

## A CHANGE OF DIRECTION

The association with Yale brought about a number of rethinkings. The first of those was to pursue a late-stage indication in ovarian cancer, as distinct from the earlier thinking of early-stage prostate cancer. The second was to see the drug as a chemo-sensitiser rather than as a monotherapy. These decisions did not lock us out of looking at other therapeutic indications, but it did mean that late-stage ovarian cancer was going to dominate our focus for the foreseeable future.

Then there was a third rethinking. And this was a big one that led to a lot of anxious nights' sleep. This had to do with the question of whether we stayed with the intravenous dosage form, or looked again at the oral dosage form. The rethinking had been caused in part because of the reluctance of the Yale clinicians to deliver **phenoxodiol** by continuous intravenous infusion, preferring instead to give the drug as a single daily injection. This essentially was a practical issue...continuous infusion requires that patients be fitted with a portable pump that delivers the drug continuously, 24-hours per day for the course of the study, which was projected to be up to 12 months in the event of patients having a response to therapy. It is a delivery mechanism generally limited to drugs where blood levels of the drug need to be kept constant. The problem was that we couldn't provide any hard evidence that it was critical that blood levels of **phenoxodiol** be kept constant. It was just an educated guess based on our understanding of how the drug worked. In the absence of such hard evidence, Yale pushed on with bolus injections delivered over about 15 minutes. That really only left an oral dosing regime as the only way we could achieve a relatively constant blood level of the drug.

But even before this, I was beginning to have nagging doubts about the rationales that had steered us down the path of an intravenous dosage form.

The first doubt had to do with the 10-15% of **phenoxodiol** in blood following intravenous injection that was in the so-called '*free*' state, not bound to a sugar or to sulphate. This had been a fairly major factor in pushing us towards an intravenous dosage form. I had decided to put this to the test because I had begun to smell a rat. The general belief was that this '*free*' **phenoxodiol** must have been attached to a protein. We couldn't find any evidence for this,

but assumed nonetheless that that was the explanation. But I began to suspect another possible explanation. And that was that the so-called 'free' **phenoxodiol** was in fact not conjugated to anything at all because it remained locked up inside its *cyclodextrin* carrier. We had assumed that all of the **phenoxodiol** that was incorporated into the *cyclodextrin* in the laboratory would leach out once the *cyclodextrin* was injected into the bloodstream. But what if it didn't? What if a small amount remained trapped in the maze that is the internal structure of a *cyclodextrin* molecule? Drugs were able to leach out of the *cyclodextrin* maze, but it surely would be unreasonable to think that it all would come out in a rush. *Cyclodextrin* did not break down in the body, so any drug remaining inside the *cyclodextrin* structure would not have the opportunity to bind to anything in the blood and thus would appear as 'free' **phenoxodiol**. When the chemists analysed blood samples for **phenoxodiol**, they were not able to distinguish between **phenoxodiol** outside of the *cyclodextrin* and any **phenoxodiol** remaining inside of the *cyclodextrin*, because the *cyclodextrin* was dissolved along with everything else.

Some intricate laboratory studies then were conducted to test this theory. The result was as I suspected. The bulk of the **phenoxodiol** leached out of the *cyclodextrin* within an hour or so of being exposed to blood. The remainder, about 10%, took many more hours to leach out. It became clear as a result of this, that what we were interpreting as 'free' **phenoxodiol** was little more than drug that remained locked up inside *cyclodextrin* and therefore was of no biological value as it would not be available to cancer tissue. The inescapable conclusion therefore was that the intravenous route of administration was no different to the oral route of administration in terms of the conjugation outcome. In both cases, all **phenoxodiol** reaching the bloodstream and available to the body was conjugated to either *glucuronide* or *sulphate* ions.

By this stage, David Brown's team also had been dosing mice bearing various types of human cancer cells with oral **phenoxodiol** and had shown that **phenoxodiol** delivered in this form was just as effective as that given intravenously. That result totally destroyed the advice from some of the world's foremost authorities on steroid biology...that conjugated **phenoxodiol** would be unlikely to work. The entire drug that was present in the blood of mice following oral administration was conjugated, and yet it very clearly was working. Suddenly the myth of conjugated **phenoxodiol** being ineffectual was exploded.

About that time, reports also were appearing in the literature of different cancers tissues, particularly ovarian cancer and prostate cancer, expressing *glucuronidase* and *sulphatase* activity. That suggested that cancer tissues probably retained the same ability to release free **phenoxodiol** from its various conjugates in exactly the same way and to the same extent as did normal tissues. We further tested this by injecting radiolabelled **phenoxodiol** conjugates into mice bearing human prostate cancer cells, and found to our delight that the cancer tissue concentrated **phenoxodiol** compared to blood levels, and that the drug was present in a free, unconjugated form. The cancer tissue clearly was capable of deconjugating the bound **phenoxodiol** and the drug was concentrating within the cancer tissue as it sought out its tNOX target.

