

OVARIAN CANCER

For better or worse, this is where we decided to focus our pivotal study efforts.

The rationale was fairly compelling. First, there was the matter of need, always a significant factor when it comes to gaining the interest and support of regulators. Ovarian cancer is an area of significant unmet clinical need. Diagnosed early, treatment is usually successful, with a 10-year survival rate of about 85%. But diagnosed later in the disease process, as most cases (about 80%) unfortunately are, treatment generally is regarded as palliative, and the 10-year survival rate for these women drops to about 10%.

Early diagnosis is complicated by the fact that the symptoms of early-stage ovarian cancer are relatively non-descript - tiredness, abdominal pain or discomfort, back pain, bloating, constipation and urinary urgency. More alarming and more specific symptoms such as pelvic pain, vaginal bleeding and ascites (build-up of fluid in the abdomen) are much less common early-stage symptoms.

The lifetime risk of developing ovarian cancer is about 1.6%, that is, about 1 in 70 women. If a woman has a mother or sister with the cancer, then that risk rises to 5%. If a woman is carrying the mutated form of the BRCA1 or 2 genes, the risk rises even higher to 40%. Ovarian cancer is the fifth leading cause of death from cancer in women and the most lethal of all of the gynaecological cancers, including breast cancer.

The prevalence of ovarian cancer meant that it was certain to get the attention and assistance of regulators, plus it represented a major market opportunity for a new drug.

The second rationale was the need to address the problem of chemo-resistance, something which **phenoxodiol** is eminently suited to do. The problem of chemo-resistance is not limited to ovarian cancer by any means, but it is a prominent feature of this form of cancer.

The standard primary therapy is platinum-based chemotherapy in combination with debulking surgery (designed to reduce the tumour mass as much as possible). The reported response rates to this approach range from 50-80%. Of patients who respond to first-line chemotherapy, less than 10 – 15% will remain in remission, and most relapsed cases are chemo-resistant, with the response rate to second-line chemotherapy being currently in the order of 20%, and even less if the disease is platinum-resistant. Drugs such as **gemcitabine**, **doxorubicin** and **topotecan** are used commonly in subsequent lines of chemotherapy, but the development of chemo-resistance to first-line drugs extends in large measure also to these other drugs. The main purpose in using these other drugs is to extend life long enough in the hope that resistance to platinum and taxanes will diminish with time.

The failure of some ovarian cancers to respond to first-line chemotherapy (chemo-insensitivity) and the development of resistance to multi-drug therapies (chemo-resistance) represent the major hurdles to effective therapy of ovarian cancer. Drugs such as the **platinums** and **taxanes** offer the ability to keep the tumours in check, but the presence of insensitivity to start with or the development of resistance with time, substantially mitigate that ability. A drug that restored chemo-sensitivity would represent a major advance for this particular cancer.

And then there was the third and ultimately compelling rationale – the enthusiasm and guidance of the Yale group working with **phenoxodiol**. Their ongoing studies made it increasingly abundantly clear that the mechanism of action of **phenoxodiol** was very well suited to ovarian cancer.

- (i) In the first place, **phenoxodiol** was equally effective against both chemo-sensitive (to **platinums** and **taxanes**) and chemo-resistant ovarian cancer cells. That is, the anti-cancer action of **phenoxodiol** was independent of any multi-drug resistance mechanisms that ovarian cancer cells may have developed. This ability is what had brought **phenoxodiol** to the attention of the Yale group originally.... the fact that it was superior to any other drug they tested against a large panel of multi-drug resistant ovarian cancer cells.
- (ii) Second, **phenoxodiol** sensitized chemo-sensitive ovarian cancer cells to the effects of platinum (**cisplatin** and **carboplatin**), taxanes (**paclitaxel** and **docetaxel**), **gemcitabine** and **topotecan**.
- (iii) Third, **phenoxodiol** restored sensitivity to **cisplatin** and **carboplatin** in platinum-resistant ovarian cancer cells.

The various anti-cancer effects of **phenoxodiol** in the case of ovarian cancer cells appeared to all stem from its inhibitory effect on the cancer cell's proton pump mechanism. Whether it was preventing the cancer cells from dividing, or causing them to undergo apoptosis, or enhancing the cytotoxic effect of other drugs, or even restoring sensitivity to other drugs, the underlying mechanisms of action had their origins in the disruption to the sphingomyelin cycle. Increased **ceramide** levels and decreased **sphingosine-1-phosphate** levels were behind these effects, up-regulating certain pro-death signal transduction pathways in the former case and shutting off pro-survival pathways (eg. Akt) in the latter.

In the case of ovarian cancer cells, a particularly important outcome of these actions was the effect on the family of **caspase** enzymes and their ability to induce apoptosis. The Yale group had shown that ovarian cancer cells over-express two important proteins that serve to keep these self-destructive enzymes from running amok in normal cells. These two regulatory

proteins are known as FLIP_{short} and XIAP. Their aim is to block the various pro-death signals from reaching and activating the main executioner enzyme, *caspase 3*. They are something like Judas goats, absorbing the death signals and sacrificing themselves so that those signals fail to reach the *caspase 3* molecules. As long as *caspase 3* is kept in check, the cell will survive; but if allowed to be activated, then beyond this critical point of no return, a series of events is set in train that results in the auto-digestion of the nucleus. By over-expressing these two proteins, ovarian cancer cells ensure their ongoing survival in the face of the body's attempts to destroy them and the best efforts of chemotherapy drugs to kill them.

