Targeted drug development by trial or error?

Anna Wagstaff

Bringing the new wave of targeted cancer drugs to the market poses new challenges for researchers and clinicians. It is not enough to design a smart drug to hit a molecular target. Researchers need to know how to test and develop the drug. The potential prizes are enormous – so is the price of failure.

The discovery of the first human oncogene in April 1982 marked an explosion of knowledge about the molecular biology of cancer. It delivered scientists their first molecular target, which had profound implications for the development of anti-cancer drugs. If patients are to reap the huge potential benefits offered by molecular targeted therapies as quickly and cheaply as possible, industry and academia alike will need to adapt their drug development strategies accordingly.

Mariano Barbacid, now head of the Centro Nacional de Investigaciones Oncológicas (CNIO) in Madrid, led one of three teams that separately, and simultaneously, isolated the mutant gene. In the same year, dubbed by *Nature* the Year of the Oncogene, he went on to show the gene was from the *ras* family, that it differed from the normal gene only in a single point mutation and that it could be found in the tissue of lung adenocarcinoma, but not in the patient's normal tissue.

What happened next is a cautionary tale that Barbacid makes a point of telling young researchers. Pharmaceutical companies rushed to find a way to inhibit the activity of the mutant *ras* gene. They identified as a likely target the farnesyltransferase enzyme, without which the *ras* gene would be unable to send its pathogenic signals. A billion dollars was sunk into the race to find the first effective farnesyltransferase inhibitors. None of them worked. Too late, it was discovered that the *ras* gene has a contingency plan. In the absence of the farnesyltransferase enzyme, a related enzyme, geranylgeranyltransferase I, is called into action, and the gene is back in business.

The researchers had identified their target correctly and had found a way to inhibit their target, but they had failed to check that hitting their target had the desired effect on the tumour. (Attempts to resolve the problem by inhibiting both enzymes at once have so far failed due to unacceptable toxicity.)

This lesson has been reinforced by subsequent history. New targets discovered in labs offer vital pointers for the development of effective anti-cancer drugs. But unless researchers explore, as early as possible, what happens in real tumours when the target is hit, they risk committing themselves to an expensive development of a drug that will not work. Worse, there is a possibility of overlooking a potentially effective drug because it was tried on the wrong patients, at the wrong dose or schedule, or without recognising its efficacy in combination with other therapies.

Development has replaced discovery as the key to getting new drugs to the market, says Jean-Pierre Armand, who set up the phase I clinical trials unit at the Gustave Roussy in Villejuif, Paris, almost 25 years ago, and was a founder of the Flims Clinical Trials Workshops. "Ten years ago there were very few drugs, but now we have many drugs that are very similar. The difference now is not in the discovery but in how you develop them."

The good news is that the possibilities for finding out what is going on at a molecular level are expanding at an impressive speed. The pathological/diagnostic imaging industry is working overtime to find improved, easier and more accurate ways to demonstrate and interpret what is happening in tissue at a genomic and proteomic level, enabling researchers to track the biological impact of their drug on the tumour and adapt their development according to what they find. The new mantra for the academic drug development community, coined by José Baselga of Vall d'Hebron University Hospital in Barcelona, is "no tissue no trial". In the words of Lex Eggermont, past President of the European Organisation for Research and Treatment of Cancer (EORTC), "The patient should guide the process," and "a properly designed clinical trial is a tool to better understand cancer biology."

It is taking a while for the message to get through. Armand tells of a more recent rerun of the farnesyltransferase inhibitor saga, this time with metalloproteinase inhibitors – a type of antiangiogenic compound. "There were five or six big companies in the game, including Schering and Bristol Myers Squibb. They had shown preclinical activity, they had a nice target, and nice concept of activity and they showed biological modifications in phase I and II. People said the clinical results will follow. They were wrong."

Armand is not at all surprised that none of these drugs turned out to be clinically active in phase III, as none had shown evidence of clinical activity in phase I or II. "We used to go into a randomised phase III trial only when we had very strong data from phase II. But now companies are so rich that they already have the money for phase I, II and III, and they believe that the phase III will tell them that the drug is active, even when they have not seen anything in phase II."

This approach, he adds, partly explains the exorbitant price of the new anticancer drugs. "It is because there are such stupid phase III trials launched, because of some minimal activity, in the hope that they will get fantastic results, when the clinical data on phase II are not encouraging. They are relying too much on serendipity."