An oral dosage form suddenly looked both attractive and realistic. And there were a number of significant benefits. One of those was that it took away the need to sterilise the product. Oral formulations still need to be free of organisms, but the trouble and expense and lengths that need to be gone into for intravenous products versus oral products are miles apart. It

meant that issues such as the endotoxin problem that had earlier almost derailed the whole program would not have occurred with an oral formulation. Another benefit was that patients could continue with treatment at home and not have to be fitted with a pump or attend hospital for injections. But the third, and main, benefit was that we could avoid the need for *cyclodextrin*, which already had produced a range of mild, but still unwanted, side-effects. Without *cyclodextrin*, we anticipated that **phenoxodiol** would be virtually free of any unwanted side-effects.

So the decision was taken, tentatively, and not without some rigorous in-house discussion, to switch over the whole **phenoxodiol** program to an oral dosage form. The effort and investment put into the intravenous dosage form to date had been so significant that there was understandable resistance within the Company to a change in direction at this reasonably advanced stage of the program. The argument came down to whether the advantages of the oral dosage form were so overwhelming as to make this the obvious way to go irrespective of the time and money that a change at this stage would incur. I certainly believed that they did, and the argument finally was won when it was shown that we would not have to repeat any of the studies done to date. Fortunately we had not gone that far that we needed to start all over again. Virtually the only thing we needed to do was to prove what pharmacologists call *equivalence*. This refers to showing that drug levels in blood are equivalent whether that drug is delivered orally or intravenously. If we could show that they were the same, then the studies done to date would not have been wasted. If **phenoxodiol** was well tolerated in the face of certain blood levels of *conjugated phenoxodiol* following intravenous injection, then it would be safe to assume that the same levels following oral dosing would be just as well tolerated. In fact, there would be every reason to believe that the oral dosage form would be even better tolerated, given that we would be able to avoid using the *cyclodextrin* carrier that carried its own small risk of side-effects.

There were understandable concerns within the Company about this move, mainly over the time that it would take to conduct a human equivalence study. That could take 12 months all up, and that would mean placing on hold the entire clinical trial program for that time. And that assumed that we could show equivalence. If the blood profiles of the two dosage forms were substantially different, it might mean having to essentially start from scratch and redo animal safety and human Phase 1 studies.

While we were contemplating this matter, a potential solution presented itself in an unexpected way. We were contacted by a doctor from a local Sydney hospital who sought access to **phenoxodiol** on compassionate grounds for one of her patients. Such requests are not unusual for experimental anticancer drugs when all else has failed, but we had managed to deflect all such entreaties up to that time. Most countries allow the use of experimental drugs on compassionate grounds providing that the attending physician assumes full responsibility for the use of the drug, that the supplying company is in agreement, and that all concerned are satisfied that no other treatment options are available. Generally, the approval process takes just a couple of days once the supplying company agrees, with a round of phone calls to the chairman of the hospital ethics committee and the central regulatory authority.

For the drug developers, this is a difficult matter. Declining heart-felt and pleading requests for a drug for loved ones is a thankless task. However, the harsh reality is that generally there are few if any potential positives in it for the company, while at all times there is the potential for serious negatives. The nature of the typical candidate in this scenario is that he or she is

close to the end, often is in precarious health with a variety of complications from the cancer, has a very substantial tumour load, and has a tumour that has become refractory to all standard forms of therapy. The likelihood that the experimental drug is going to work under those conditions is remote. But people not unnaturally will say, well why not try? What is there to lose? What's to lose is that the patient dies while being treated from heart failure, or renal failure, or has an epileptic seizure.....all of which had to do with the terminal stages of the disease and nothing to do with the treatment. Nevertheless, it still goes on the drug's record as a reportable adverse event that is a potential blemish on the drug's character that carries all the way through to an application to the FDA for registration approval. And of course, there is always the problem of not being able to say 'yes' to everyone. The floodgates are a considerable size.

Adverse events are always going to occur with any experimental drug as it goes through its clinical development, but in a formal clinical trial program, the risk of events occurring is managed. You do this by only recruiting patients with an approved health status; and then those patients must be managed according to strict guidelines that limit such things as the use of other drugs and therapies. When an adverse event occurs under such tight conditions, then the likelihood of the drug being responsible for a particular adverse event is fairly readily decided. With compassionate use, no such control exists. The patients usually are in poor health and the doctor is free to use any combination of drugs that is deemed appropriate. So, from a company perspective, a lot of downside for very little upside.

As I have said, we had been receiving requests for compassionate access to **phenoxodiol** on a fairly regular basis, and we had managed to maintain a polite but firm refusal. But the latest request was different. First, because of the timing of the request. It came right as we were agonising over the issue of changing the dosage form. We knew that the oral dosage form was absorbed in mice, and was an effective anti-cancer treatment, but that was using *cyclodextrin* as a carrier for the **phenoxodiol**, and we were keen to avoid that in the human dosage form. What we wanted to use was a simple dry formulation of **phenoxodiol** in a capsule. Before embarking on an expensive equivalence study, it would have been useful to get some indication from just a few patients that we were on the right track with such a formulation. Supplying drug to a few compassionate use patients was an opportune and quick way to obtain that information.

