Astex shapes CDK4/6 inhibitor for approval

Kisqali (ribociclib), a frontline treatment for metastatic breast cancer developed by Basel, Switzerland–based Novartis, won approval from the US Food and Drug Administration (FDA) on March 13. The small-molecule drug approval is a success for the Swiss pharma but also throws the spotlight on Astex, a drug development company based in Cambridge, UK, and its drug-fragment screening platform.

Kisqali inhibits a pair of cyclin-dependent kinases, CDK4 and CDK6, which regulate various parts of the cell cycle. It is the second CDK4/6 inhibitor to reach the market, following New York-based Pfizer's first-in-class Ibrance (palbociclib), which the FDA greenlit for use in advanced breast cancer in 2015 (*Nat. Biotechnol.* **33**, 323–324, 2015).

The drug's go-ahead is for use in post-menopausal women with hormone receptor positive and human epidermal growth factor receptor-2 negative ($HR^+/HER2^-$) breast cancer. Last year, a phase 3 trial had shown that Kisqali combined with letrozole, an anti-estrogen drug, could shrink tumors. The CDK4/6 inhibitor helps to stop tumors becoming resistant to letrozole, as estrogen receptors also depend on the CDK4/6 pathway to drive cell proliferation. Compared with letrozole alone, Kisqali plus letrozole reduced the risk of disease progression or death by 44%, and caused tumors to shrink by 30% or more in 53% of patients.

The approval was cause for celebration at Novartis—analysts forecast annual peak sales of $1.5 billion for Kisqali—and at Astex, which partnered with Novartis to optimize the lead structure that resulted in Kisqali.

Astex is a leading proponent of fragment-based drug discovery (FBDD), an approach with a couple of market approvals already under its belt that has become increasingly popular over the past decade (*Nat. Rev. Drug. Discov.* **16**, 225–226, 2017). Drug hunters are increasingly using fragments as a technique for finding hits for a target of interest, eschewing the vast libraries of millions of drug-like molecules used in conventional high-throughput screening (HTS), the longstanding workhorse of big pharma's drug discovery programs. Instead, FBDD screens thousands of molecular fragments, much smaller than typical drug molecules, for their ability to lock into the pockets of target proteins. And whereas HTS tests each compound for its biological activity against proteins or cells, FBDD screening typically relies on biophysical techniques like X-ray crystallography and nuclear magnetic resonance to assess binding.

Although Kisqali was not developed from a fragment, Astex's rapid crystallography screening system played an important role in developing the drug, says Christopher Brain, group leader at the Novartis Institutes for BioMedical Research in Cambridge, Massachusetts. When Novartis found the initial hit that led to Kisqali, the molecule inhibited CDK1 but not CDK4/6, so researchers had to change the molecule's structure to invert that selectivity. "There was quite substantial modification," says Brain. To enable that process, Astex solved the crystal structure of CDK4 for the first time (*PNAS* **106**, 4166–4170, 2009), which then allowed them to fine-tune the initial hit by studying how various fragments interacted with the protein. "We were able to understand how those compounds were binding," says Harren Jhoti, CEO of Astex, "and using that information we were able to build the selectivity of the lead compound for CDK4/6."

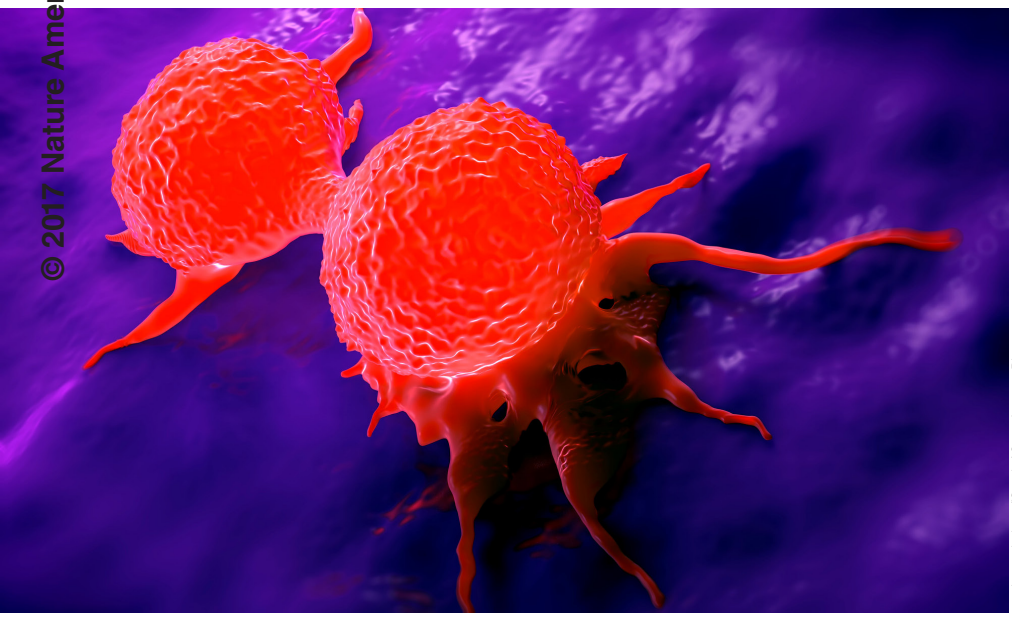
X-ray crystallography is usually a slow business, not least because of the time needed to grow protein crystals, and then acquire and analyze X-ray data. So Astex began to automate much of this process when it was founded in 1999, using computational software and robotics. "There was huge skepticism for the first couple of years," Jhoti remembers. These days, Astex can typically generate 80 different crystal structures over one weekend (a feat that might take conventional laboratories a few weeks to complete, reckons Jhoti), and this speed was crucial for shaping Kisqali. "We were able to quickly study lots of [protein plus fragment] complexes, so we could understand how many different compounds bind to the same target," says Jhoti. Unlike conventional crystallography, "we were able to solve the structure much more rapidly, and solve many more of them, so it helped to guide the chemistry in real time."