He accepts, however, that lessons are being learnt, and that pharmaceutical companies are far more cautious about committing themselves than they were five years ago, "sometimes too cautious". Surprisingly, perhaps, it is the smaller 'biotechs', for so long hailed as the creative engines of the drug industry, who are now branded as the main culprits – and their problem is too little money, rather than too much.

Eggermont says "The culture in biotechs is to try to hit a home run. They have a budget that depends on very quick decisions and offers the chance for only a very limited amount of study, and one phase III trial. If they don't score on the phase III, they usually are dead. So you see a lot of wishful thinking and jumping into phase III trials, where you take the whole population into your study, hoping you are going to be lucky, rather than working with more selected patient populations where your chances of being successful may be enhanced, because at least you have proved that the patients have the target, and that you have reached the target. Some biotechs may be simply too small to have the time and money to go through all these steps, and they make a tremendous push to try to make the home run without having done all these studies."

There has also been a failure among clinicians and statisticians involved in designing trials to appreciate just how unlikely it is that the traditional trial protocols used for the old-style cytotoxics would work for a drug designed in the laboratory to hit a very specific molecular target.

Traditional trials do not work

Clinical trials have traditionally been designed to answer only minimal

Too late, it was discovered that the *ras* gene has a contingency plan

questions, the key one being: does this drug work better (prolong survival, prolong disease-free progression, etc) than a given alternative or placebo, with secondary questions about side-effects. Phase III, usually a large randomised controlled trial, was designed to answer this question, while phases II and I were merely designed to clear the way. Phase I tested for toxicity in humans and tried to identify the maximum tolerated dose; phase II looked for signs of efficacy – usually tumour shrinkage – and was used to judge whether it would be worth investing in a phase III.

The whole point about the new targeted agents, however, is that they are targeted. Some are precisely targeted against a particular molecule such as the epidermal growth factor (EGF) or HER-2 receptor, others, notably the kinase inhibitors, often hit a number of proteins similar to their intended target, but they are nonetheless highly selective compared to the blanket bombing approach of traditional cytotoxics. They can therefore be expected to work only in patients in whom the target is a significant driving force behind their cancer.

Sadly, with an experimental drug, it is rarely possible to identify in advance who the responders will be. Though targeted drugs by definition aim at a target believed to be involved in driving the cancer in question, only in the more 'simple' cancers such as chronic myeloid leukaemia, has shutting down that target proved sufficient to get a response in the vast majority of patients. Even Herceptin, hailed as a huge step forward, is only effective in half the breast cancer patients with HER-2 overexpression. A key part of developing a targeted drug therefore has to be finding predictive 'markers' that differentiate responders from the non-responders. Rushing into phase III trials without doing the necessary work to identify the patients likely to respond is therefore likely to be a recipe for failure.

In addition, there is also the 'old' problem that experimental drugs are usually tested first for use in advanced disease. In early disease the molecular mutations are likely to be implicated in driving the cancer, and are therefore potential targets. Over time, however, the tumour will usually mutate further as a result both of the natural history of the disease and the effect of treatments given earlier in the disease, making it very hard to interpret what is going on.

For this reason it is becoming common, before going into phase III trials, especially with less toxic drugs, to explore how they function in a small group of patients with earlier disease, if a 'window of opportunity' can be found. Typically this will be in a neoadjuvant setting, for instance in women with locally advanced breast cancer in the run up to surgery, or perhaps prostate cancer patients who have undergone surgery or radiotherapy with curative intent, who still show rising levels of prostate specific antigen, but are without sufficient symptoms to warrant hormonal therapy.

Neoadjuvant/adjuvant studies also have the advantage that pre- and post-treatment tissue is readily available from preoperative biopsies and later from the excised tumour. In studies of metastatic cancer, by contrast, it can be difficult to harvest tissue, for instance when the lesions are in the bone, liver or lung.

These trials may be designed as non-randomised single-arm studies, but very often they will move on to a randomised controlled phase II trial. They may be stratified to refine understanding of the best dosing schedule, or to check whether a potential marker of response really can differentiate patients who are likely to respond from those who are not, or even to look at the effect of using the drug in combination (each combination must of course have been tested preclinically and in a phase I to establish toxicity). Phase I/II trials now often comprise a series of studies, each step adapted to the findings of the previous one.

