NETWORKING FOR NEW DRUGS

e-Therapeutics plc

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Many of today's most celebrated drugs are designed to hit only one biological target with great precision. But a novel clinical trial aims to turn this idea on its head by using 'network pharmacology' to more effectively tackle a common neurological disorder affecting limb movement. **Claire Ainsworth** looks into how medicine's proverbial 'magic bullet' might soon give way to a more sophisticated arsenal.

At first sight, it seems like a rather perplexing experiment. In several hospitals across France, some 60 patients are taking part in a clinical trial to test a combination of three drugs. The first striking thing about this trial is that the drugs have already been approved for human use. The second is that each drug is being given at doses between 10 and 100 times weaker than those usually prescribed. Finally, and most surprising of all, none of the patients suffer from the conditions for which these drugs were originally developed. They all have Charcot-Marie-Tooth disease type 1A, a currently incurable neurological condition that causes muscle weakness and loss of touch sensation, and this trial is the result of a radically new approach to understanding and treating disease.

The approach, called network pharmacology, is very different from the current 'targeted' method of drug discovery. To design targeted medicines, researchers typically identify a single protein they believe causes the disease and then design a small molecule that binds exclusively to this target. Network pharmacologists take a very different tack. Rather than focusing exclusively on single proteins or individual biochemical pathways, they look at the bigger picture and target the myriad networks of interactions between the molecules in our cells.

"If we want to increase our range of potential drug targets or increase our chances of having a greater clinical effect, then we need to consider multiple modes of perturbation," says Andrew Hopkins, who researches medicinal informatics at the University of Dundee, UK.

Network pharmacology is only a few years old but has already yielded promising results in preclinical studies, leading to new possibilities for anticancer drugs and antibiotics against multidrug-resistant bacteria. A number of human clinical trials of drugs created using network pharmacology are also underway, such as the Charcot-Marie-Tooth disease trial, which is the brainchild of the biotech company Pharnext, located just outside Paris. The disease affects one in 2,500 people, and current treatment merely manages symptoms with physiotherapy and devices such as leg braces.

Proponents say that drugs developed this way will be more effective, will have fewer side effects, and will be faster and cheaper to develop than conventional treatments because they sometimes incorporate already approved medicines. But it won't be easy: there are large gaps in biological knowledge, and many developers are still heavily geared toward supporting the single-target, reductionist approach. "This is a difficult time for the field, which is under a lot of pressure to rapidly deliver new, safe, efficient and affordable drugs," says Daniel Cohen, head of Pharnext. "It is hard to question the current single-target paradigm, which has probably reached its limits.

The uncomfortable truth is that the single-target–based model of drug discovery simply isn't living up to its promise. Of the few drugs that make it through early clinical trials, 50% fail in the phase 3 to launch stages (*Nat. Rev. Drug. Discov.* **10**, 87, 2011).

To make things worse, many of the exquisitely honed magic bullets offered by modern medicine fragment into scattershot, as new research reveals that they act on far

more than just the single protein at which they were originally targeted. The successful antileukemia drug Gleevec, for example, physically binds four other proteins in addition to the one intended, a property known as 'promiscuity' or 'polypharmacology'. "There is a big hole sitting in the bottom of the assumptions of conventional [drug] discovery," says Malcolm Young, chief executive of the UK biotech company e-Therapeutics. When it comes to traditional compounds, "there is no effective drug that only sticks to one protein."

It's complicated

The complex physiological networks that keep cells running have evolved over billions of years to withstand all manner of disruptions. So even though our state-of-the-art targeted drugs might successfully disable one node in a disease pathway, backup processes can kick in to compensate.

Some of the most basic staples of the medicine cabinet serendipitously reveal the power of taking the network approach. Penicillin, discovered by the Scottish chemist Alexander Fleming almost a century ago, can bind about a dozen proteins in a bacterium. The broad action of the drug is important; *E. coli* can survive even when scientists delete eight of these protein targets (*J. Bacteriol.* **181**, 3981–3993, 1999).

To understand more about these complex networks, researchers have turned to systems biology, an approach that studies how the sum of all the interactions between the molecules in a cell creates a greater whole. Over the past decade, new technologies, such as microarrays, whole-genome sequencing and high-throughput proteomics, combined with greater computing power, have allowed the field to flourish and create new disciplines such as network pharmacology. Researchers can now gather vast reams of these data to construct complex diagrams representing how cellular molecules are connected to one another to form a network.