FBDD got its start in 1996 when researchers led by Stephen Fesik, then at Abbott Laboratories, used NMR to screen small molecules for their ability to bind to proteins. Small companies like Astex, Vernalis, in Winnersh, UK, Vertex in Boston and Plexxikon in Berkeley, California, subsequently pioneered the growth of FBDD. "Now all big pharma companies do it," says Jhoti. Indeed, several have bought up existing expertise: Plexxikon is now part of Tokyo's Daiichi Sankyo, and Astex was acquired by Otsuka Pharmaceutical in Tokyo for $886 million in 2013.

The attraction was that FBDD offered to solve a problem that had long dogged HTS. Despite the enormous size of HTS libraries, they often yield vanishingly few bioassay hits. This is partly because complex, drug-like molecules have a greater number of potential interactions with a target protein, some of which can actually stymie the binding process.

In contrast, FBDD uses chemical compounds that are around half the size of most small-molecule drugs. Based predominantly on key elements such as carbon, nitrogen and oxygen, they usually contain fewer than 20 non-hydrogen atoms. Many can be bought from chemicals suppliers, although about 40% of the fragments in Astex's library are proprietary compounds synthesized in-house or with collaborators.

A library of just a few thousand fragments contains enough chemical variety to ensure that some stand a good chance of binding to a protein, albeit much more weakly than a typical HTS hit. Medicinal chemists can then develop



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Kisqali stops breast cancer cells dividing by blocking the CDK4/6 cell cycle enzymes.

UC granted CRISPR patents in Europe, appeals in US

The University of California (UC), the University of Vienna and Emmanuelle Charpentier have filed an appeal to overturn a decision by the US Patent and Trademark Office's Patent Trial and Appeal Board (PTAB), which ruled that patents covering the use of CRISPR–Cas9 in a cellular setting, issued to the Broad Institute, Harvard University and the Massachusetts Institute of Technology, did not interfere with patent applications filed by the UC group. In February the PTAB found that although UC's patent application and the Broad's patents and patent application overlapped in scope, the claims in the interference are separately patentable. The appeal by the UC group, filed on April 12 in the US Court of Appeals for the Federal Circuit in Washington, seeks to have the PTAB reinstate the interference. The UC group's appeal will be bolstered by a recent decision by the European Patent Office (EPO). In March, the EPO said it would grant a patent covering the broad use of CRISPR–Cas9 in both cellular and non-cellular settings to Dublin-based ERS Genomics, the University of Vienna and UC, a finding that puts the EPO at odds with the USPTO. Charpentier is a cofounder of ERS Genomics, which holds the rights to her intellectual property covering all CRISPR applications other than human therapeutics. "It is gratifying to see the EPO recognize with this broad patent the contributions of Dr. Charpentier and her colleagues on the invention of this important technology," said Eric Rhodes, CEO of ERS. "[W]e are hopeful that we can expect similar outcomes throughout the roughly 80 countries that use a first-to-file system like Europe."

> **"I'd much rather fight Ebola in West Africa than in West Dallas. Defense is generally important. These things are part of the defense of the country and its development too."** Congressman Tom Cole (R-OK) makes a point on the priorities of the current administration in favor of defense spending while scaling back funding for science. (*Bloomberg*, 30 March 2017)
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> **"Do you pray for an iPhone? God forbid, I've had situations with my parents, my loved ones, my children, when they have a serious disease, that's when we pray, and that's why as a society, we have to make some decisions about what to invest in."** George Yancopoulos, co-founder of Regeneron of Tarrytown, New York, speaks about the consequences of reducing funding for science. (*Bloomberg*, 30 March 2017)
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> **"Cancer relates to our fundamental constitution as multicellular organisms, our limited lifespan, the epidemiology of the aging population, socioeconomics and the future of society. Those who believe that the problem of cancer can be solved by killing or reprogramming cancer cells need to take a step back from the molecular technicalities and take a look at the bigger picture."** Jarle Breivik, of the University of Oslo, is skeptical that the hacking analogy, in vogue in Silicon Valley, will work with cancer. (*Nautilus*, March 2017)

FBDD hits into a more potent drug-like molecule by linking together two hit fragments that bind to different sites on the target protein, or by blending key chemical characteristics of several hits.