A third problem with relying exclusively on traditional phase III trials is that many targeted drugs are expected to be effective mainly through halting disease progression (cytostatics) rather than by killing tumour cells (cytotoxics). For example, anti-angiogenesis agents choke tumours by inhibiting their ability to grow new blood vessels.

Though angiogenesis inhibitors are now proving effective in what had hitherto been some of the hardest cancers to treat, such as renal cell cancer and metastatic colon cancer,

"They had a nice target and nice concept of activity. People said the clinical results will follow..."

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Genetically destined to develop cancer. By engineering genetic changes in mice that mimic mutations known to cause specific cancers in humans, researchers can now breed strains of mice that will, for instance, develop a particular type of breast cancer 'like a clock', 6 months from birth, and whose offspring will do the same

Mouse models can tell you if a drug is hitting its target, and if the target is indeed driving the cancer

they are not natural performers in clinical trials. The tumour shrinkage shown with cytotoxics is a strong indicator of activity; it is harder to prove activity where the aim is non-progression. However studies that show the scientific concept of the drug works in live tumour tissue – in this case, that vascular growth is inhibited – increase its medical plausibility, and can help to convince regulators that a cytostatic drug is clinically effective.

Doing the science preclinically and in phases I and II is crucial to success at phase III. It is also essential to the early identification of those early drugs that are enticing in their scientific concept but do not actually work in human tumours.

The return of the mouse

Clinical trials are costly, they take time to accrue patients and they are heavily restricted by ethical consideration safeguarding the best interests of the patients. One way to avoid wasting time on inactive drugs and quickly to find out as much as possible about active drugs is to subject them to a thorough examination before allowing them into the clinic. The era of molecular medicine has opened up new possibilities in this area that are often not fully exploited.

The mouse model, criticised for its shortcomings in predicting human responses to drugs, is making a comeback in mutant form. Instead of injecting mice with carcinogenic agents, 'transgenic mice' are genetically engineered to model specific mutations known to be driving particular cancers. These 'transgenic mice' can be used to examine the pathogenic mechanisms involved, to try out the efficacy of targeted drugs and to look for surrogate markers for anti-tumour activity.

Pier Paolo Pandolfi, of the Sloan Kettering in New York, sits on the US National Cancer Institute's Mouse Model of Human Cancer Consortium, which is dedicated to extending the range of mouse models and making them available for research. He stresses that when it comes to predicting toxicity or even efficacy in humans, mice models are not much help. However, they can tell you whether a drug is actually hitting its target, and whether the target

is indeed a driving force for the cancer in question.

The story of the farnesylase inhibitors, he says, is a case in point. Researchers used a mouse model engineered with a mutant *ras* gene, and then inactivated the farnesylase enzyme. "They proved that knocking out this enzyme, which was what the farnesylase inhibitors were designed to do, did not affect the tumorigenesis driven by *ras* at all, and they even came up with an explanation, which is that *ras* uses another enzyme to be activated." Sadly, this was only done after millions had been wasted on the drug.

Pandolfi says that such tests are essential before jumping into the clinical arena and testing for efficacy and toxicity in labour-intensive, expensive and long-term experiments in humans. "You cannot quote me a single example where a drug that should work in a mouse and does not, has been shown to work in humans. If they would first assess whether the target, once inhibited or taken out from a mouse, would impact at all on the tumour, they could save an enormous amount of money."

The transgenic mouse is a far more powerful investigation tool than its predecessors. "Technologically speaking we are in a position to literally recreate a tumour with the genetic makeup that we want, because we are able to inactivate genes, activate genes, mutate genes in a specific tissue at a specific given time."

Of course models can only be made after the genetic make up of the equivalent human tumours have been profiled – and scientists have only just scratched the surface of that work. But as Pandolfi points out, the benefits increase as more profiles are defined. The more that genetic subtypes of a given cancer are identified, the smaller the patient population for each becomes, which makes it more difficult to accrue patients in trials. Genetic subtypes are not a problem with mice, which can be bred for each strain of the relevant mutation. In this way, a potential breast cancer drug can quickly be screened across a variety of genetic subtypes, while a drug aimed at a particular mutation can be screened across a variety of tumour sites. The information can be used as a guide to stratify patients in early clinical trials to help refine the target patient group.

