectors will offset the weakness in the semiconductor

Along these lines, he sees "a trend toward minienvironments, following the trend in the semiconductor market," says McIlvaine.

"There's also an increasing trend toward the use of fan-filter units, with many such units being used to replace a

Worldwide pharmaceutical forecast (\$ millions)

Application	2000	2001	2002	2003	2004	2005	2006
Employees (1000s)	128.00	136.47	145.59	155.43	166.05	177.54	190.02
Room revenues	311.54	332.16	354.35	378.30	404.16	432.11	462.48
Space additions*	1.48	1.58	1.68	1.80	1.92	2.05	2.20
Space in use*	12.80	13.65	14.56	15.54	16.61	17.75	19.00

* Millions of square feet

sector for the cleanroom hardware and consumables marketplace," says Robert W. McIlvaine, President, The McIlvaine Co., a cleanrooms market research concern in Northfield, IL.

McIlvaine forecasts that by 2004, sales of cleanroom hardware and consumables worldwide will rise above the 2000 peak of \$6.5 billion and will continue rising through 2006 to \$9.1 billion. These figures are cited in his continuously updated report, "Cleanrooms: World Markets." The most recent update to the report was posted in mid February.

McIlvaine forecasts the worldwide cleanroom consumables market (clothing, wipes, furniture, laundry services, etc.) to rise to \$4.7 billion in 2005, up from the actual of \$3.3 billion in 2002. Concerning orders for cleanrooms, he expects the market to reach \$3.9 billion in 2006, rising from \$705 million in 2002.

Chief among the pharmaceutical/biotech drivers contributing to cleanroom market growth is an increasing trend toward the use of isolators, in part as a result of the higher potency of some drugs and the corresponding need for containment. Other factors driving the growth, according to McIlvaine, are stringent regulatory constraints. Both pharmaceutical and biotech companies are "upgrading and enhancing their facilities, not just to help meet regulatory requirements, but also to protect workers," says McIlvaine.

big plenum in the ceiling and multiple filters." McIlvaine expects that in some pharmaceutical facilities, use of perhaps 1000 or more fan-filter units has now become economically feasible.

"It's an amazing production feat that the price point of fan-filter units has gone so low," he says. "Their use in pharmaceutical and biotech facilities is a steadily increasing trend that we've been watching over the past five years."

The cleanroom industry had been accustomed to double-digit growth increases. However, for the period 2000 to 2006, annual growth in hardware sales will be just 6% and consumables sales will be just 5%. The lack of growth in the semiconductor sector will offset substantial growth rates in the other sectors. Most of the growth will be in Asia, according to McIlvaine.

Suppliers to the industry will benefit unevenly from future growth. Those emphasizing sterility and microbial control will continue to experience rapidly growing markets, according to the report. Those focused on particulate and inanimate contaminants will experience slower growth through 2006.

Thereafter, the potential in microelectromechanical systems, wireless communications and nanotechnology could put the particulate and inanimate sectors on the fast track.

George Miller

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"PTK inhibitors that are currently in clinical trials show limited toxicity," said Thrash, "and in animal models we have tested, there has been no evidence of toxicity that we have seen. Generally, inhibitors that are specific for a particular kinase are more likely to express a favorable toxicity profile. Because Signase's thienopyrimidines are specific for the Src kinase family, they are essentially nontoxic."

Signase's principle contribution to the joint research project is its knowledge of the Src family of enzymes. "Our expertise is a chemistry-based approach to drug discovery," Thrash said. "We do a large quantity of our chemical syntheses in house such that we make the drug in house, and we test it against our enzyme targets in house. We then are able to very rapidly turn around a chemistry result to a biochemical result. We will leverage our expertise in drug discovery with Tripos's expertise in drug design. Tripos also provided us with a LeadQuest Volume I chemical library of 10,000 compounds for initial screening." When Signase identifies the lead compounds for testing, Tripos will step in to develop a predictive molecular model to help determine the efficacy of the molecule.

Tripos will use many different software technologies during the collaboration, according to Mike Lawless, senior project manager at the company. To visualize the target proteins and ligands, the company's Sybyl system will read the

3D coordinates—obtained from an X-ray crystal structure—of the kinase and display the atoms and bonds on the computer screen. Using the modeling software, "we can rotate the protein, create different displays of the protein, and compute surfaces of the protein," Lawless said. "This helps us identify pockets in the protein where ligands, cofactors, and substrates can bind. It also allows us to investigate potential hydrogen bonds and salt bridges."

When the system docks the kinase inhibitor in the crystal structure of the kinase, the program automatically generates potential conformations of the ligand as it interacts with the kinase. "The more interactions, then the stronger the ligand binds to the kinase," said Lawless. The techniques will show how known inhibitors bind and rank synthetic candidates for their potential as inhibitors. This narrows the field so that compounds with greater potential can then be synthesized.

A Tripos software program will model large datasets of molecules to determine similarities between compounds. The system will provide structure-activity relationships, examine the high-throughput screening results produced at Signase to identify false positives and negatives, and create a 2D distribution of the screened molecule. By highlighting the active compounds and their neighbors to quickly obtain structure activity relationships, the researchers can decide which molecules will go into lead generation and optimization.

