THE FULL CYCLE

Results can be thrust from bench to bedside, but there is also much to be learned by pushing the other way. **Heidi Ledford** tells tales of clinical trials that have prompted a change in tack.

In April this year, Nobel laureate Sydney Brenner brought the crowd to its feet at the American Association for Cancer Research meeting in San Diego, California. Brenner pioneered the use of the nematode *Caenorhabditis elegans* as a simple model for studying growth and development. But in his talk, he championed experiments on a more complicated creature: *Homo sapiens*. "We don't have to look for model organisms anymore because we are the model organism," he said.

Brenner is one of many scientists challenging the idea that translational research is just about carrying results from bench to bedside, arguing that the importance of reversing that polarity has been overlooked. "I'm advocating it go the other way," Brenner said. Bedside to bench means that clinical trials and patients' unexpected responses are valuable human experiments, and failed trials can stimulate new hypotheses that may help refine the experiment in its next iteration.

Reverse translation of this type comes with its own challenges, such as gaining access to clinical samples. And only certain trials, for example those the research community has already invested in, tend to get this type of scientific scrutiny. Below, *Nature* recounts three stories in which results from human experiments inspired new avenues of research.

ILLUSTRATIONS: B. MELLOR

"We don't have to look for model organisms anymore because we are the model organism."
— Sydney Brenner

A moving target

It was a front-page article in *The Boston Globe* that first caught cancer researcher Daniel Haber's attention. The 2003 article[1] described the strange results from an experimental cancer drug called gefitinib, one of the first generation of 'smart drugs' designed to target a specific protein, in this case one called epidermal growth factor receptor (EGFR).

Several types of tumour churn out higher than normal levels of EGFR, and gefitinib was designed to block the receptor and prevent further tumour growth. In 2003, the US Food and Drug Administration conditionally approved the drug, marketed by AstraZeneca as Iressa, to treat a severe lung cancer called non-small-cell carcinoma. And in some cases — typically non-smokers, women and Asians — the drug yielded dramatic results, seemingly eradicating signs of the disease from patients whose illness was thought to be terminal. "The response was magical," says Haber, director of the Massachusetts General Hospital Cancer Center in Boston.

But in other patients, as the *Globe* article explained, gefitinib was ineffective. Trials completed after the drug's approval showed that it failed to improve patients' survival when averaged across all individuals[2]. Regulators took the drug off the US market in 2005, although it is still approved in some other countries, and for certain exceptional cases in the United States.

To many, the drug's failure was a bitter disappointment. But Haber was intrigued and wondered whether there was a molecular explanation for the discrepancy in responses between one patient and the next. Down the road at the Dana Farber Cancer Research Institute, Matthew Meyerson and his colleagues were wondering the same thing. Neither group initially thought to look for answers in the sequence of the *EGFR* gene: the experiment was so obvious that both assumed it had already been done. "It was naivety," says Meyerson.

In 2004, Haber, Meyerson, and William Pao at the Memorial Sloan-Kettering Cancer Center in New York, independently published results showing that most tumours that responded to gefitinib harboured mutations in *EGFR* that rendered the protein more sensitive than usual to the drug[3–5]. As a result of these findings, researchers have developed genetic tests for *EGFR* mutations, and several clinical trials are now under way to determine whether the drug is effective when it is given only to the patients with a mutated receptor.

But there was another puzzle to solve. Early trials had shown that even those who did initially respond to the EGFR inhibitor soon become resistant, many within a year of starting treatment. By genetic analysis of tumour samples and cancer cell lines, researchers have found that these resistant tumours acquire secondary mutations that render the drug impotent.

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Sometimes, the culprit is a second mutation in EGFR that stops the drug from binding to the protein; at other times, a gene called *MET* is also amplified, which allows the tumour cells to multiply even when EGFR is not working[6]. Occasionally both EGFR mutations and *MET* amplification arise in different cells within the same patient, illustrating how cancer cells will use every genetic trick in the book to continue growing. Findings such as these partly inspired the Cancer Genome Project, which aims to sequence genes from multiple cancers to reveal their genetic idiosyncrasies. And early clinical trials are under way to test whether gefitinib-resistant tumours that have accumulated a secondary mutation can be tackled with alternative drugs, such as EGFR inhibitors that bind more tightly to the protein and are unaffected by the mutations.