Eggermont from EORTC also believes that mouse models enhance understanding of targeted drugs. "With all the technology we can now knock out and knock in genes, which greatly enhances your biologic understanding of what a certain target means. It would be a failure of understanding of how biology moves forward to say that mouse models are not important. Mouse models can be very important, but they are part of a much bigger picture."

He cites work by Craig Jordan, of the Fox Chase Cancer Center in Philadelphia, into tamoxifen resistance in mouse models. "The tumours were originally sensitive, then after treatment with tamoxifen become insensitive. Then you give second-line and third-line hormonal therapies and you can end up with tumours that are tamoxifen sensitive again. Those mouse models give great insight into what actually may be happening in the subset of patients with hormonal sensitive tumours who 'live on for ever' despite having metastatic disease. It's a new thing to even conceive that you could go back to tamoxifen after a number of lines of hormonal therapy."

Phase 1 trials

Although a lot can be learnt from preclinical trials about a target, its role in driving a cancer and the ability of the drug to hit that target, it is only by trialling it in patients that it is possible to discover its toxicity, activity, and optimal dosage and to identify in which

THE MUTANT MICE MARKET

Mice models of human cancer have only recently been liberated from a highly controversial all-encompassing 'oncomouse' patent held by Harvard University. Patents are still in force covering the use of these mice or cell lines derived from them for testing drugs. However, Pier Paolo Pandolfi, who sits on the US National Cancer Institute's Mouse Model of Human Cancer Consortium (MMHCC), says that in his experience there is a lot of scope for academic exploratory studies without running foul of the patent.

So far, mouse models are available from the MMHCC for around 64 genetic mutations, with types of leukaemia, lymphoma, skin, breast, lung, gastrointestinal, prostate and brain tumours being the most frequently modeled. They can be purchased over the Internet, as live mice or in frozen embryo form at http://mouse.ncifcrf.gov/. Though Europe does have a consortium, the European Mutant Mouse Archive (www.emmanet.org) dedicated to archiving and distributing 'relevant mutant strains essential for basic biomedical research', it does not have the same focus on generating engineered models of human cancers. There are individual institutions, such as the CNIO in Madrid, that are heavily involved in this sort of work.

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The ultimate investigative tool. The diagnositic imaging industry is working flat out to develop sophisticated techniques that show what is happening to a tumour at a molecular level. But feedback from patients may still offer the most reliable – and most speedy – indication of whether a drug is active or not

"Tell me a blood test that can predict activity quicker than the clinic. I don't have one"

patients it works best. Phase I trials were traditionally limited to establishing maximum tolerated dose, but Armand from the Gustave Roussy, who did the phase I trials on sunitinib [Sutent], says this has all changed. Phase I trials are where you begin to learn about how the drug impacts on the tumour, and how best to measure that impact to find out the most effective dose and schedule, and maybe tease out some pointers to what differentiates responders from non-responders, which can be tested in phase II.

"I believe this phase I moment is very critical. You should do more than one phase I trial and you should explore more than 40 patients, maybe 60 or 80 at this level, because that is the moment you do the fine tuning to optimise the dose and recognise a few signals of activity."

Armand uses a variety of techniques to see what the drug is doing, including expensive high-tech methods like genomic profiling, cheaper quicker functional imaging techniques and, above all, his clinical experience as a doctor.

His advice to clinicians involved in clinical trials is not to get blinded by the technology. "When you are a clinician, you should remain a strong clinician. You cannot see your patients through a chart. You should see your patients every week during development, because the patients have tools which tell you, before you can read it anywhere, that there is some activity. So see the patient and measure what they say."

Everyone, he says, is rushing around desperate to find 'surrogate markers' of activity that can quickly and reliably predict whether a treatment will be clinically effective, in order to speed up the phase I and II studies. "The real activity, and this is one of my latest discoveries, is the clinical benefit," says Armand. "When I have patients telling me, 'Dr

"When there is no change in the genes, most of the time I have no responders"

Armand, I am happy with this drug,' even if the surrogate markers are not significant, for me it is a real drug.

"We are back to the old story: let the patient tell you whether they are happy or not with the treatment, and then you have something in your hands. On the other side, you have people who would prefer to say: 'We don't need the patients. We only need a sample of blood to say this is a good drug for the patient'."