When a set of ligands with experimental IC₅₀ values is input into a Tripos molecular modeling program, a 3D grid is placed around each ligand, and the steric and electrostatic interaction between each grid point and the atoms in the molecule are computed. These interaction points are used as descriptors. Next, a statistical analysis of the ligands' activities and the grid points is performed that correlates to the molecule's IC₅₀ with its grid-point values. "If the program creates

a good correlation, then the model can be used to predict the potency of other molecules," said Lawless. "This again allows us to rank synthetic targets to decrease the hit to lead time."

Tripos's virtual library technology will enable the researchers to search virtual libraries containing trillions of structures "typically in less than 12 hours," according to Lawless. The company then will build virtual libraries of combinatorial reactions using various searches, including using a known kinase inhibitor as the query molecule. The system will find molecules that have shape and features that are similar to the known inhibitor, a procedure that produces potential kinase inhibitors composed of various structural chemotypes as the query molecule. The company's technology also can "predict the biological activity of molecules in large virtual libraries to quickly optimize a lead candidate," says Lawless.

Signase and Tripos have developed a set of benchmarks for their compounds and have synthesized all leading Src tyrosine kinase inhibitors from published and patent literature to be placed in clinical trials. "We would like to choose a clinical candidate this year," Thrash said. An oral formulation of the thienopyrimidines is in development. Although two PTK inhibitors are already on the market—Gleevec, Novartis's BCR-ABL protein inhibitor for the treatment of chronic myeloid leukemia, and Iressa, AstraZeneca's selective epidermal growth factor receptor-tyrosine kinase inhibitor—no Src tyrosine kinase inhibitors are in clinical trials or on the market. "Src is a gatekeeper enzyme that regulates many PTKs," Thrash said. "If Src can be regulated, you will be able to keep all the PTKs in regulation as well. This represents a rich field in possibly regulating many disease states."

The eight members of the Src family are being investigated by major pharmaceutical companies for treatment applications that include cancer, osteoporosis, cardiovascular and autoimmune diseases, the management of viral infections, and the control of graft rejection after organ transplantation.

Cheryl Mikkola

Condolences

We at *Pharmaceutical Technology* magazine wish to express our sympathies to the families, friends, and loved ones of the four people who lost their lives at the Kinston plant tragedy on 29 January 2003.

James Byrd—contractor; Kevin Cruiss—contractor; William Gray—molder, employed with West since 1985; and Lenni Wilkins—material handler, employed with West since 1986.

West Pharmaceutical Services, with headquarters located in Lionville, PA, used the Kinston plant to manufacture syringe plungers, intravenous components, and compound rubber materials for use in West facilities. The critically damaged building housed the Automated Compounding System (ACS), where the bulk of rubber materials are mixed into formulations for molding into medical device components.

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healthcare costs for senior citizens. "That's a big driver at FDA right now," says Bianchi.

Bianchi adds that the completeness of the Abbott submission also probably had a lot to do with the speed of approval. "Abbott has a lot of experience with FDA applications. That's one of the reasons Cambridge would want to work with Abbott—for that expertise."

Another factor was the size and scope of the clinical trials, which were large by biologic standards. Humira was studied in 2300 patients around the world in 23 clinical trials, with some patients receiving the treatment for more than three years. The customized syringe was used in some of the trials. Clinical data in the regulatory submission included data evaluating efficacy based on inhibition of the progress of structural joint damage and on improvement in rheumatoid arthritis symptoms.

The European Agency for the Evaluation of Medicinal Products accepted

Abbott's submission for Humira in April 2002. Approval is anticipated in mid-2003. "The EU is tracking right on schedule," says Aruffo.

Aruffo says he was "pleasantly surprised" by the speed of the FDA approval but was prepared to ramp up manufacturing quickly once approval came. Concerning the speed of the approval, Aruffo says that many factors influenced it: "We gave FDA a very complete package," he says. "Also, manufacturing is done at a state-of-the-art facility. And we provided evidence that the process is reproducible."

Abbott's Bioresearch Center contains a 90,000-ft² production area for Humira and other products in various stages of development. The center features multiple manufacturing suites with as much as 6000-L fermentation scale for products. Antibody capabilities include a technology platform that incorporates proprietary and in-licensed technologies, including phage antibody display technology and mammalian cell expres-

sion systems to produce fully human monoclonal antibodies and small-molecule angiogenesis inhibitors. The center also accommodates yeast and bacterial production of other therapeutic proteins.

Manufacturing of Humira may eventually shift to a new biotech facility that Abbott is building in Barceloneta, Puerto Rico, but will remain in Worcester for the foreseeable future. Construction of the \$350-million Barceloneta plant started in December 2002 and is scheduled to be completed in 2006. The expansion will create approximately 200 jobs and approximately 800 temporary jobs as a result of construction-related activities.

George Miller

USP Establishes Admissions Criteria for Dietary Supplements

The United States Pharmacopeia's (USP's) Council of Experts Executive Committee has approved new safety admissions criteria for dietary supplement monographs. Published in the January–February 2003 edition of the *Pharmacopeial Forum*, the criteria call for an assessment of proposed monographs based on human data (e.g., clinical studies and postmarketing surveillance), pharmacological data (e.g., pharmacokinetics), contemporaneous extent of use in the United States and worldwide, and historical usage.

According to David Roll, director of dietary supplements at USP, concern over the safety of dietary supplements prompted the organization to establish a four-part classification system that identifies articles according to the safety issues deemed "present when the article is used and formulated appropriately," which could prohibit a monograph from being developed.

Maribel Rios