The other matter that made this request different was because of who made the request. I didn't know the oncologist, but she introduced herself as Max's oncologist, Max being the patient with renal carcinoma who had been the first cancer patient to receive **phenoxodiol**. She had been so impressed with Max's response to **phenoxodiol**, that when her next patient with renal carcinoma reached the point of becoming unresponsive to chemotherapy, she contacted Novogen.

The request caused a lot of soul-searching and angst within the Company, with the risk of opening up the floodgates for compassionate use being the main concern. Fortunately the argument for quick action prevailed and along with a promise to limit the extent of compassionate use to no more than a couple of patients, the request was approved.

We quickly filled some capsules with **phenoxodiol** and dispatched them off to the hospital. The actual process of formulating the final oral dosage form was a task that went on over most of the following year. Working out the excipients (all the non-active ingredients that have to do with bulking up the material, allowing it to flow for filling purposes etc) took

some months, followed by the need to conduct accelerated stability tests to determine the shelf-life of the product. But for the purpose of this urgent compassionate use, hand-filling some empty capsules sufficed.

We picked a dose of **phenoxodiol** (200 mg, or approximately 3 mg/kg body weight) that we thought from our experience in mice would deliver the sort of blood levels we wanted, and again on the basis of mouse data, repeated this dose every 8 hours. Blood was collected and analysed and we were able to confirm that this dosing schedule roughly mimicked the sort of blood levels and conjugation profile that we had achieved with continuous intravenous infusion. This was repeated and confirmed on a further two patients, giving us all the confidence we needed to commit to a change in dosage formulation.

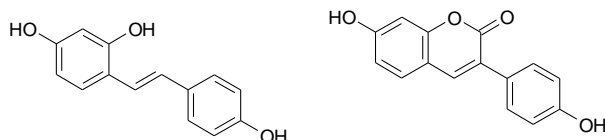
The next step was to conduct a formal pharmacokinetic and acute safety study. This was to be done in patients with haematological cancers. We had been under pressure from a local clinic to try **phenoxodiol** in patients with refractory leukaemias, and had already started down that path with the intravenous dosage form. But with the news that we had decided to discontinue with the intravenous formulation, the investigators were amenable to adjusting the trial, and so we had a ready-made Phase 1 pharmacokinetic and safety study.

Ultimately, 7 patients were treated with oral **phenoxodiol** at dosages up to 58 mg/kg for 15 days. The pharmacokinetic profile of the drug mirrored that of the intravenous dosage formulation in humans and that of the oral dosage formulation in mice. This provided all the evidence that we needed that the oral dosage formulation was an entirely suitable way to administer the drug in terms of its bio-availability (particularly in the absence of *cyclodextrin*), and was well tolerated both locally (eg. no gastric irritation) and systemically.

Yale were duly informed about the decision to switch to an oral dosage form and the Company set about applying to the FDA for a new IND for this change of formulation. That IND eventually was granted in June 2003.

For the pharmacologically-inclined, the key details of the oral dosage form are as follows.

- **Phenoxodiol** appears in blood within 20-30 minutes of dosing, and peak plasma levels occur at 2-3 hours, indicating absorption mainly from the stomach and upper small intestine.
- The absorption rate varies between 20-30%.
- With the recommended dosage of 400 mg 8-hourly, steady-state plasma levels are reached within 48 hours with levels ranging between 10-60 ug/mL.
- **Phenoxodiol** appears in blood as both single and double conjugates of *glucuronide* and *sulphate*.
- The half-life of **phenoxodiol** in blood is 8.4 hours.
- **Phenoxodiol** is excreted primarily in urine (as conjugates), although animal studies also indicate a modest amount is excreted through the bile.
- Two metabolites of **phenoxodiol** have been identified (shown below), but these are very minor only. The bulk of the drug remains intact.



I don't look back on this part of the story, and the extended detour that we took, with any sense of embarrassment or regret. It was the consequence of dealing with a drug that was first in its class. There was no manual on the bookshelf or published experience of others to rely on when it came to developing an isoflavonoid drug. The analytical techniques and blood assays all had to be developed from scratch, with no reference points to guide the chemists. And there is no better example of the poor state of knowledge of the field than the advice of the international experts that we consulted regarding the conjugated state of **phenoxodiol**, much of which ultimately proved to be wrong. We were breaking new ground, and with new ground in any area of medical research, not every educated guess turns out to be right. The important thing is that we did eventually get it right, and in the end I am not sure that it cost us all that much time. The lessons coming from the early learning experience with the intravenous dosage form were able to be applied in large part to the oral dosage form without any need to repeat them. For that reason we were able to segue from the intravenous dosage form to the oral dosage form with minimal delay to the overall clinical program.

