Molecular Targeted Therapies in the Treatment of Ovarian Cancer

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ABSTRACT

The outcome of treatment for patients with advanced ovarian cancer, despite recent improvements, remains poor. New therapeutic approaches are urgently required. Biologic agents in the form of monoclonal antibodies and small molecular targeting agents (e.g. tyrosine kinase inhibitors) appear promising and many of these are currently undergoing early clinical evaluation. However, these agents are mostly cytostatic and this has implications for their clinical use as well as in assessments of efficacy in pre-clinical models. These agents generally have relatively low single-agent activity and therefore may be most effective either as modulators of activity of other agents including cytotoxics and other biologic agents or as maintenance therapy. We have learnt that carefully matching the choice of therapy to patient characteristics and tumour biology is essential for this approach to be successful, reflecting the molecular heterogeneity of ovarian cancer; indeed, the search for more effective tumour predictive biomarkers is ongoing. In ovarian cancer, the role of maintenance therapy has not been established and it is in this setting that we think these agents may be particularly helpful, in addition to their possible roles as adjuvant therapies and in relapsed disease. Ultimately, the hope is not just to increase progression free survival but to improve overall survival by devising strategies to prevent and overcome resistance to treatment.

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INTRODUCTION

Epithelial ovarian cancer is the fourth leading cause of can- cer death among women in western Europe with the overall lifetime risk of developing the disease estimated at 1 in 75 (Howe *et al.* 2001). Most patients present with evidence of

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disease spread beyond the ovaries, necessitating treatment with surgery and chemotherapy. Although response rates with first-line chemotherapy may exceed 80%, patients with advanced disease invariably relapse with a median progression-free survival of only 18 months (Greenlee *et al.* 2001).

Improvement in the treatment of ovarian cancer has been achieved mainly through the introduction of platinum-based chemotherapy and more aggressive cytoreductive surgery (Balvert-Locht *et al.* 1991). Randomised-controlled trials have established the combination of carboplatin and paclitaxel as the standard first-line chemotherapy for advanced ovarian cancer (McGuire *et al.* 1996; Ozols *et al.* 2003). Patient outcome in recent years has continued to improve, probably due to a better appreciation of the management of recurrent disease and this involves the empiric and sequential use of different classes of chemotherapeutic agents.

Notwithstanding the aforementioned progress, we still have limited understanding of the molecular aetiology of ovarian cancer and treatment is still largely administered with a palliative intent for patients with advanced disease. The overall 5 year survival averages 30 to 40%. Moreover, the standard of care in first-line systemic treatment of ovarian cancer has remained unchanged since its establishment about a decade ago; several recent trials have failed to demonstrate a significant impact on outcome measures by the addition of conventional cytotoxic agents active in recurrent disease (including pegylated liposomal doxorubicin, gemcitabine, topetecan and etoposide) to the carboplatin/paclitaxel doublet (de Placido *et al.* 2004; Bookman 2006). This is clearly unsatisfactory and needs to be addressed.

We believe one of the ways of overcoming this impasse to an effective curative treatment is through the development of effective molecular targeted (biologic) therapies, and this will be the focus of our discussion. As the scope is extremely wide, we shall limit the discussion to areas of research that, in our opinion, have shown promise for the treatment of ovarian cancer. Data from pre-clinical models and clinical trials (as single agents or in combination) will be discussed.

BACKGROUND TO OVARIAN CANCER

Although the aetiology of epithelial ovarian cancer is unclear (Cramer 2000), certain epidemiological and clinical observations might prove helpful in delineating the pathogenesis of this disease.

Firstly, a range of epidemiological variables have been correlated with ovarian cancer (Cramer 2000). For example, case-control studies have identified several risk factors that predispose to a higher probability of developing ovarian cancer, including caucasian race, high fat diet, prior talc use and reproductive history, amongst others. It is interesting to note that the accumulated number of menstrual cycles correlates with the risk of development of ovarian cancer. Moreover, the use of the oral contraceptive pill and parity seem to exert a protective effect. These observations have, therefore, led to the incessant ovulation hypothesis that suggests ovulatory rupture and repair might predispose the ovarian surface epithelium to mutations that eventually lead to the oncogenic phenotype (Fathalla 1971).

Secondly, epithelial ovarian cancer primarily develops and spreads within the peritoneal cavity. Clinical studies suggest a transcoelomic metastatic route that involves tumour shedding followed by dissemination via the peritoneal or ascitic fluid current (Buy *et al.* 1988; Carmignani *et al.* 2003). This is a complex process, which, although facilitated by the circulation of peritoneal fluid, also involves adaptations by the epithelial ovarian cancer cell. Various molecular mediators have been implicated in transcoelomic metastasis, but the molecular mechanisms behind this process remain unclear (Tan *et al.* 2006).

Thirdly, most epithelial ovarian cancers are sporadic with hereditary patterns accounting for approximately 10%

of cases (Diamond *et al.* 1998). The latter category has led to the elucidation of mutations affecting BRCA-1 and 2, as well as MSH-2, MLH-1, PMS-1 and 2 (of Lynch II syndrome) being contributory to ovarian carcinogenesis. In addition to these, many other genetic events have been associated with ovarian cancer but it is likely that few are causal (Aunoble *et al.* 2000; Havrilesky *et al.* 2001).