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Instead of thinking about single, mutated genes or proteins, network pharmacologists talk of damaged or corrupted networks—damage that will need to be tackled on several different fronts to correct. For example, Eric Schadt, director of the Institute for Genomics and Multi-Scale Biology at Mount Sinai School of Medicine in New York, and his colleagues have modeled networks underlying the metabolic traits associated with obesity, diabetes and heart disease. Working in mice, Schadt and researchers in the US and France built a network of genes on the basis of their co-expression in liver and adipose tissue and then cross-referenced it against genetic variations associated with metabolic diseases such as obesity. This allowed them to identify a part of the network whose function was perturbed in these diseases. Intriguingly, many of these genes were associated with immune function, and the team was able to use their network to pinpoint three new genes involved in obesity (*Nature* **452**, 429–435, 2008). Additional work by Schadt and his colleagues in the US and Iceland identified a similar network in humans (*Nature* **452**, 423–428, 2008).

Young's team at e-Therapeutics has modeled networks that give cancer cells their ability to evade apoptosis, the suicidal self-destruct mechanism that healthy cells trigger when irretrievably damaged. Using computer modeling, they screened another database detailing interactions between about 15 million compounds and 2.6 million proteins to look for potential drugs that would have either direct or indirect effects on the target proteins.

Ultimately, the analysis pointed them to the drug ETS2101. The compound, although previously shown to be safe, failed phase 3 trials for lack of efficacy in treating severe brain injury. Yet it turned out to be a potent killer of cancer cells, including melanoma, breast cancer and colon cancer cells, in the lab, says Young.

Network pharmacology could also tackle another challenge of drug development: the fact that many drugs work on only a subset of patients, says David de Graaf, president and chief executive officer of the Cambridge, Massachusetts-based biotech company Selventa. "I think the key to this is understanding interindividual variations of disease-driving mechanisms," he says. Selventa performs network analysis on individual patients for commercial partners such as pharma companies and research organizations to identify the faulty networks underlying the patients' disease. This allows clinical trial organizers to group patients who share the similar disease-driving mechanisms together, thus ensuring that they are testing a therapy on people with the same underlying molecular condition.

Something old, something new

Earlier this year, Philip Bourne and his colleagues at the University of California–San Diego used their computational platform to show that the anti-HIV medication nelfinavir exerts anticancer effects by weakly inhibiting a broad range of proteins called kinases (*PLoS Comp. Biol.* **7**, e1002037, 2011). "This is directly in the face of traditional drug discovery," says Bourne. He and his team have also used network analyses to screen 274 existing drugs and showed that 92 of them, including some anti-HIV drugs, might work against tuberculosis (*PLoS Comp. Biol.* **6**, e1000976, 2010).

One key advantage of screening for combinations of existing drugs, as Pharnext has been doing since Cohen founded it in 2007, is that such drugs have already been safety-tested and approved, meaning that researchers can skip some of the expensive and time-consuming research and development stages. Pharnext's aim is to target the diseases with the largest unmet clinical needs. Charcot-Marie-Tooth disease type 1A (CMT1A), the most common inherited disease affecting peripheral nerves, seemed an obvious one to tackle. Pharnext's combination treatment for Charcot-Marie-Tooth disease has only been three years in the making, compared with the usual eight required for new compounds. A patent application from the company describes tests on a rat model of CMT1A in which the drug combination resulted in a marked improvement in motor function and muscular strength in the rats. The company has been able to go straight into phase 2 efficacy trials

John Soares/Selventa

Linked in: David de Graaf of Selventa, which offers molecular network analyses for drug companies. Facing page: A network of proteins involved in DNA repair in neurodegeneration, as modeled by e-Therapeutics.