The greatest cause of delay had more to do with trying to work out where to focus our efforts in terms of therapeutic indication. With the benefit of hindsight, a clearer focus from the start would almost certainly have cut 3-4 years off the program. But I am sure NASA could say the same thing about its space program.

What is more important, however, is that the lessons learnt from this experience were applied later to the **triphendiol** drug program, saving considerable time and money. The clinical application of **triphendiol** was identified very early on and not detoured from.

## MISCELLANEA

By 2006 we had finally settled on the primary (ovarian cancer) and secondary (prostate cancer) indications for **phenoxodiol**. But before then, a number of tentative directions were tried which we can address here just to tidy up the story.

The first tentative direction was that of haematological cancers. We had succumbed to the entreaties of a local haematology clinic to look at the potential therapeutic benefit of **phenoxodiol** in cancers of the blood because of some strikingly good anti-cancer effects of the drug in the laboratory with leukaemic cells. We had known about this effect from the early days, when we had included acute lymphoblastic leukaemic and chronic myeloid leukaemic cells in the early screening batteries of cancer cells. **Phenoxodiol** showed strong cytotoxicity against those cell lines.

The drug also showed a separate ability to induce terminal differentiation in HL60 cells. This cell line was established by the National Cancer Institute from a 36-year old woman with acute promyelocytic leukaemia. **Phenoxodiol** caused these undifferentiated cancer cells to differentiate into monocytes,

This news, once published, attracted the attention of a number of haematology clinics, one of whom persisted to the point where we agreed finally to cooperate. At the time there was no great interest on our part in haematological cancers for no other reason than you can't do everything, and our initial focus was on solid cancers such as prostate cancer.

This particular Sydney clinic took the drug and tested it against a library of highly chemo-resistant cell lines that they had accumulated over the years from failed cases. **Phenoxodiol** showed remarkably good killing activity against most of these cell lines, where no other chemotherapy showed any effect. Not surprisingly they wanted the drug for their patients who had become refractory to standard therapies.

We started with the intravenous dosage form, because that was our preferred dosage form at the time. Seven patients received rising dosages for about 2 months. Then we shifted to the

oral dosage form, with a further 7 patients receiving incremental dosing up to about 58 mg/kg per day for about 2 months again. We saw no sign of any response in these patients, and called a stop to the study at that point once we came to better understand the nature of the blood conjugation process. The *glucuronide* and *sulphate* conjugates of **phenoxodiol** were biologically inactive. The bond between **phenoxodiol** and the sugar molecule or the *sulphate* ion needed to be cleaved by the *glucuronidase* or *sulphatase* enzymes respectively in order for the drug to be released and able to attack cancer cells. The problem was that those enzymes are only present in solid tissues. They are not present in blood, because if they were, the whole process of conjugation would be prevented, destroying the body's vital system of transport of water-insoluble chemicals. Once we came to understand this simple biological fact, it was clear that **phenoxodiol** would find no application in a cancer that was confined to the body's blood compartment and which was devoid of de-conjugating enzyme activity.

While this automatically steered **phenoxodiol** down the path of a treatment for solid tumors only, even there we encountered some anomalies. We were relying on the fact that solid cancer tissue expressed *glucuronidase* and *sulphatase* activity in order to release **phenoxodiol** from its conjugated state. Conversely, some cancers do the opposite to this and over-express the capacity to make conjugates. This is not that surprising in the case, for example, of bowel cancer. *UDP-glucuronosyltransferase* is the enzyme present in the cells lining the bowel wall that are responsible for attaching glucuronide to water-insoluble chemicals (such as **phenoxodiol**) in order for them to be passed into the bloodstream. When these cells become cancerous, it is not unusual for them to over-express that activity. That leads to a situation in those tumours where any activity of the two de-conjugating enzymes freeing up **phenoxodiol** is more than offset by the ability of the cancer cells to re-conjugate the drug.

The two cell types that are known to be capable of over-expressing *UDP-glucuronosyltransferase* activity are colon cancer and pancreatic cancer. We certainly encountered this, with **phenoxodiol** showing much reduced killing effect against some of these cell types. It was not, however, a universal effect, with some colon and pancreatic cancer cell lines showing the same sensitivity to **phenoxodiol** as any other cancer types, from which we assumed that over-expression of *UDP-glucuronosyltransferase* does not occur in all forms of those two cancers. [As an aside, the curious thing is that **triphendiol**, a derivative of **phenoxodiol**, appears to be almost universally effective against pancreatic cancer cell lines. **Triphendiol** is conjugated in blood in the same manner as **phenoxodiol**, so just why it should be able to circumvent excessive *UDP-glucuronosyltransferase* activity in solid cancers when **phenoxodiol** cannot is not immediately clear. **Triphendiol** has a slightly different mechanism of action to **phenoxodiol**, and it is possible that in this difference, *UDP-glucuronosyltransferase* activity is switched off.]