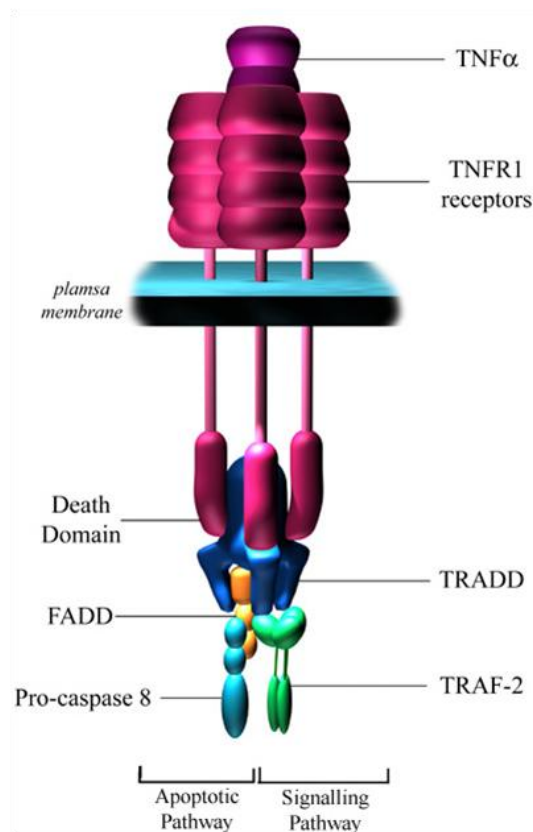
The *ying* and *yang* of cell survival in relation to *caspase 3* goes something like this. [The following is an over-simplification admittedly, but essentially a summary of what is going on in our bodies continuously. We have touched on this subject earlier, but it is worth repeating here in a bit more detail because of its particular relevance to ovarian cancer.] The *ying* is the efforts of the body to ensure the cell's survival and the *yang* the counterbalancing efforts to kill the cell.

The *ying* is due mainly to chemical messages such as growth hormones and dozens of different cytokines. These reach the cell via the bloodstream from distant cells and neighbouring cells and even the individual cell itself. Their role is to activate a range of growth receptors scattered around the surface of the cell. One of the main growth receptors is *sphingosine kinase*. Activating this receptor triggers a wide range of pro-survival responses such as cell enlargement, growth, proliferation and migration. The primary response of *sphingosine kinase* is to produce the sphingolipid, *sphingosine-1-phosphate*, which then goes on to activate various pro-survival signalling pathways such as Akt. One of the effects of activating Akt is to lead to the production of FLIP and XIAP.

Ovarian cancer cells have increased *sphingosine kinase* activity that leads to increased Akt function that leads to increased XIAP and FLIP levels. The end result is that the amount of *yang* required to activate *caspase 3* is grossly enhanced.

The *yang* is coming via the cell's death receptors. These are structures located on the cell surface that respond to chemical death signals. This situation is the counterpart of the pro-survival chemical messages stimulating the growth receptors ... chemical signals designed specifically to stimulate receptors that promote death.

There are different types of death receptors, the main ones going by the titles of TRAIL, TNF and Fas. The following diagram shows the structure of the TNFR1 death receptor interacting with its specific activating death factor, TNF α , but the structure is essentially the same across the different death receptors.



[Death receptor function is nicely illustrated on the site www.sgul.ac.uk/depts/immunology/~dash/apoptosis/receptors.htm].

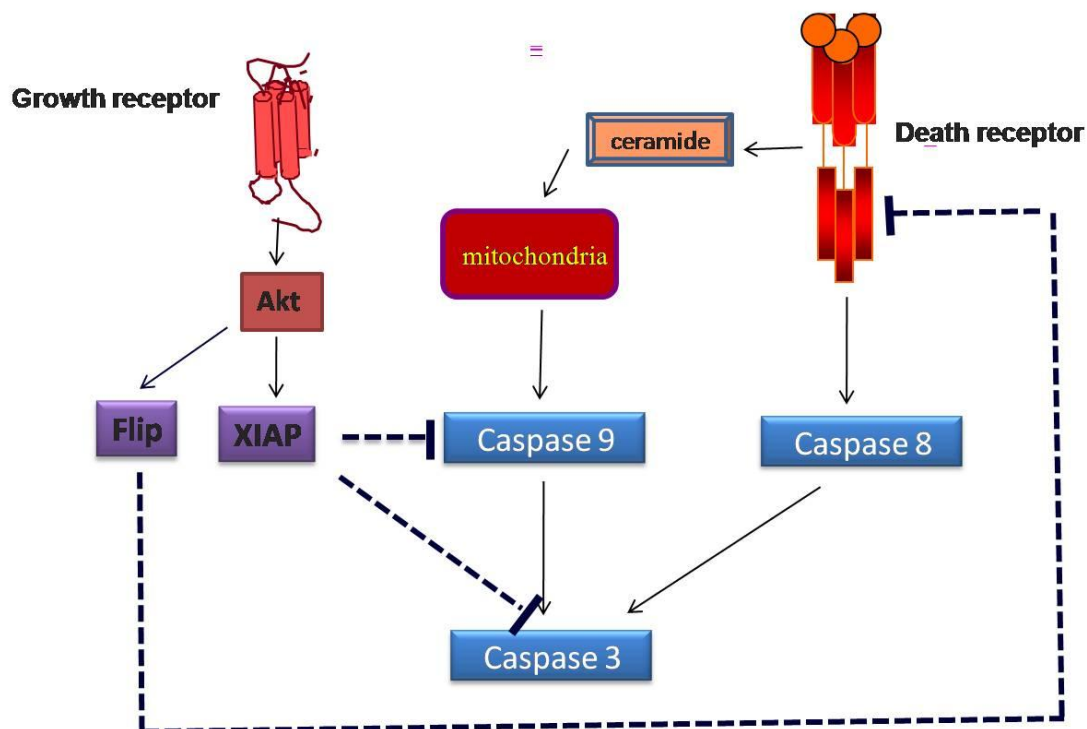
The most relevant death receptor for **phenoxodiol** appears to be Fas. That doesn't mean that it doesn't affect the function of the other death receptors, but the Fas death receptor is preferentially involved, and the relevance of that is that Fas appears to be the predominant death receptor active in ovarian cancer.