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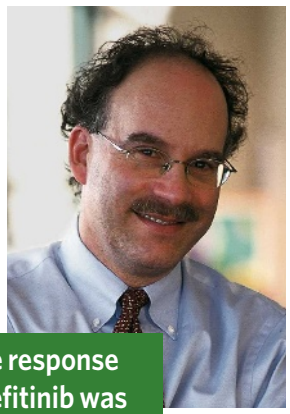

"The response to gefitinib was magical."
— Daniel Haber

Few failed drugs receive so much scrutiny. Gefitinib was a special case because it had been designed specifically for a target, and because it produced such a 'magical' response in some patients.

One of the major obstacles in bedside-to-bench research is obtaining high-quality tissue samples from trials. Few physicians are willing to collect the multiple invasive biopsies that are needed to determine the molecular changes in a tumour as it evolves, but that offer no direct benefit to the patient. Meyerson had to track down the investigators who had tested the drug and apply for separate approval to use the samples from the many ethics committees who approved the gefitinib study. "I didn't understand how hard it was to get samples from a clinical trial at that time," he says.

M. STEPHENS / PA PHOTOS

Tale of the unexpected

When leukaemia first developed in a child given gene therapy, there was still hope that it was just a coincidence. "We didn't know what to make of it," says Brian Sorrentino, who directs the gene-therapy programme at St Jude Children's Research Hospital in Memphis, Tennessee. "Then the second case came, and it was clear this was going to be a recurring problem."

Since 2002, 5 of the 21 children who received a high-profile, experimental gene-therapy treatment for a disease called X-linked severe combined immunodeficiency (X-SCID) have developed leukaemia. X-SCID is caused by a mutation in a gene, called *IL2RG,* that is required for the immune system to generate working T cells and other cells in the immune system. The condition is commonly called 'bubble boy disease' because babies with X-SCID must live in sterile environments to avoid lethal infections.

At first, the two X-SCID trials, in France and Britain, were heralded as the clearest success in the controversial gene-therapy field. Most patients developed a functional immune system, and the first recipients now live a normal life, says Marina Cavazzana-Calvo, a researcher at the Neckers Children's Hospital in Paris, France, who helped conduct the French trial. The technique relied on a retrovirus to shuttle a functional copy of *IL2RG* into the patient's bone marrow stem cells, from which immune cells are generated. Researchers expected that the retrovirus would integrate into the patient's genome at random. But shortly after the two trials started, Christopher Baum of Hannover Medical School in Germany, and his colleagues published a short report indicating that the virus preferentially inserted itself next to a cancer-causing gene, causing leukaemia in mouse models[7]. "The initial reaction was that our mouse model would not be relevant to the clinics," Baum says.

Once the third case of leukaemia came to light in 2005, the French trial was put on hold, sending a chill through the entire gene-therapy community. "It was a difficult time," says immunologist Frank Staal, who studies gene therapy at the Erasmus University Medical Center in Rotterdam, the Netherlands. For many clinical trials, such a disaster would mark an immediate end to the research. But because this therapy had looked so promising and because the disease is so devastating, researchers were anxious to find out what had gone wrong.

Gene therapy helped some children who were born without a working immune system.

Using a method to isolate and amplify only the regions of DNA that surrounded the virus, scientists have found that the insertions were far from random in the bone-marrow stem cells. The virus had multiplied and slotted into hundreds of different sites, preferring to settle near highly expressed genes[8,9]. In the French trial, some of the patients with leukaemia had viral inserts near possible cancer-causing genes such as *LMO2*, which is involved in blood-cell formation[10]. The researchers suspected that genetic elements in the virus that were used to activate *IL2RG* also stimulated expression of *LMO2* and other genes nearby the virus' insertion site. This probably caused the proliferation of T cells that caused the leukaemia.

Since then, researchers have been trying to get around this by modifying the viruses used to transfer the gene. Adrian Thrasher at University College London has designed a vector with genetic control regions that are less likely to activate nearby genes and that contains a 'kill switch' to prevent it from replicating once it has inserted into the genome[11]. Pending final approval, this vector will be used in the next round of gene-therapy trials for X-SCID, says Thrasher, who is one of those leading the trial.

Of the five children who developed leukaemia, four were successfully treated and their gene-therapy-repaired immune systems remained intact. Sorrentino notes that if the gene therapy itself had not been so successful, the community might not have rallied so readily to fix the leukaemia problem. "At the time it felt terribly depressing," he says. "But we've worked through that and now I feel very enthusiastic."