A decision to abandon any haematological cancer indication for **phenoxodiol** wasn't difficult. We hadn't sought it at the outset, so nothing was lost. In fact, it had been a very useful learning experience, helping us towards an understanding of the drug conjugation process, as well as serving as a pharmacokinetic and acute safety study for the new oral dosage formulation.

But that wasn't the end of the matter. In the next breath we were contacted by oncologists at the Children's Hospital of Los Angeles. This is the largest children's hospital in the world, and a leader in the fight against childhood cancers such as leukaemia and neuroblastoma. The group that contacted us had a long-standing interest in the use of **ceramide**-like drugs to treat

childhood leukaemias. The rationale was that apoptosis could be induced in the cancers by mimicking the action of the pro-death signalling pathway that was associated with **ceramide**. With the publication of data showing that **phenoxodiol** was operating via the sphingomyelin pathway and killing cancer cells by shifting the ratio of **ceramide** to **sphingosine-1-phosphate**, it was only natural that they would want to follow up with us.

We came to learn that this group had accumulated the largest library in the world of leukaemic cancer cells obtained from children. This was an extraordinary resource of hundreds of cell lines that were resistant to all forms of chemotherapy. And just as the group of Sydney haematologists had found with their much smaller library 2 years earlier, **phenoxodiol** was highly effective at killing these cells, almost universally. As with the Sydney group, the Los Angeles group's enthusiasm for looking to put the drug into patients was infectious and persistent. But by this time we knew that **phenoxodiol** was not the answer. If **phenoxodiol** or any of a hundred or so analogues of **phenoxodiol** that Novogen chemists had created by that time was to work clinically, we would need to find a way to circumvent the conjugation problem.

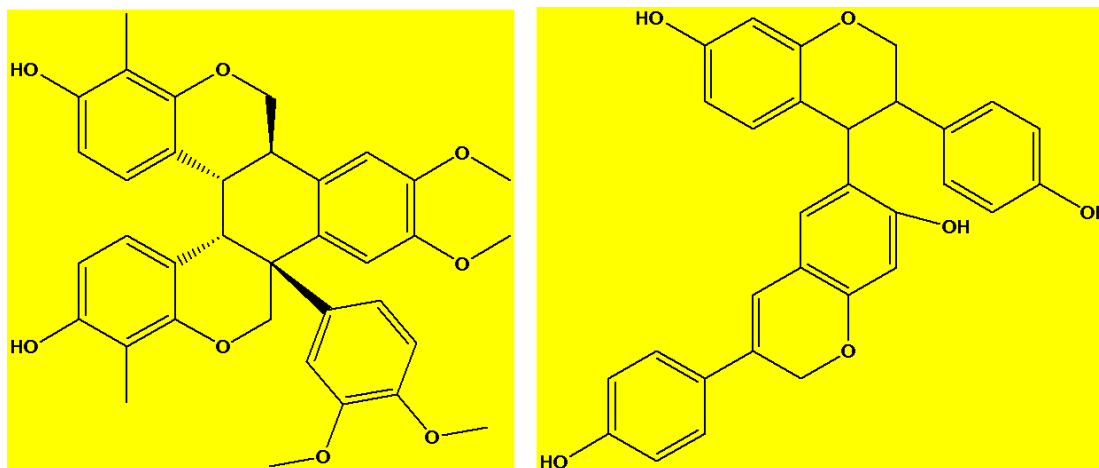
One potential solution lay with the way the body handled steroids. As we have looked at earlier, steroids made in the body (or injected) are transported around the body attached mainly to the blood protein, *sex hormone-binding globulin (SHBG)*. When this complex passes through the liver, a proportion of it is uncoupled and then re-conjugated to *glucuronide* and *sulphate*. The purpose of this is to ensure that steroid levels don't build up excessively. Steroids attached to blood proteins can't leave the body because the kidney can't excrete protein into the urine. Steroids in the form of *glucuronide* and *sulphate* conjugates on the other hand can pass into urine. The conjugation process is acting like an overflow pipe, thereby ensuring that steroid levels can be regulated. Something like one-third of the steroids in our bodies is conjugated in this way.

The steroid-protein bond is relatively weak and can be broken quite readily without the need for any enzyme to cut it. In this way, steroids are able to provide a biological response in blood as well as in solid tissues. The steroid-conjugate bond on the other hand is very strong and can only be broken by enzymes. Those enzymes (*glucuronidase* and *sulphatase*) are only present in solid tissues, and the lack of them in blood means that steroid-conjugate complexes are inactive in blood.