Most phase I trials are conducted with patients reaching the end of their disease, when they are getting worse by the week. Armand cites the example of Glivec (imatinib) in gastrointestinal stromal tumour [GIST]. "You just give the patient the drug, and one week later they are playing tennis, when before they spent all day in bed. Tell me a blood test that can predict activity quicker than the clinic. I don't have one."

Patients can also give vital information about dosage. "I treated the first 15 patients in the world with Sutent, and I can tell you data about the activity even the company doesn't know. Some of my patients have enough drug for one month, and they take it in one week. And they say, 'You know your dosage is not enough, so I increased it and I feel a lot better.' There is critical clinical information available when, as a good clinician, you know how to listen to a patient."

That is not to say that biological markers of activity and predictors of response are not essential, but merely that highly relevant information from patients is too often ignored in favour of charts of assay results and tumour genomic profiles.

Surrogate markers

Surrogate markers, the indicators of anti-tumour response, can give an idea, in a relatively short time, about a drug's anti-tumour activity. Good markers are key to exploring the most effective dosing schedule and whether a drug will work better in combination or alone. Crucially, they can also be used to sort patients into responders and non-responders (sometimes more of a continuum than a 'yes/no' variable). This information is then used to look for 'predictive markers' that can prospectively identify the target patient group.

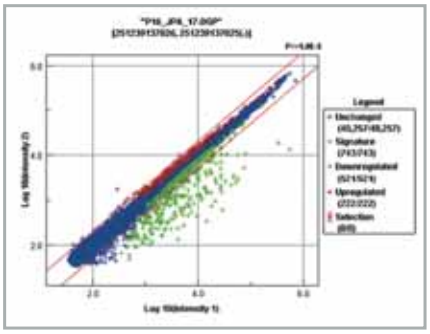
As individualised therapy is increasingly tailored to the exact phenotype of a cancer, the ideal is to find biological markers (biomarkers) of response based on key changes in the tumour's genetic expression profile, or even better, changes at the proteomic level, as demonstrated in biopsies taken before, during and after treatment. This can be difficult and expensive, and is by no means a requirement for getting a drug through to approval.

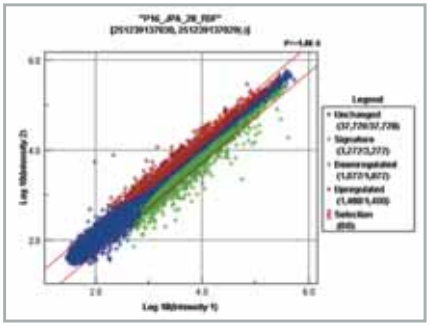
The key to an effective surrogate marker is simply that it reliably indicates anti-tumour activity (evidence of biological changes is not enough), that it reveals itself within weeks, days or hours of initiation of treatment, and that it can be easily and reliably measured. There is a huge international research effort underway to find and validate new biomarkers that can be used in this sort of research. Currently prostate specific antigen (PSA) for prostate cancer and CA125 for ovarian cancer are the only strong candidates, and neither of these have yet been fully validated.

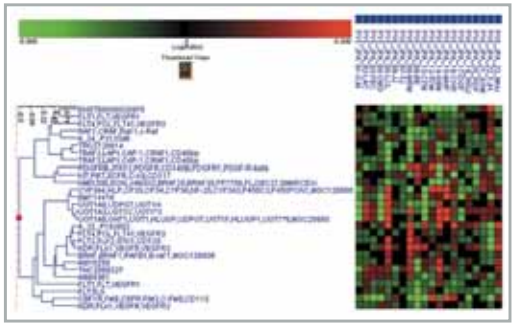
THE SEARCH FOR BIOMARKERS

Hopes that the new molecular imaging techniques would quickly deliver reliable surrogate 'biomarkers' that correlate with anti-tumour activity and predict clinical response have so far not been realised – prostate specific antigen (PSA) for prostate cancer and CA125 for ovarian cancer are the closest so far, and neither have yet been validated.

This is not for lack of effort. The US National Cancer Institute is allocating millions of dollars to teams looking for protein biomarkers using transgenic mice. In Europe there are proposals to set aside funding for research into biomarkers as part of the initiative to promote a European Technology Platform proposed for the European Union research programme, FP7, set to run from the beginning of next year. Eggermont of the EORTC is confident that finding biomarkers is only a matter of time, and says – in a reversal of the advice given to people investing in uncertain financial activities – "Failure in the past is no guarantee of failure in the future."