Because fragment libraries are modest in size, they are also much easier to assemble and screen than HTS libraries, allowing many more researchers to use them. "For a group with limited resources, it's relatively simple to put together a high-quality fragment library of about 1,000 compounds," says Justin Bower, head of chemistry at the Cancer Research UK Beatson Institute in Glasgow, UK, whose team uses the strategy. Over the past five years or so, academia has begun to play a much greater role in FBDD, and research collaborations with industry are thriving. "It's actually really connected," says Angelo Romasanta at the Free University of Amsterdam, who is studying FBDD research networks as part of an EU project called Fragnet. "They're learning from each other."

Because fragments interact with and bind proteins of interest more readily than do typical drug-like compounds. FBDD also improves the chances of identifying new pockets in proteins. This can reveal novel functions; or uncover a hit against difficult targets, such as protein–protein interactions that involve fairly shallow pockets. "You're more likely to find a hit on a target that might be deemed 'undruggable,'" says Bower. One particularly hot target for FBDD is RAS, an annoyingly smooth protein that helps to transmit signals in cells. RAS mutations are found in some of the most intractable cancers, but it has been particularly difficult to find drugs that bind and block it.

As FBDD has matured, researchers have added more screening methods to their arsenals, including surface plasmon resonance (SPR), which measures how the refractive index of a protein changes when a fragment binds to it. Each technique has its own strengths and weaknesses, says Bower. SPR, for example, can provide a measure of the fragment's binding affinity, but unlike X-ray crystallography it cannot reveal the precise geometry of the binding site. "You need a suite of techniques to do fragment screening effectively," he says. Astex is now considering adding cryo-electron microscopy to the mix. The technique is already taking structural biology by storm, and relies on diffracting beams of electrons through frozen biomolecules to image their structure. "There are two key benefits: the amount of protein is minuscule, and you don't need crystals," says Jhoti.

So far, more than 30 fragment-derived drugs have entered clinical trials, and two have made it to market. First across the line was Zelboraf (vemurafenib), a BRAF inhibitor developed by Plexxikon that was approved by the FDA in 2011 for the treatment of late-stage melanoma. Plexxikon created Zelboraf using a variant of FBDD called scaffold-based drug design. "You can consider scaffolds as bigger fragments," says Chao Zhang, senior vice president of research at Plexxikon. The researchers use libraries of up to 100,000 compounds, and screen them using a mixture of enzyme assays and biophysical measurements, making the technique something of a halfway house between FBDD and HTS.

But FBDD-derived drugs have also faced some recent setbacks. Recruitment to a phase 3 trial of Plexxikon's pexidartinib, aimed at treating a rare type of cancer, was suspended in October 2016 after two cases of serious liver toxicity. Plexxikon still hopes to seek approval in 2018. And in February, Merck of Kenilworth, New Jersey, decided to end a phase 3 Alzheimer's disease trial of its FBDD-derived verubecestat, an inhibitor of beta-site amyloid precursor protein cleaving enzyme 1, after data suggested that it offered no benefit.

Meanwhile, Astex is hoping that lanabecestat, a beta-site amyloid precursor protein cleaving enzyme (BACE) inhibitor that aims to treat Alzheimer's, will soon be its first FBDD-derived product to market. The company expects initial results from its phase 3 trial next year.

As for Kisqali, Seamus Fernandez, managing director and senior pharma analyst at Leerink in Boston, says that it might struggle to persuade some physicians and patients to switch from market leader Ibrance, despite Kisqali's lower price. "From an efficacy perspective, the two products are almost identical," he says. But Fernandez notes that Kisqali's clinical trial revealed a risk of cardiac side-effects, so patients will have to undergo periodic electrocardiogram monitoring.

Both drugs may soon face competition from Indianapolis-based Eli Lilly's CDK4/6 inhibitor abemaciclib, whose phase 3 trial is already showing positive results in treating $HR^+/HER2^-$ breast cancer, which accounts for about three-quarters of all breast cancer cases. To tap into what Fernandez thinks could be a $10-billion global market for this kind of treatment, Lilly intends to submit a new drug application for abemaciclib later this year.

All three of these CDK4/6 inhibitors—Kisqali, Ibrance and abemaciclib—are currently in clinical trials for other indications, which could significantly expand their use. "Over the next 5 years, I think we'll see a lot of exploration of these drugs in other cancers," says Matthew Ellis, director of the Lester and Sue Smith Breast Center at Baylor College of Medicine, Houston.

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