When we compared in the laboratory the behaviour of **phenoxodiol** to that of steroids, we found that **phenoxodiol** also binds readily to blood proteins. The difference is that it attaches mostly to albumin, the dominant protein in blood, with only a small proportion binding to sex hormone-binding globulin. Steroids have the reverse of that situation. That difference probably wouldn't stop **phenoxodiol** in theory from being able to detach itself from the protein when it comes across a leukaemic cell in the bloodstream or in the bone marrow, delivering a killing punch to the cancer cell. However, the problem is that this situation does not occur in the body. When **phenoxodiol** is given orally, it all ends up being conjugated during its passage across the gut wall, so binding to any blood protein doesn't come into the equation. But when given intravenously, the **phenoxodiol** that leaches out of the *cyclodextrin* into the blood must obviously attach to something in order to be solubilised and that 'something' almost certainly is albumin. The problem is that the **phenoxodiol**-protein complex doesn't stick around long enough to work. With steroids, the liver uncouples about one-third of the steroid-protein combination, converting it to conjugates. With **phenoxodiol**, the rate of uncoupling and conversion to conjugates by the liver is close to 100%. If we were

ever to have an isoflavonoid drug like **phenoxodiol** capable of killing blood-borne cancer cells, then we were going to need to find a way of either blocking or avoiding the ability of the liver or the gut wall to conjugate the drug.

Novogen head chemist, Andrew Heaton, came up with the potential solution. The *UDP-glucuronosyltransferase* enzyme that conducted the conjugation step was only able to perform that step on chemicals of a certain size. The question then was, if we somehow increased the size of the drug, would that block the conjugation step? And so was born the concept of dimers, or the joining together of two isoflavonoid molecules as per the following two examples.



A variety of combinations was manufactured .... various joinings of **phenoxodiol**, **equol** and many of the dozens of the novel isoflavonoid compounds that the chemistry team had synthesised. Most dimers showed no anti-cancer activity, some showed some activity, and one showed stand-out activity – this was a dimer code-named **NV5063**. **NV5063** was at least as potent in the test-tube as **phenoxodiol** against most forms of cancer, but most importantly, was effective against those colonic and pancreatic cancer cells that were resistant to **phenoxodiol**.

The first question needing to be asked was whether this dimer would resist the action of conjugating enzymes such as *UDP-glucuronosyltransferase*? To test this, the drug was given either orally or by intra-peritoneal injection to mice and blood then collected for analysis. One hour following dosing, levels of unconjugated **phenoxodiol** in blood were 2 and 80 uM respectively. The dimer apparently wasn't escaping conjugation as it crossed the gut mucosa, but it appeared to be preventing in large part the ability of the liver to uncouple the **NV5063**-protein bond. The outcome of the intra-peritoneal injection looked very similar to the situation with steroids. To my simple mind, this looked like a highly promising drug, not just for colorectal and pancreatic carcinomas and leukaemias, but as a more general anti-cancer drug. True, it would need to be given intravenously, but that would have a very small price to pay for an effective drug.

Regrettably, we probably will never know. The Company canned the project. **NV5063** proved a difficult drug to manufacture. Not impossibly difficult, but difficult enough to make the chemistry team very reluctant to devote time to it. At the time, they were heavily committed to maintaining **phenoxodiol** supplies, as well as being charged with designing new drugs (such as **triphendiol** and **NV128**), and the challenge of committing significant

manpower resources to producing a very tricky drug was understandably not particularly welcome.

The argument that **taxol**, ultimately to become a major new drug, provided a similar manufacturing challenge, carried little sway. It took chemists about 8 years to crack that problem, so that argument was cold comfort, although no-one argued that the **NV5063** problem was quite in the same league as **taxol**. Nevertheless, with a lot of cajoling, we managed to scrounge from the chemists enough drug to conduct a couple of preliminary studies in mice. The first step was to see if the drug could provide a significant anti-cancer effect in its own right in vivo. We did this by injecting it intra-peritoneally into mice bearing human ovarian cancer cells. It produced an anti-cancer effect similar to that of **phenoxodiol**. So we had our confirmation that it was an effective, injectable, anti-cancer drug.

With the ultimate goal being the use of the drug in colorectal cancer and leukaemia, we then set about looking at the best way to use it. By this time we had come to the realisation with **phenoxodiol** that there was little joy in going down the route of monotherapy in late-stage disease. If we were going to be using the drug in late-stage disease, then we needed to look at combination therapy, with the aim of either synergising a sensitive tumour to a second drug, or reversing chemo-resistance in a resistant tumour.

**NV5063** proved highly effective in the test-tube at reversing resistance to **gemcitabine**, **paclitaxel** and **doxorubicin**. The **doxorubicin** effect was particularly interesting, in part because that is the drug of choice for many forms of leukaemia, and in part because it was the opposite effect to that of **phenoxodiol**.

In conjunction with the Children's Hospital of Los Angeles, we decided to focus on acute lymphoblastic leukemia (ALL), a disease responsible for about one-quarter of all cancer diagnoses in children under the age of 15. It also was decided to focus in the first instance on **doxorubicin** as the second chemotherapy. The first study was an in vitro study looking at the effect of **doxorubicin** and **NV5063** on ALL cell lines. The cell lines that we looked at were particularly insensitive to standard chemotherapies. **Doxorubicin** and **NV5063** alone had little or just a modest effect across those cell lines. But when combined, the two drugs produced a dramatic killing effect.