Doing the science. In his phase I exploratory work, Armand of the Gustave Roussy Institute in Paris, is looking at how an exploratory combination of two drugs changes the signalling pattern of 40,000 genes in a variety of cancers. He compares patterns of change in responders (e.g. patient DGP *top left*) with non-responders (e.g. patient RDF *bottom left*) and tries to identify the genes of relevance *(bottom right)*. With proper analysis, this exercise can reveal a wealth of information about the disease and the drug. Armand hopes to be able to identify a genomic signature that will predict who is likely to respond and who is likely to be resistant to the combined therapy. Source: Jean-Pierre Armand, Gustave Roussy Institute, Paris. Reproduced with permission

However, while evidence-based validation will probably be required if the regulators are ever to licence a new drug on the basis of such a surrogate biomarker, the level of validation for surrogate markers used in exploratory phase I and II trials can be a lot less robust. As Eggermont points out: "Most surrogate markers are not able to have a direct 100% outcome correlation, certainly not with survival. But you need target validation and some surrogate marker in order to make rational decisions about the next step in your drug development plan. Without that the chance of failure is greater."

The most commonly used markers remain changes in the size or rate of growth of lesions, which have been codified into a set of 'rules' with the acronym RECIST (Response Evaluation Criteria In Solid Tumors, see www.eortc.be). These define when cancer patients 'respond', remain 'stable', or 'progress' during treatments. Others include markers derived from functional imaging measuring changes in metabolism or blood perfusion in the tumour, and measures of apoptosis or proliferation.

Armand believes in keeping things as simple as possible for both the patient and the technician, and he is very proud of the novel technique of dynamic contrast-enhanced Doppler ultrasound, invented and validated at the Gustave Roussy Institute, which shows changes in perfusion of the tumour tissue (*Ann Oncol* 16:995–996; 1054–60).

"It is a fantastic predictor of activity or resistance. In GIST for instance it shows the effect of Glivec [imatinib] three months before a CAT scan can tell you what is going on. I use this tool in phase I trials, because it helps me to optimise the schedule and the dose. We need to have a tumour where we can inject bubbles, for instance the liver, but not the lung. We see the angiogenesis by injecting bubbles and exploring the ultrasound. We see how it is before treatment, and we see it seven hours later. It is a dynamic exploration of the new vessels, which showed, for instance, that the perfusion of the tumour had decreased by 30% or 40%. And if there is a change, this predicts clinical activity later on.

"It is very cheap, because you need minimal software, and you don't need to schedule a scan weeks in advance as you do with CT. We are very strong believers in this type of tool."

One of the key goals of phase II trials is to identify which patients are likely to respond

He also works with expensive high-tech micro-array techniques for genetic profiling, which can profile the expression of 40,000 genes in tumour tissue. This has to be done off site at the cost of around US $2000 a throw.

"I do a tumour biopsy before the treatment, and I do the same tumour biopsy one day, one week or a little longer after. And I see the change in the tumour as it is manifest in 40,000 genes. When there is no change in the genes, most of the time I have no responders. When there is massive change, very quickly, then I have some clinical activity. This is not yet validated. I just use it in a very experimental way."

At this stage, Armand is using the before and after data, simply to identify the changing patterns that correlate with clinical response, to help him "fight for the drug".

"Then we will move to phase II and confirm what we have seen, and *maybe* try to see if we can select the responding patients with the special profile, and identify from the 40,000 genes, the 50 or so that can be applicable in a microchip in any type of patient.

Phase II trials

Well-designed phase II trials ensure that a potentially valuable drug is given the best chance to show its worth and so be included in a phase III trial, and that complete duds are rejected as early as possible.

Traditionally, phase II trials checked for activity, usually by estimating the proportion of tumours that shrink by 50% or more when the drug is administered (singly or in

DOING THE SCIENCE

Susie Stanway of Imperial College, London, has spent the last two and a half years researching STX64, the first in a new class of drug, sulfatase inhibitors, which may help patients with hormone-responsive breast cancer.