The death receptor straddles the cell membrane, comprising an external part (that receives and attaches to the death signals) and an internal part known as the death domain. Activation of the death receptor leads to the recruitment within the cell of a protein known as FADD (Fas-associated death domain protein). This event then triggers two separate reactions.

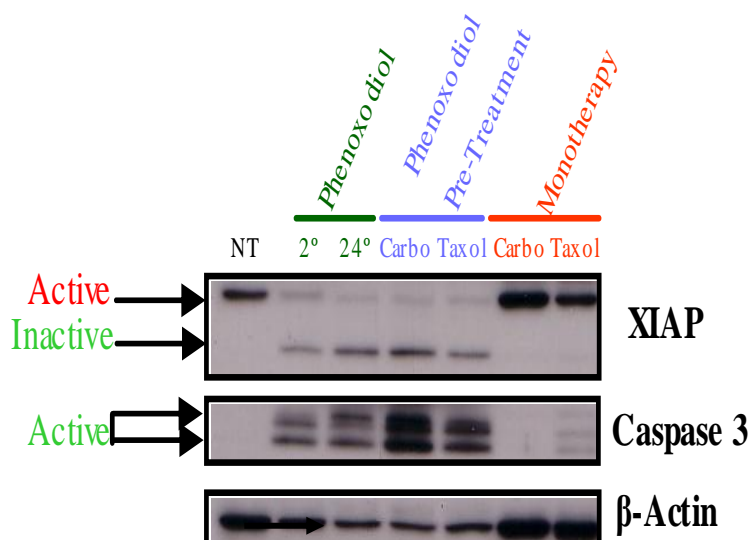
The first reaction is the recruitment of *caspase 8* to the complex and its subsequent activation. *Caspase 8* then goes on to activate *caspase 3*, thus setting in motion the apoptotic process.

The second reaction is to shift the sphingomyelin cycle into the production of **ceramide**, a pro-death factor. Ceramide acts on the cell's mitochondria, disrupting their function and leading to the activation of *caspase 9*, which like *caspase 8*, goes on to activate *caspase 3*.

The following diagram summarises this series of events, with the dotted lines showing the points at which FLIP and XIAP block *caspase* activation.



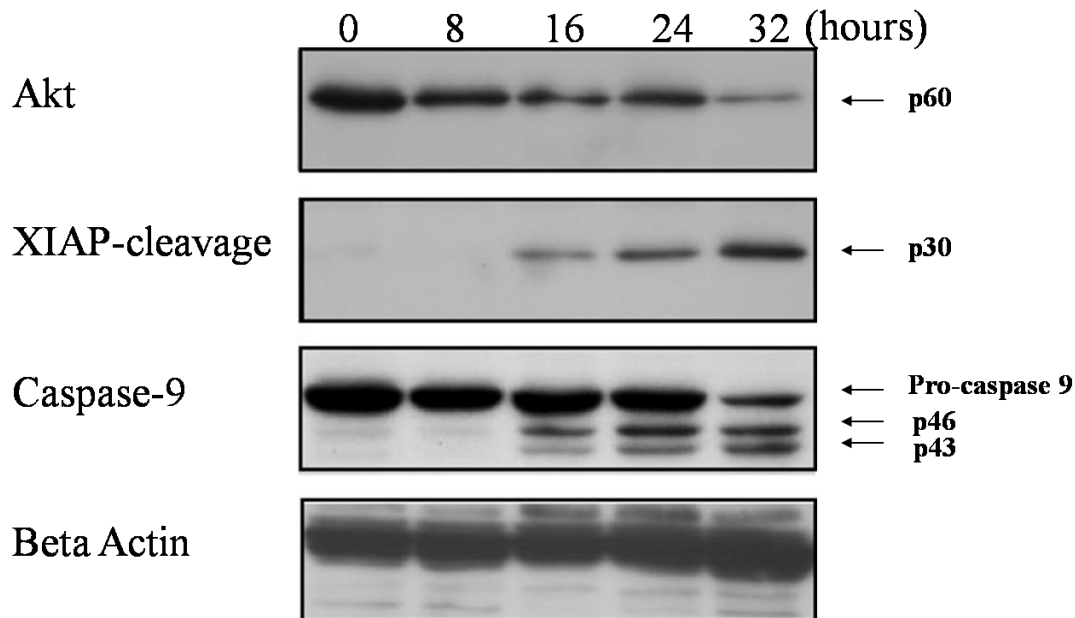
The following two diagrams show the effect of **phenoxodiol** on the activities of *caspases* and FLIP and XIAP in chemo-resistant ovarian cancer cells.



In the above diagram, the black bars represent activity, so that the darker the bar, the greater the level of activity. These particular ovarian cancer cells can be seen to be expressing a high level of active XIAP and negligible *caspase 3* activity. This is accounting for their lack of apoptosis. The addition of either **carboplatin** or **taxol** has no effect on either parameter and therefore no effect on apoptosis. **Phenoxodiol**, on the other hand, drops the level of active

XIAP, retaining it in its inactive form and greatly increasing the level of active *caspase 3*. The combination of **phenoxodiol** + **carboplatin** led to even greater active *caspase 3* levels.

Again, the following diagram shows, **phenoxodiol** over a 32 hour period progressively leads to a fall in Akt function, increasing breakdown of XIAP, and an increase in the active forms of *caspase 9*.



All of this biochemical data went to confirm the Yale belief that **phenoxodiol** was a highly promising candidate for the treatment of ovarian cancer. The drug showed a high ability to kill ovarian cancer cells that were resistant to all other drugs, and the biochemical data provided a ready and logical explanation for that ability.