Test shot

By the time Merck's HIV vaccine candidate made it into a phase II trial, there was no shortage of strong opinions about its probable fate. Over the past few decades, the

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HIV vaccine community had seen one failed attempt after another. Merck's approach — to stimulate a T-cell response, rather than one from antibodies — was new. So some voiced hope for the vaccine's success. Others pointed to the raging HIV epidemic as a reason to move any promising candidate into clinical testing. And yet more were downright pessimistic.

But no one expected the vaccine to make some study participants more susceptible to infection. "That really shocked the field," says Barton Haynes, from Duke University in Durham, North Carolina, and director of the Center for HIV-AIDS Vaccine Immunology (CHAVI).

Researchers remain at a loss to explain what went wrong with the trial, called STEP, which was halted last September. But they have been galvanized to find that explanation, and are lining up to access samples and trial data. Part of the willingness to explore the failure lies in the nature of the HIV vaccine field, which was born of 'bedside-to-bench' research, says Bruce Walker of Massachusetts General Hospital in Boston. "In the beginning, we didn't have any idea of what this disease was," he says. "It required going from the bedside back to the bench to figure it out." Plus the research community was already heavily involved in developing the vaccine and the trial was co-sponsored by the National Institutes of Health.

The fact that this vaccine increased the rate of infection injected a sense of urgency into the trial's post-mortem because of concern that other vaccines might do the same. "There is no more important question than determining what happened," says Mark Feinberg, Merck's vice-president of medical affairs and policy. "The implications for the field are enormous."

R. ESPINOSA/AP PHOTO

Problems with Merck's HIV vaccine trial inspired researchers to examine what went wrong.

Some have cautioned that another failure like the STEP trial could spell the end of the HIV vaccine quest altogether.

Preliminary analysis of the trial data has revealed that those who became more susceptible were typically uncircumcised males and carried pre-existing immunity to the virus used in the vaccine. That virus, a disabled form of a common cold virus called adenovirus serotype 5, was used to ferry HIV genes into the patient to elicit an immune response. But researchers do not yet know whether the pre-existing immunity was important in reducing resistance to HIV, or whether it is merely a surrogate for some other factor.

One hypothesis is that the increased susceptibility could have a genetic cause, and researchers in the CHAVI consortium have put forward a proposal to scan the genome of trial participants to look for such a genetic signature. Others want to determine whether immune responses to the vaccine's vector may have rendered participants more vulnerable to infection by fuelling an increase in the subset of T cells that HIV infects.

The HIV vaccine community has formed a scientific review panel to evaluate proposals for research that will use STEP samples, and Merck is planning to create a central repository of samples from the trial to facilitate their distribution. So far, more than 25 investigators have proposed projects, says Julie McElrath, an HIV researcher at the Fred Hutchinson Cancer Research Center in Seattle, Washington, who is helping to coordinate the effort. The challenge, McElrath says, lies in recruiting new basic researchers with fresh ideas to work with the samples because many do not know that they are available or how to go about getting their hands on them. "It seems like this huge barrier," she says.

Prompted by the failed trial, the US National Institute of Allergy and Infectious Diseases made it clear at an HIV vaccine summit in March that the institute will shift the balance of its vaccine funding away from clinical trials and towards basic research, in an effort to stimulate new approaches to vaccination. Those involved say that the shocking scale of the HIV epidemic demands a dogged approach that is sometimes absent from other areas of research. "In academia, we'll come up against a brick wall and we'll tend to move to a different area," says Haynes. "With the HIV vaccine, we have to keep looking at that problem."

> **"In academia, we'll come up against a brick wall and we'll tend to move to a different area."**
> — Barton Haynes

Learning lessons

Francesco Marincola, a cancer immunologist and advocate of translational medicine at the National Institutes of Health Clinical Center in Bethesda, Maryland, says that the level of organization and persistence found in the HIV vaccine community is rare. "There is a lack of that in other, more complex diseases," he says. One reason is that HIV researchers have a clear target — the disease-causing virus itself. Another is that industry and government-funded researchers collaborated on the vaccine's development, so the project wasn't just abandoned when the results turned out negative.

Other fields may begin to catch up. The current emphasis on developing biomarkers to monitor disease progression is encouraging more investigators to collect patient samples. Without them, it is difficult to look back and evaluate what went wrong if the trial fails. "That's the reason why we haven't learned from these failures," says Marincola. "Instead, we have gone from one failure to another." ■

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