After that, we had just enough drug remaining to set up an in vivo study of combined **NV5063** and **doxorubicin** therapy in mice bearing human ALL cells. That study was inconclusive, pointing to the obvious need, as we later learnt from the **phenoxodiol** experience, to look at different ways of using different drug combinations to determine the optimal combination. Nevertheless, by this time we had used up our stockpile of drug and that was where the program ended. **NV5063**, in my humble opinion is one of the Company's greatest assets and the start of a rich vein of potential anticancer drug candidates that richly deserves to be mined. I very much hope one day that it will.

The other source of potential therapeutic indications was the compassionate use trial. What had begun as an offer to supply drug to 1 or 2 patients with renal carcinoma, largely as a means of proving the credentials of the new oral dosage formulation, eventually turned into an informal study involving 40 patients. It grew mainly because, once started, we realised that it offered an ideal opportunity to explore the drug's usefulness in a range of cancer types beyond the prostate and ovarian cancer indications that we were homing in on at that time.

But, in truth, we were still very much in the dark as to the optimal way to use the drug, so any opportunity to extend our experience with the drug was welcome.

Any clinical outcomes from a compassionate use scenario were always going to be subject to the usual caution that these patients were outside of the usual formal clinical trial controls. But we had decided to mitigate this by limiting any compassionate use to the one clinic and to the one clinician, and by working closely with that clinician, ensure that the drug was used in a restricted and controlled way. Ultimately this arrangement came to involve 40 patients, by which time I had run out of excuses, trade-offs, threats etc to keep the program running. But by the time that program ended, it had yielded invaluable information on the use of the drug. The trial ended because it became a victim of its own success. Enough patients responded to the treatment that the ongoing supply of drug became a serious issue. At the time, we were manufacturing **phenoxodiol** in-house. The manufacturing process was still being refined and the process was underway with external contractors to move into large-scale manufacture. But all drug at the time was being made in-house. The Company had commissioned at considerable expense a sophisticated small-scale synthetic plant to meet the growing need for drug, but the limited scale of the operation meant that it was laborious and expensive.

One of the conditions of testing a drug in a formal clinical study (or supplying it on a compassionate use basis), is that the Sponsor has to commit to continue to supply the drug indefinitely in the event of a response. With a growing number of people from the compassionate use program being supplied with the drug, the financial team were becoming alarmed and the drug production team were complaining of their stocks being raided. The second patient to be given the drug on a compassionate use basis (with pancreatic cancer) was still being supplied with drug some 3 years later, with his cancer in remission and no end to treatment in sight. It was obvious that a line had to be ruled under the program, and that was at the 40<sup>th</sup> patient.

Patient confidentiality and the fact that no formal report will ever be published on the patients in the compassionate use program, limit our ability to talk about the outcome in any detail. However, a clinical report was published on one case, and so we can look at that in some detail.

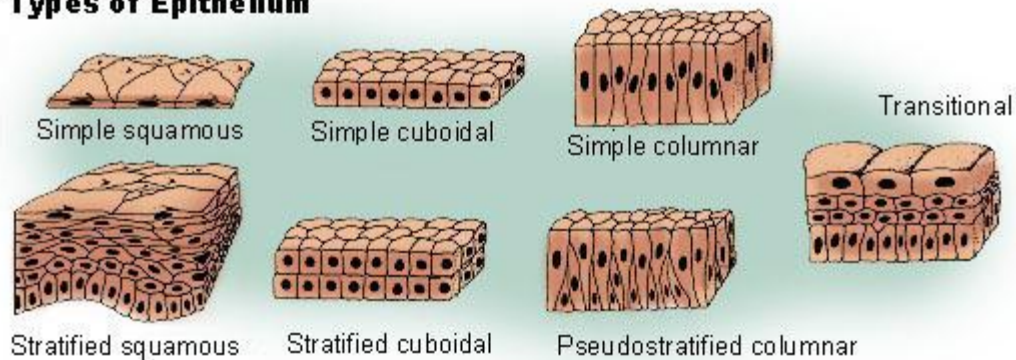
This case involved a male patient with highly aggressive squamous cell carcinoma (SCC) of the skin. The patient some years earlier had accidentally been exposed to a very high dose of ultraviolet irradiation to his legs. The result was the recurrent development of multiple SCCs that were aggressive in the extreme, requiring regular and prompt surgical resection. Various chemotherapies had been tried to no avail. The response to **phenoxodiol** was dramatic and sudden. Existing tumours disappeared in a matter of days and no new ones appeared while treatment was maintained.

This prompted us to commence a small formal study of **phenoxodiol** monotherapy in patients with aggressive SCC, but that study eventually had to be abandoned because of problems with patient recruitment. Patients with a primary SCC of the skin (usually the face) are referred generally immediately for surgery. Patients with recurrent SCC of the extent that we had seen in the compassionate use program patient, turned out to be hard to recruit, despite the initial optimism of the hospital oncologists who had supported the study.