This led to an initial belief that the drug could be used as a monotherapy to treat end-stage ovarian cancer. Yale was in the same boat as almost every other oncology unit in having a list of patients with end-stage ovarian cancer who had run out of treatment options. **Phenoxodiol** offered hope where nothing else existed.

That prompted the establishment of a Phase 1b/2 study at Yale using the intravenous dosage form in 40 patients who had failed second-line therapy. These patients displayed a high level of chemo-resistance having had a median number of 5 (range 2-11) previous lines of therapy. There were no objective responses, although 10 patients (25%) showed stabilized disease at the end of the study (3 months). The lack of objective responses (tumour shrinkage) came as no great surprise to us within Novogen given that the drug in our view had been under-dosed administered on only 2 days per week plus given on a sliding dosage basis (1, 3, 10 and 20 mg/kg). Even so, by the time that this study had finished, the thinking within the team had shifted from using the drug as a monotherapy, to using it as a chemo-sensitiser in combination with either a **taxane** or a **platinum** drug, so there was little enthusiasm for repeating the study with a more appropriately intensive dosing schedule.

That shift in thinking had come about for several reasons. One was a growing understanding of the powerful chemo-sensitising ability of the drug. Another was an acceptance that the drug probably lacked the knock-out punch required as a stand-alone drug to treat an aggressive, rapidly-growing cancer that was capable of doubling its mass within a month or two. A drug such as **cisplatin** or **taxol** certainly had that ability, so the opportunity of using **phenoxodiol** to restore sensitivity to either of these two more powerful drugs became an infinitely more attractive proposition. This change in thinking certainly was helped along in no small part by a fascinating observation of the fate of 8 of the patients who had participated in the Phase 1b/2 study. Ten patients at the end of the study (that is, who had shown disease progression) were given salvage therapy comprising a **taxane**. Eight of these 10 patients showed an objective tumour response (partial or complete response) including 4 who had been classified as taxane-resistant or taxane-refractory. This was far from being a controlled trial outcome, but it still was hard to avoid the conclusion that **phenoxodiol** had restored the sensitivity of these end-stage tumours to **taxane** drugs.

Running with a chemo-sensitising effect in late-stage ovarian cancer came with one concern, and that was a possible competitor. The US biotech company, Telik Inc, was conducting trials of the drug, **Telcyta**, as a chemo-sensitizer for **carboplatin** in platinum-resistant and –refractory ovarian cancer in a clinical study known as ASSIST-3. One concern was how their patient recruitment would affect our ability to recruit patients. But the other more important concern was the potential effect on our plans for **phenoxodiol** should **Telcyta** be successful and be approved by the FDA. If that proved to be the case, then suddenly the ground would have shifted and the standard of care would have become **Telcyta + carboplatin**. Any trial we would need to conduct to get **phenoxodiol** approved would need to compare it to **Telcyta** and to provide a benefit to patients compared to **Telcyta**. The two drugs had completely different mechanisms of action, so we would not be comparing like with like, but regulators still only want to approve new drugs that provide both clinicians and patients with different options such as by way of an improved tumour response or a better or different safety profile or better management practice.

The only information we had on **Telcyta** was what was in the public domain, and that was scanty. Various investment bank analysts that we spoke to were very bullish about the drug, and the share price certainly reflected strong support. However, I was less confident. The little amount of clinical data available did not give me a strong sense that the drug had promising data behind it, a sense that was confirmed in early 2007 by the release of the ASSIST-3 trial data showing no clinical benefit from the use of the drug. But at the time we made the decision to pursue a chemo-sensitising role for **phenoxodiol**, we were yet to have our suspicions about **Telcyta** confirmed and so there was a significant element of risk involved. Such is the nature of drug development.

The next decision we had to make was whether we were going to use **phenoxodiol** to re-sensitise tumours to **platinums** or **taxanes**. We could only pursue one drug combination if we were to do this efficiently. In the test-tube and animal models, there was little to separate the two effects. Both drugs appeared to have their sensitivity in ovarian cancer cells restored equally by **phenoxodiol**. We knew at the time that the mechanisms of action of restoration of chemo-sensitivity probably differed between the two drugs, but that knowledge didn't help with the decision.

To answer this question, a Phase 2 study was set up involving 40 patients in Australia and the US with refractory disease. Twelve patients who had progressed on previous **platinum** therapy (platinum-free interval <6 months) were assigned to the **phenoxodiol + cisplatin** treatment groups. Ten patients who had progressed on previous **taxane** therapy (taxane-free interval < 6 months) were assigned to the **phenoxodiol + paclitaxel** treatment group. There was an additional 18 patients who had progressed on combined platinum/taxane therapy and these patients were randomised equally to the two treatment groups. Treatment consisted of **phenoxodiol** being administered intravenously (3 mg/kg) on Days 1 and 2 each week followed by either **cisplatin** (40 mg/m²) or **paclitaxel** (80 mg/m²) on Day 3. This continued weekly until patients either responded or progressed or were withdrawn. The outcome of this study is summarised in the table below.

	PXD + Cisplatin	PXD + Paclitaxel
n	21	19
Stabilised disease	9	11
Partial response	6	2
Complete response	0	1
Disease progression	6	5

Taking the partial responders + complete responders as the best overall response rate, the **cisplatin** arm delivered a 29% rate versus 16% for the **paclitaxel** arm. This was a relatively small sighting study, and the data needs to be seen in that light. Nevertheless, the response rates were seen as meaningful and significant in view of the refractory nature of the disease in these patients and provided the justification we needed to commit to a pivotal study. We were confident that a 29% overall best response rate, if maintained across a considerably larger patient sample size, would deliver a clinical benefit likely to meet the FDA's guidelines.