The scientific concept

More than three-quarters of breast cancers are oestrogen-receptor positive (ER+) and it is known that suppressing oestrogen is an effective treatment. Current treatments include drugs such as tamoxifen and Fulvestrant, which block the activity of oestrogen at the receptor level, and aromatase inhibitors, which inhibit the conversion of androgens to oestrogens. However, another pathway exists, the steroid sulfatase (STS) pathway, which is responsible for the conversion of sulphated steroids, such as oestrone sulphate and dehydroepiandrosterone sulphate to biologically active oestrogenic steroids. This pathway may account for resistance to aromatase inhibitors. STX64 is a potent non-steroid-based irreversible inhibitor of the STS enzyme.

Preclinical trials

Was it likely to work? STX64 was tested in rats in which breast cancer had been induced by nitrosomethylurea, and whose ovaries had been removed. The tumour was stimulated with oestrone sulphate and the drug was then given orally, resulting in regression of the tumour.

Surrogate markers? Taking successive biopsies to measure levels of STS activity in the tumour tissue would not be feasible. Studies were done to see whether STS activity in blood correlated with that in tumour tissue, and the correlation was seen to be very strong. STS activity in peripheral blood lymphocytes (PBL) was therefore accepted as a valid surrogate marker, and its validity was later checked in human tissue.

Phase I translational research

A two-centre (London and Belfast) single-arm, dose-escalation design was used. Fourteen post-menopausal patients with metastatic ER+ breast cancer who had undergone at least one form of systemic (endocrine or chemotherapy) treatment were recruited over a period of two years.

Each patient was studied for seven weeks, using an intermittent dosing schedule – one week on, one week off.

Stanway saw her patients every day during the weeks they were on the drug, and one day in each of the intermittent weeks. Every two weeks she did a formal examination, including clinical measurement of the lesions where possible. Tumour response

combination) to patients with advanced-stage tumours of a specific primary site. Only if the proportion was high enough – and in most cases it was not – would the drug continue to a full-scale randomised phase III trial.

Under the new paradigm, the idea is that by the time a drug moves into phase II, a lot is already known from translational work in phase I about its activity in humans. Surrogate markers of activity will have been established and validated, allowing more nuanced studies into dosing schedules and to find ways to identify patients most likely to respond.

Randomisation is being increasingly used in early trials to tease out relevant information to guide the development process. As cytostatic drugs find it hard to impress over a short time-scale, and would never pass the traditional 50% tumour shrinkage criteria, randomised controlled studies can be used to provide the confidence needed to proceed to a phase III, or provide the evidence of lack of activity needed to condemn the drug. Such studies can use significance levels set far below the requirement for approval, say $p<0.1$, thus requiring far fewer patients.

Randomised controlled phase IIs were used in the development of both sorafenib and sunitinib – two of the first multi-kinase inhibitors to reach the market. In a "randomised discontinuation phase II trial" patients were given the drug, and those whose disease stabilised were then randomised to either continue or stop taking it.

Randomising to different doses or schedules (including sequential or combined administration with other

was measured according to the RECIST criteria using pre- and post-treatment bone scans and plain films, CT or MRI scans, depending on the nature of the lesion and where it was.

Every week she took a blood sample. At the end of the seven weeks, all blood samples were assayed at the same time.

Was the drug safe? Patients spent the night under observation in hospital the night after taking their first dose. They were assessed for side-effects using the US National Cancer Institute Common Toxicity Criteria version 2 at every visit and in a formal examination every two weeks. The only adverse events that were thought to be drug related were mild – grade 1 or 2.

Did the drug hit its target? Stanway measured changes in the level of STS activity in PBLs and in selected patients' tumour samples. Median STS inhibition in PBLs was 98% and in tumour samples 99% of baseline activity. This confirmed that the target was being hit, and also that STS activity in PBL is indeed a reliable surrogate marker for STS activity in the tumour.

Did inhibiting the target have the desired biological effect? The study aimed to cut down the concentration of oestrogenic steroids, oestrone, oestradiol and androstenediol, which are substrates for the aromatase enzyme. Stanway compared the concentrations pre- and post-treatment and found a significant decrease. Unexpectedly a decrease was also found in androstenedione and testosterone concentrations.

Did inhibiting the target have evidence of anti-tumour activity? She compared pre- and post-treatment scans using the RECIST criteria for disease response, stabilisation or progression. Four patients, all of whom had previously progressed on aromatase inhibitors, showed evidence of stable disease for 2.75-7 months, with a further patient showing stable disease in target lesions only.