One message that we did take out of this experience was the likelihood that SCC may be a type of cancer this is particularly sensitive to **phenoxodiol**. While SCC of the skin may not be a therapeutic indication we would want to pursue because it is not a strong candidate for chemotherapy, SCC is not limited to skin.

Squamous cells are a type of epithelium. Epithelium is the tissue that lines any part of our body that comes in contact with the external world, along with collecting ducts for glands and hollow organs such as the bladder. There are a number of different types of epithelium as shown below.

### Types of Epithelium



Squamous epithelium is found in skin, the lining of the mouth and throat, oesophagus, trachea, lungs, cervix and vagina.

It was entirely possible that our case of skin SCC had responded to **phenoxodiol** for specific reasons related to that one individual (a particular genotype) and to the circumstances that had caused that particular cancer (UV damage), and was not necessarily applicable to SCC in general. But we didn't believe so, because SCC cell lines from sites other than skin (eg. lung and vagina) also showed high sensitivity to **phenoxodiol** in the test-tube.

It made sense therefore to shift focus away from SCC of the skin to SCC of another site where chemotherapy, and not surgery, was a more standard form of therapy. Lung cancer and oesophageal cancer came immediately to mind because of their prevalence and lack of effective chemotherapy with any enduring effect. But we settled in the end on cancers of the cervix and vagina. There were a number of reasons behind this decision. One was our involvement with the Yale group. The ovarian cancer connection meant that we were already dealing with a department focused on gynaecological cancers. The Yale physicians made the case that there was a significant unmet clinical need for cervical and vaginal cancers that are inoperable and which typically show little or no responsiveness to chemotherapy. They had more than enough cases to establish a clinical study, and so one was started. It was what is known as a *neoadjuvant* study, meaning that the test therapy was used prior to the initiation of standard therapy, which in this case was surgical resection. Women were recruited who had SCC or adenocarcinoma of the cervix, vagina or vulva. The strategy was to treat them with **phenoxodiol** for 4 weeks, which is the usual time between diagnosis and surgery. We would have tissue specimens collected both before and after treatment, allowing us to look closely at the effect of drug therapy on the cancer process, as well as analysing the cancer tissue for **phenoxodiol** levels. There were to be 10 women in each of three groups receiving 50 mg, 200 mg or 400 mg **phenoxodiol** 8-hourly.

A final report on this study has yet to be published. An interim report that was made public after the first two dosage groups were recruited made the following observations:

- (i) there were no tumour responses (as defined by RECIST criteria), although most patients showed a reduction in the sum longitudinal diameter of their tumour by between 20-27%;
- (ii) 2 of 6 patients in the 50 mg dose group, and 8 of 8 in the 200 mg dose group were considered to have stabilised disease by RECIST;
- (iii) all patients in the 200 mg dose group showed histological evidence of an anti-cancer effect evidenced by an increased apoptotic index and a decreased mitotic index.

Within the confines of a 4-week treatment window, this outcome is highly encouraging, and of course we await with interest the outcome of the highest dosage group. But what this interim data confirms to me is the greater effectiveness of **phenoxodiol** as a monotherapy in early-stage cancer. These women did not have large tumour loads, the tumours were relatively early in their life-cycles having been detected by colposcopy, and they were naive in terms of chemotherapy.

This view is significant because it goes to the heart of the real reason why we agreed to set up this study in the first place. And that was because it offered a unique opportunity to shift the use of **phenoxodiol** from the typical late-stage scenario that we were locked into for regulatory reasons, back to early-stage cancer. Cancer of the cervix is arguably the only cancer where it is possible to detect cancer with ease in its formative stages, and where progression of the disease is readily and accurately monitored. The PAP smear detects not just early-stage cancer, but even further back into pre-malignant disease. From that point onwards, colposcopy and ongoing PAP smears allow progression of the disease to be readily monitored.

The study as it was designed meant that we were exposing **phenoxodiol** to cancers at a far earlier stage than ever before. If that provided evidence of efficacy, then the opportunity was there to take it even further back into the cancer process...at the first sign of cancer cells, or even before that, to the first appearance of premalignant cells, something that a PAP smear would pick up. To the best of our knowledge, no cytotoxic chemotherapy had ever been used at such an early stage, and for good reason, because of the unwanted side-effects. Using **phenoxodiol** under those circumstances would be breaking entirely new ground in cancer therapy. Whether it would ever lead to early cancer therapy beyond that of cervical cancer was not the issue. The issue was that it would establish the bona fides of the drug in the treatment, almost prevention, of cancer.

The full scenario imagined was a trial of the drug on a neoadjuvant basis in patients with early-stage cervical cancer prior to laser treatment. The good folks at Yale were supportive and keen to progress the concept. We watch with interest to see if this concept comes to fruition.