It is worth noting also at this point that the dose of **phenoxodiol** used in that Phase 2 study was relatively modest compared to the dose we came eventually to settle on as our preferred dose. A dose of 3 mg/kg intravenously is only slightly higher than an oral dose of 400 mg (about 6-7 mg/kg), allowing for a 30% absorption rate from the gut. However, relatively high blood levels following intravenous dosing only last about 8 hours, whereas oral dosing on a continuous 8-hourly basis (as is the current preferred dosing schedule) delivers steady-state, high blood levels on a 24-hourly basis.

And so to the pivotal study. What a convoluted process this turned out to be. The process began by consulting gynaecological oncologists and surgeons across the US, UK and Australia. From those discussions we hoped to have a general consensus on the best way to use **phenoxodiol** in late-stage ovarian cancer. That was to lead to the appointment of a clinical panel which would have the task of finalising a clinical protocol that would be taken eventually to the FDA. Under the terms of the Fast-Track status that **phenoxodiol** had with the FDA, we then assumed that we would modify the protocol in collaboration with them on a Special Protocol Assessment (SPA) basis. The only problem with that plan was that virtually every oncologist that was consulted had a different view of the world and held fast

to that view, that those views often did not take into account such practicalities as commercial imperatives and regulatory requirements, and that the FDA held their own view of the world that was driven by regulations and did not necessarily go hand-in-glove with the clinical world.

The first stage of this plan, to seek a consensus on the best use of **phenoxodiol**, led to a variety of clinical plans varying from using the drug in combination with **cisplatin** to treat patients who failed to respond to first-line therapy, through to using it in combination with a **platinum + taxane** in refractory patients. Even before any discussions were held with the FDA, it was clear that all of these approaches held regulatory and practical issues that may have been insurmountable. The questions that go into such a decision are:

- which indication is likely to deliver the best and most significant clinical response?
- what is the FDA's likely view of this indication in terms of community need and existing therapies?
- is this use going to be competing with other large trials that probably will have the effect of reducing patient recruitment?
- how large will the trial need to be to deliver a statistically significant outcome, an important factor in determining how readily the trial can be filled?

The effect of these various questions is that the need of the sponsoring company to get a study completed as quickly and as cheaply as possible, the practical realities of the clinician in volunteering to direct patients to particular studies, and the strict regulatory requirements of bodies such as the FDA, don't necessarily run parallel.

In the end, a meeting was called in Orlando, Georgia in 2006 to make a final decision. Twelve gynaecological oncologists from around the world were put into the same room and asked to provide a consensus. By this stage, we had obtained some guidance from the FDA, so that advice was put on the table to guide the discussion. In the end, the decision was reached that the target patient population would be patients with platinum-refractory disease who had received at least 2 lines of platinum therapy. These patients were to be randomised to either **phenoxodiol + carboplatin** or to salvage therapy comprising a panel of 5 chemotherapies (**carboplatin, paclitaxel, etoposide, gemcitabine, doxorubicin**). The decision also was reached to use **carboplatin** at a standard dose of AUC = 2.

Further discussions then took place with the FDA who declined the general plan, insisting that if we were to be claiming the therapeutic indication that **phenoxodiol** was restoring sensitivity to **carboplatin** (as that was the basis of the mechanistic action), then we would have to provide absolute certainty of this by running a comparator arm of **carboplatin** alone. It counted for nothing that all patients were platinum-refractory and that we were only using patients with a platinum-free interval of < 6 months, where we would be hard-pressed to find an oncologist anywhere in the world who would claim that any more than 5-10% of such cancers would respond by to further **platinum** therapy using the same dosing schedule. And that any such response would be any more than stabilised disease on a short-term basis.

The main problem for the FDA was that there was no published data on the response of platinum-refractory ovarian cancer to re-challenge with platinum therapy. And for very good reason oncologists knew from experience that it was highly unlikely that there would be any response, so why bother testing it? But without this validation the FDA was put into a

position that if our goal was to show that **phenoxodiol** restored sensitivity to **platinum** therapy, then there only one way that we were going to prove that in an unequivocal way, and that was to compare **platinum** therapy alone to **platinum** therapy + **phenoxodiol**.

Such a stringent view of the world had an upside, and that was that approval meant that **phenoxodiol** would be officially recognised as a chemo-sensitiser. Admittedly the approval would be for the restricted use as combination treatment (**carboplatin** + **phenoxodiol**) in the treatment of platinum-resistant or platinum-refractory ovarian cancer, but as with any new drug, marketing approval necessarily is limited to the indication tested in the pivotal study. Once approved, the new drug can be prescribed for any chemotherapeutic situation by the process known as ‘off-label’ use. For **phenoxodiol**, the obvious extended steps of off-label use would be, in the first instance, to use in combination with **cisplatin** or **carboplatin** in second- or third-line therapy of ovarian cancer patients in order to seek an improvement in the 20% response rate. Beyond that, use in first-line therapy in order to seek a response in the 20% of cases that fail to respond initially seemed a logical extension. Beyond ovarian cancer, the common use of the **platinum** drugs across a broad range of cancer types (sarcomas, germ cell tumours, lung cancer, and head and neck cancers) where the development of platinum-resistance remains a major hurdle, suggested a broad application for **phenoxodiol**.

Our clinical advisers reluctantly accepted this pronouncement. Their concerns were understandable how could they in all conscience recommend their patients enrol in the study when there was a 50% chance that they would receive **carboplatin** + placebo, with virtually no promise of any result. They were the first to acknowledge that there was almost no chance that any other drug therapy would provide any benefit at this stage, but they pointed out that re-treatment with carboplatin carried a significant risk of a platinum hypersensitivity reaction and neuropathic toxicity, all with little prospect of an anti-cancer effect.