The future

Dose. Stanway now intends to explore optimal biological dose using a continuous daily oral dosing schedule.

Target population. She also wants to identify which patients are most likely to benefit from this new therapy. To do this, an 'enrichment strategy' will be employed (which restricts recruitment to those deemed most likely to respond), exploring the use of potentially predictive biomarkers. Over-expression of STS is known to correlate with a poorer survival, and Stanway is particularly interested to see whether this predicts a stronger response to STX64. She also wants to find out about the clinical benefit rate in a homogenous enriched population of patients who have previously been treated with aromatase inhibitors.

Other indications. Ultimately, she hopes STX64 will show a significant clinical benefit rate and a favourable risk/benefit ratio in metastatic disease. If it does, the next step may be to seek approval for it to be used in the adjuvant setting. Because the drug shuts down a pathway that is common to the production of many hormonal agents, she also thinks it would make sense to test it in other hormone-dependent tumours, such as prostate and endometrial cancers.

drugs) can also yield important information about the most effective way to use the drug, which could be used to further develop a promising drug that might have failed a traditional approach using a single protocol.

One of the key goals of phase II trials is to identify which patients are likely to respond, so that the phase III trial can exclude patients known not to respond to the drug (this is known as patient 'enrichment'). Stratified phase II trials test for differences in response between patients stratified according to potentially relevant criteria – be they genomic/proteomic criteria, age, ethnicity, history of smoking, or even history of previous cancer therapies.

Very often phase II trials involve a number of different studies, some of them in patients with early-stage and some with advanced disease, looking to refine knowledge about the drug and its use. In what is known as an 'adaptive trial', as more is found out about dosing schedules and target patient groups, the patient selection in the relevant trial arms can be progressively enriched and ultimately can form the basis for a larger phase III trial, thus minimising the number of new patients who have to be accrued at this stage.

Discourse on appropriate designs for phase I/II trials of targeted drugs is still at a very early stage. A good introduction to the subject can be found in papers by Marc Buyse, Elizabeth Eisenhauer and Karen Gelmon, and Richard Simon, which can be found under the clinical trials heading of the ASCO 2006 educational book. The US Food and Drug Administration also recently conducted a workshop on this issue – Accelerating Anti-cancer Agent Development and Validation – slides from which can be found at www.fda.gov/cder/genomics/presentations/anticancer.pdf.

Approval: the final hurdle

At the end of the science, there is still phase III, where every drug has to show clinical effect in a large randomised trial – if not on survival, then at least improved disease-free survival, time to progression, or possibly decreased side-effects compared to existing therapies. The dream is that progress in validating surrogate endpoints will allow this cumbersome procedure to be dispensed with. We're not there yet, but being able to show medical plausibility can still clinch it for a drug that can only show marginal benefit in a phase III trial.

It is therefore essential not just to get the science right, but to keep the regulators on board throughout the development process. They need to be convinced that the chosen biomarkers and the assays for those biomarkers are valid, and researchers are well advised to discuss these issues, as well as the phase I/II trial design, with the regulators as they go along, to avoid spending years going down one particular path, only to be told at the end of it to go back to the drawing board. Storing tissue according to accepted standards is also vital, as regulators may well ask the researchers to go back and do further tests before their application can proceed.

Undoubtedly, developing drugs in the era of molecular biology is a complex process. The upside is that with every new anti-cancer drug developed, we learn a great deal more about the disease itself. Eggermont talks of moving from one level of complexity to the next, and is confident that Europe has the resources to meet the challenge. "The infrastructure, the institutes, the basic science and translational research labs are there and we perform very well in the translational research field. In terms of tissue legislation we are on a footing that actually has more opportunities and is simpler than in the US, where exchange of tissue between institutes is almost impossible because of the HIPAA [Health Insurance Portability and Accountability] Act."

He warns, however, that for a successful trial, researchers must take the time and money to do the necessary science. He offers the following advice: "Make sure that you have done sufficient early phase II studies with translational research components to be convinced of the potential efficacy of your drug, that you reach your target and that you can narrow down the patient population you want to study in a phase III trial. If you don't raise enough funds to take those steps, the likelihood is that you will fail in phase III. In the process you may actually kill your own drug."

With every new anti-cancer drug developed, we learn a great deal more about the disease itself