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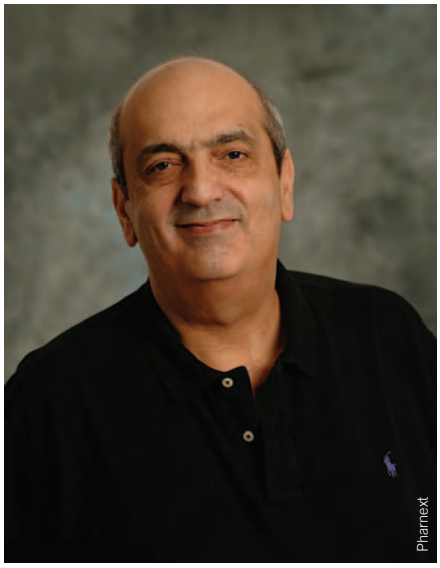
with the mix, currently dubbed PXT3003.

PXT3003 is a combination of the generic drugs baclofen, normally used to treat spasticity, naltrexone, usually used to treat opiate and alcohol addiction, and sorbitol, a laxative. The trial began in December 2010, involves 80 participants and results are expected at the end of next year.

"CMT1A is a relatively 'mild' disorder. From an ethical point of view, this reduces the risk doctors and patients are willing to take when testing candidate drugs," says neurogeneticist Klaus-Armin Nave of the Max Planck Institute of Experimental Medicine in Goettingen, Germany. As such, "the approach of starting a drug screen with already approved compounds and known side effect profiles is very appealing," explains Nave, who has consulted Pharnext on the disorder. "The overall risk to the patient seems minimal in comparison to the risk associated with completely new drugs, although the interaction of drugs is unknown and must still be carefully tested."

Pharnext used its own in-house bioinformatics platform to build its Charcot-Marie-Tooth disease network, the details of which remain confidential until the company publishes the trial data next year. As well as using the network to screen for potential drugs *in silico*, the company used it to predict a profile of proteins and other molecules it would produce when diseased or when healthy. This is allowing the company to screen samples (such as blood samples) from patients to look for early signs that the therapy is pushing their diseased networks into a healthy configuration. Pharnext is also planning to launch further phase 2 trials for CMT1A with combinations of different drugs to look for the most effective one.



Mixing it up: Daniel Cohen's Pharnext approach combines approved drugs.

Net benefit: Malcolm Young of e-Therapeutics, which is using network pharmacology against cancer.

The Pharnext approach also has the advantage that the drugs are being used at far lower dosages than those used for the original indications and should therefore run a lower risk of generating unwanted side effects. Under typical circumstances, single-target drugs aimed at one protein often need to be used at relatively high doses to cripple enough of that protein to overcome the backup systems in the cell. Network-based drugs, in contrast, target multiple proteins and other molecules and so are needed in lower amounts.

Bringing network pharmacology drugs to the mass market, however, is not necessarily going to be easy. Regulatory agencies such as the US Food and Drug Administration will need to adapt to evaluate novel multiple-target, network-based drugs instead of the usual single-target models they currently work with. Although such agencies are engaging with network pharma, say scientists, this process is likely to take some time. And what happens if one pharma company has a drug that is predicted to synergize with one produced by a competitor? There will be all sorts of legal, economic and business issues to be worked out before such a drug combination can be marketed.

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Despite these potential hurdles, some of the big pharma companies are taking notice of the network approach. Miguel Barbosa, who heads the Immunology Research division at Centocor Research and Development, a subsidiary of Johnson & Johnson, says his team has been working with network pharmacology for the past four years, having witnessed the explosion in biological data and the development of the technology to use it. "We as a field and as an industry are poised to begin to use those diverse data sets in a more integrated fashion," he says. "We are applying this type of advanced methodology and approach day to day in how we do immunology."

For network pharmacology to succeed, biologists will have to work across disciplines and at an unprecedented scale, akin to how "big physics" is done, says Schadt. He and his colleagues have established Sage Bionetworks, an open-access online resource that helps researchers of all stripes access network biology. Encouragingly, the marrying of public, private and academic data—a crucial step toward modeling networks of cellular proteins—is already starting to happen. Barbosa says that Johnson & Johnson is forming consortia for network pharmacology with other big pharma companies, biotech and academic labs—although, as these are at a very early stage of development, the precise details remain confidential for now.

Network scientists say that perhaps their biggest challenge is persuading 'old school' biologists, chemists and drug regulators to embrace their way of thinking. Things are changing, albeit slowly. "It used to be the case that it was a pretty lonely vigil," says Young. "But now members of our church are cropping up everywhere."

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