And so was born OVATURE (OVarian TUmour REsponse study). The official title of this study (relevant because it defines the indication being sought) is:

Multi-Center, Randomized, Double-Blind, Phase III Efficacy Study Comparing Phenoxodiol (Oral Dosage Form) in Combination with Carboplatin versus Carboplatin with Placebo in Patients with Platinum-Resistant or Platinum-Refractory Late-Stage Epithelial Ovarian, Fallopian or Primary Peritoneal Cancer Following at Least Second-Line Platinum Therapy.

The following Protocol Synopsis summarises the key points of this study.

Study Sites:	Multiple sites in Australia, USA, UK and Europe.
No. of patients:	470
Indication:	Epithelial ovarian, fallopian or primary peritoneal cancer that is resistant or refractory to 2-weekly or 3-weekly platinum drug therapy.
Test Treatments:	Patients to be randomized to one of the following two treatment groups:

(a) Treatment Group 1. Phenoxodiol (400 mg, 8-hourly, daily) + carboplatin (AUC=2, weekly);

(b) Treatment Group 2. Placebo (400 mg, 8-hourly, daily) + carboplatin (AUC=2, weekly).

Patients showing disease progression on therapy to receive salvage therapy which will be any therapy or combination of therapies from the list comprising liposomal doxorubicin, docetaxel, gemcitabine, etoposide, topotecan and paclitaxel.

Duration of Treatment: Treatment cycle = 4 weeks.

Treatment to continue until disease progression, dose-limiting toxicity, or patient withdrawal.

Study Objectives: The primary efficacy objective is:

to compare the effect of a treatment regimen of (i) daily phenoxodiol in combination with weekly carboplatin, versus (ii) weekly carboplatin therapy in combination with a placebo, on progression-free survival in patients with platinum-resistant or platinum-refractory, late-stage epithelial ovarian, fallopian or primary peritoneal cancer.

The secondary efficacy objective is:

to compare the effect of a treatment regimen of (i) daily phenoxodiol in combination with weekly carboplatin, versus (ii) weekly carboplatin therapy in combination with a placebo, on overall survival in patients with platinum-resistant or platinum-refractory, late-stage epithelial ovarian, fallopian or primary peritoneal cancer.

The tertiary efficacy objectives are:

to compare the effect of a treatment regimen of (i) daily phenoxodiol in combination with weekly carboplatin, versus (ii) weekly carboplatin therapy in combination with a placebo, on overall response rates, duration of response, clinical status (Karnofsky Performance Score) and quality of life in patients with platinum-resistant or platinum-refractory, late-stage epithelial ovarian, fallopian or primary peritoneal cancer.

The primary safety objective is:

to compare the effect of a treatment regimen of (i) daily phenoxodiol in combination with weekly carboplatin, versus (ii) weekly carboplatin therapy in combination with a placebo, on drug-associated toxicity and intolerance in patients with platinum-resistant or platinum-refractory, late-stage epithelial ovarian, fallopian or primary peritoneal cancer.

Methods of Assessment: CT-Disease Burden, Tumor Marker (CA125), Clinical Status, Drug Safety, Drug Tolerability.

Population:	Patients \geq 18 years of age with progressive or recurrent, late-stage, epithelial ovarian, fallopian or primary peritoneal cancer that has become resistant or refractory to at least 2 nd line platinum therapy.
Investigational Product:	Phenoxodiol (oral dosage form) in combination with carboplatin.
Reference Therapy:	Weekly carboplatin therapy plus an oral placebo (containing phenoxodiol capsule excipients only).
Safety Criteria:	Standard safety monitoring.

One of the key aspects of patient selection was in relation to previous **platinum** therapy. The main requirements here were:

- The patient must have responded to a primary course of platinum therapy (cisplatin or carboplatin) as determined by either RECIST or GCIG criteria. *[So ensuring that the patient had a pre-existing chemo-sensitivity capable of being restored];*
- The patient must have shown disease progression within 6 months of a subsequent course of platinum therapy (cisplatin, carboplatin) including patients who relapse while receiving platinum therapy, as determined by either RECIST or GCIG criteria, taken from the last day of platinum therapy. *[So ensuring that the patient had developed platinum-resistance];*
- The patient must have had the most recent platinum therapy in either a 2-weekly or 3-weekly regimen. *[So ensuring that the patient had the potential to respond to a dose-dense regimen of weekly platinum therapy];*
- The patient must have undergone at least two (2) courses of platinum therapy; and the patient must have had a platinum-free interval of no longer than 6 months, taken from the last day of platinum therapy until the day of enrolment. *[So ensuring that patients were enrolled with minimal risk of restoration of chemo-sensitivity with time].*

Inclusion and exclusion criteria.

(a) Inclusion Criteria

Patients needed to meet the following criteria to be considered for admission to the study:

Disease type

- ✚ histologically-confirmed ovarian, fallopian, or primary peritoneal carcinoma of epithelial origin;
- ✚ recurrent or persistent advanced disease;
- ✚ have measurable disease. [Measurable disease being defined as at least one lesion that can be accurately measured in at least one dimension, allowing a response to be determined by the RECIST criteria. Each lesion must be \geq 10 mm when measured by spiral CT and \geq 20 mm when measured by conventional CT];

Treatment response history

- ✚ undergone at least two courses of therapy with a platinum drug (cisplatin or carboplatin) and have responded to the first of those courses of therapy as determined by either RECIST or GCIG criteria;

- ✚ shown disease relapse as determined by either RECIST or GCIG criteria within 6 months of completion of the second or greater course of platinum therapy using a 2- or 3-weekly regimen and
- ✚ have a platinum-free interval of no greater than 6 months at the time of enrolment, being the time taken from the last day of platinum therapy;

Treatment history

- ✚ can have any number of previous courses of platinum therapy or non-platinum therapy;

Clinical status

- ✚ be considered likely to survive at least 3 months;
- ✚ have a Karnofsky Performance Score of at least 60%;
- ✚ have adequate physiological function without evidence of major organ dysfunction;
- ✚ have adequate haematological function;

Other

- ✚ be aged ≥ 18 ; and
- ✚ be able to understand the risks and benefits of the study and give written informed consent to participation.

(b) Exclusion Criteria

Patients presenting with any of the following were not eligible for the study:

- ✚ patients with mucinous histological type of ovarian cancer;
- ✚ patients who had failed to show a clinical response (RECIST or GCIG criteria) to at least 1 prior course of platinum therapy;
- ✚ patients with active infection;
- ✚ patients with concurrent severe and/or uncontrolled medical disease (*e.g.*, uncontrolled diabetes, hypertension, ischemic heart disease, congestive heart failure, etc.);
- ✚ patients with a history of chronic active hepatitis or cirrhosis;
- ✚ patients with HIV;
- ✚ patients with active CNS metastases. Patients with known CNS metastases must have received prior radiation therapy, and CNS metastatic disease must be stable for 4 weeks;
- ✚ patients who had not recovered from the acute effects of any prior anti-neoplastic therapy; and
- ✚ patients with known hypersensitivity to platinum drugs.

Efficacy end-points

The definitions of the efficacy end-points were:

The primary efficacy end-point was progression-free survival (PFS). PFS being the time from randomization until disease progression or death.

The secondary efficacy end-point was overall survival (OS). OS being the time from randomization until patient death from any cause.

The tertiary efficacy end-points were:

- overall response rate
- duration of response
- clinical status (Karnofsky Performance Score)

- Quality of Life (QOL FACT-O and FACT-BRM).

Disease progression was to be based on CT-scans. These were obtained at the time of screening and then after every 2nd treatment cycle (= 8 weeks), or more frequently where disease progression or tumor response was suspected. To confirm a tumor response according to RECIST criteria, a follow-up CT-scan had to be performed no less than 4 weeks after a partial or complete response was achieved. Disease progression could be determined on the basis of a single CT-scan.

CA-125 levels also were measured every 2-weeks and used both as a guide to the appropriate timing of CT-scans, and in the determination of a complete response by RECIST.

Sample size and statistical analysis

Consultant statisticians, along with the FDA statisticians, determined that the appropriate sample size was 470 patients. This was based on projected median PFS values of 5 and 8 months for the control and test arms respectively, and the need to meet the statistical outcomes set by the FDA. It allowed for a 25% drop-out rate due to drug toxicity and platinum hypersensitivity.

There was no historical data to support a median FS value of 5 months for the control arm. All available clinical advice was that almost all patients could be expected to have experienced disease progression by the end of the 2 treatment cycles (4 months), suggesting a median PFS somewhere between 3 and 4 months. However, we decided to adopt a conservative approach and set a higher value of 5 months.

The median PFS of 8 months for the **phenoxodiol** arm also was an estimate based on the Phase 2 data where we saw a significant proportion of patients showing stable disease or better.

The advantage of choosing a conservative control PFS value (5 months), we believed meant that a more realistic and lesser value in the control arm would relieve the ultimate statistical pressure on **phenoxodiol**.

The study was powered such that the log-rank test for the equality of PFS survival curves with a 0.05 two-sided significance level would provide approximately 90% power to detect the difference between the PFS survival curves of the two treatment arms.

The FDA approved an interim analysis once all 470 patients had been accrued and when there had been at least 95 events of disease progression. The interim analysis was to compare the PFS outcomes in the two treatment groups. If this analysis showed that the difference between the two groups met a pre-agreed level of significance, then the FDA under the terms of the SPA could grant the drug marketing approval.

The final analysis was to be conducted when the last patient had completed at least 18 months since the start of the study and would have PFS as its primary efficacy end-point, with overall survival as the secondary efficacy end-point. Overall response rate, duration of response, Karnofsky Performance Score and QOL would be tertiary efficacy end-points.

Study logistics

A pivotal study is a significant logistical operation. For those unfamiliar with the process, I will try and summarise the main activities here.

Site recruitment. The process starts with the recruitment of study sites. We had set a target of 60 sites worldwide on the basis that each site would be called on to contribute between 8-10 patients, a target that we considered realistic. Each site (generally a major hospital or cancer centre) requires a visit and presentation to capture the interest of the clinical staff. Almost always you are competing with other studies for the same patients, and perversely the larger clinics with the greatest patient pools generally have the greater number of competing studies. This tends to drive sponsors towards smaller centres where the competition tends to be less. Eastern European hospitals in particular are an attractive source of patients because of their greater reliance on clinical studies to fund expensive chemotherapies.

Having recruited the interest of a site, the task then is to seek the approval of the local ethics committee or investigational review board (IRB) to conduct the study, along with negotiations over study costs and budgets. For studies such as OVATURE, it is not unusual to budget for between about \$15,000 – 25,000 per patient.

The whole process of site recruitment and finalisation of contracts is a protracted exercise typically taking up to 12 months per site. Multiply that across 60 sites and the whole exercise becomes very labour- and time-intensive.

Medical Monitor. The role of the Medical Monitor is to provide an independent authority for the study. This person, in the case of OVATURE, a senior oncologist based in Europe, had sole authority to approve the recruitment of patients and the overall conduct of the study from a clinical perspective. Thus, determinations of disease progression and withdrawal of patients due to safety issues were discussed with the Medical Monitor.

Patient randomisation. For OVATURE, a computerised patient allocation service was used. This is a contract service provided to the pharmaceutical industry where a hospital on recruitment of a patient calls a telephone line and is allocated a patient number. The contractor is aware of whether that patient is randomised to either the control or the test treatment arm, but all other parties remain blinded to that information.

The contractor then dispatches to that hospital the coded treatment comprising either placebo or active **phenoxodiol** capsules.

Radiology review. A US-based independent contractor was retained to review all CT scans. Each CT scan was reviewed separately by at least two radiologists, with a third providing a decision in the event of disagreement.

Independent Data Management Committee. The IDMC plays a crucial role in allowing the data to be reviewed on a progressive basis at arms length from the Sponsor, so allowing the Sponsor to remain blinded to the data as it accumulates and to avoid accusations of bias.

Safety is a key role of the IDMC, alerting the Sponsor to any potential side-effect issues and advising the Sponsor of the need to abandon the study in the event of unacceptable toxicity.

The second role this group plays is to assess the merits of continuing with the study. There are no strict guidelines as to when they can do this, but it is usual for them to consider the efficacy data before the study has recruited half of the proposed number of patients when there would be a meaningful amount of data. By reviewing this data, they should be in a position to determine if it would be futile to continue with the study (no possibility of reaching the statistical target) or if there is a reasonable prospect of reaching that target with further recruitment.

Data review and collection. This activity is at the heart of study's integrity. Studies like OVATURE generate an enormous amount of data and it is vital that all such data be validated. The inadvertent incorrect transcribing of what might ultimately prove to be a barely relevant piece of data to the final data log has the potential to jeopardise the whole study. Regulators such as the FDA often drill down into the data on a random basis just to check on the validity of the data recording process, and even the most minor transgression can be fatal to an application, or if not fatal, then at least resulting in a major delay while all the data is reviewed again.

The role of data checking is in the hands of Clinical Research Associates, usually people with nursing or science training who on a regular basis validate the collected data with original source documents, as well as ensuring that all data collection points are being filled.

Study progress

It is not unusual for pivotal studies to undergo modifications as the study proceeds, even studies under an SPA protocol. In the case of OVATURE, it appears that a major modification was driven by the realisation shortly into the study that patient recruitment was going to fall well short of expectations. This appears to have arisen as a result of a shift in thinking about the way **carboplatin** could be used in late-stage ovarian cancer patients. There was growing evidence that by condensing the normal dose of **carboplatin** given over 3 weeks into 1 week (so-called 'dose-dense' therapy), some patients with platinum-refractory disease would show a tumour response. This meant that from a situation where patients eligible for OVATURE had little if any other options, the ground had shifted to a situation where there was something, no matter how slight, that might provide some prolongation of life. That became increasingly attractive compared to the prospect of half of all patients on OVATURE being allocated to a placebo arm with virtually no prospect of response.

In May 2008, the Company announced that as a result of slow patient enrolment, the FDA had allowed an adjustment to the SPA in reducing total enrolment from 470 to 340 patients, and extending the patient accrual time. As a result of a smaller sample size, the interim analysis was amended to be conducted after completion of 95 events, down from 195 events.

On 9 June 2008, the Company announced that after reviewing available safety and efficacy data, the IDMC recommended continuation of the study. The sample size was not revealed, but presumably by that time there were sufficient events to enable the IDMC to determine that the 'futility' barrier had not been crossed. A follow-up announcement on 12 November

2008 when presumably further data had been analysed, confirmed that the study should continue to enrol patients.

On 14 April 2009, the Company announced that it was undertaking an un-blinded review of data from the 141 patients enrolled to date. Clearly patient enrolment must have ground to a halt by this time to force such a decision as this effectively meant that the study was closed to any further enrolment. The time that it would take to enrol the remaining 200 patients very clearly would have extended past the accrual time agreed between the Company and the FDA.

On 17 June 2009, it was announced that the IDMC had undertaken a review of 117 patients who had completed the study, and that there were a further 25 patients ongoing. No outcome of that review was released publicly, presumably because it would have been based on data yet to be 'locked' and 'cleaned'. Interestingly, mention was made of advice from the IDMC on the 'disposition' of remaining patients, which essentially means that if the IDMC is satisfied that the clinical benefit of the drug has been established, then they have the power to recommend to the Sponsor that all remaining patients on study should be put onto the **phenoxodiol** treatment arm. Again, we have not been informed of that advice.

At the time of writing, we await the imminent release of the data analyses. Leaving aside the burning question of whether **phenoxodiol** has delivered a benefit or not, the other key issue is what the FDA will make of any positive outcome in light of the smaller study sample size. Under the terms of the SPA, the results of the interim analysis can only be considered by the FDA once the study was fully enrolled, that is, when all 340 patients were enrolled. This requirement was not always the case. Up till several years ago, a significant number of anti-cancer drugs were allowed onto the market on the basis of a positive interim analysis before the studies were fully enrolled. In almost every case, the Sponsors then failed to complete enrolment. Why? Because why would you want to jeopardise an approval by continuing with the study when you had it in the bag? To stop this, the FDA some years ago made it mandatory that interim analysis data would only be looked at as part of a regulatory submission once the study was fully enrolled.

There are a couple of important and very relevant factors operating here with OVATURE in this respect. The first is the nature of the primary and secondary efficacy end-points. In the case of those earlier studies that failed to go on to completion, the interim analysis was based in almost every case on a secondary end-point (usually progression-free survival or tumour response rates), but that when those patients were followed for their primary end-point (usually overall survival), no benefit was seen. IN the case of OVATURE, the interim analysis is based on the primary outcome of progression-free survival, meaning that the interim outcome is not subordinated by other outcomes.

The second relevant factor is that the study has been forced by circumstances beyond the Company's control to be incomplete.

We now can only wait with interest to see the outcome of the interim analysis and the attitude of the FDA to that outcome.