Currently, a range of high-throughput techniques are being utilised to provide molecular profiles of ovarian cancer; attempts are then being made to correlate these with biological behaviour and response to therapy. It is expected that an improved understanding of the molecular basis of ovarian cancer with its attendant complexity and heterogeneity will provide a platform upon which rational therapies could be designed and applied.

OVERVIEW OF MOLECULAR TARGETED THERAPIES

Conventional chemotherapies possess limited tissue selectivity with low therapeutic indices. Cytotoxic agents act by principally preventing cell growth at the level of DNA replication, by inhibiting the synthesis of DNA precursors, damaging the DNA template or disrupting the mitotic apparatus. In contrast, molecular targeted therapies are directed against cancer-associated molecules and pathways and the hope is that a broader therapeutic window is offered with less toxicity.

The rational design of biologic therapies capitalises on the knowledge of specific biochemical processes and molecular mechanistic pathways occurring within the cancer cell and its microenvironment, and attempts to tailor combinations of these to fit a treatment profile. The acquisition of more sophisticated genetic and molecular tools over the past few decades has facilitated the development of biologic therapies for the treatment of malignancy; deeper insight into the molecular genesis of cancer is made alongside the identification, characterisation and manipulation of potential molecular targets.

The success of this approach is exemplified by the evolution of treatment for chronic myeloid leukaemia (CML); Imatinib (STI 571, Gleevec), the tyrosine kinase (TK) inhibitor of the bcr-abl fusion product, is highly active without the common toxicities associated with conventional chemotherapy.

Therapeutic agents aimed at molecular targets have efficacy in a variety of malignancies and are being investigated in ovarian cancer. For the sake of simplicity, two broad approaches can be identified, i.e. small molecule drugs and monoclonal antibodies (mAbs), and we shall structure our review along these lines. These agents are being used as single agents or as modulators in combination with other drugs including cytotoxics and other biologic agents. Both approaches are rational, and they should be pursued in parallel.

Both mAbs and small molecule compounds can cause receptor down-regulation and signalling pathway interruption. However, broadly speaking, these two classes of compounds have markedly different pharmacokinetic and pharmacodynamic profiles and these properties have contributed to an increasing interest in biological therapy in which mAbs and small molecules are given together. These differences are as follows: firstly, mAbs generally target extracellular epitopes which are normally components of signalling pathways upstream of intracellular molecules targeted by small molecular agents. In addition to their impact on intracellular signalling, mAbs can also sequester growth factors and cytokines found in the extracellular compartment in the setting of neoplastic growth. Secondly, mAbs generally demonstrate greater target specificity. The clinical significance of this is not fully understood and has implications for their use especially in combination with other anticancer agents. On the other hand, the broad spectrum of activity of some recent examples of small molecular agents may translate into increased efficacy as there is a high deg-

ree of redundancy and cross-talk between the pathways they target. Thirdly, as large molecules (normally IgG with molecular weights of approximately 150 kDa), mAbs potentially have poorer tumour and blood brain barrier penetration, suggesting that they may have more limited roles in the treatment of patients with solid and central nervous system tumours. Fourthly, small molecular compounds tend to be available in oral preparations and have shorter half-lives (typically hours) compared to mAbs (typically days to weeks). The oral preparations allow for convenience whilst the short half lives may be better for patient safety but also require that patients be dosed more frequently (once or twice daily). Fifthly, small molecular compounds, such as TK inhibitors, are generally metabolised by the P450 enzyme system and have a greater potential for drug interactions than mAbs. Lastly, mAbs (generally synthesised as humanised antibodies) may generate an immune response although its contribution to efficacy is poorly understood.

Work has centered on the various biochemical processes and signalling pathways contributing to the phenotypic hallmarks of cancer. These include growth factor signalling pathways, intracellular signal transduction mechanisms, angiogenesis, cell cycle regulation, apoptosis and metastasis. As these pathways continue to be elucidated, more potential therapeutic targets are identified.

MONOCLONAL ANTIBODIES

The use of mAbs is gaining acceptance in a variety of chronic inflammatory and malignant conditions. Derived from cultured cells, these are clonal immunoglobulins that have been amplified and selected with homogenous specificity for particular antigens. In the context of biologic therapy for cancer, their mode of action could be divided into three categories: (1) receptor or ligand targeting, (2) immunomodulation, and (3) carrier of radioisotopes or other drugs.

Receptor or ligand targeting

Growth factor signalling pathways are implicated in oncogenesis and *in vitro* evidence indicates that inhibitors of these pathways can induce apoptosis independently of cytotoxic chemotherapy. The growth factor receptors, ERB-B2 and EGFR, have been most extensively evaluated as therapeutic targets in ovarian cancer and other solid tumours.

Monoclonal antibodies to ERB-B2 (HER2)

Trastuzumab is a humanised anti-ERB-B2 mAb that has demonstrated single agent response rates of up to 26% in individuals with breast cancer whose tumour over-expresses ERB-B2 (Vogel *et al*. 2002). In combination with conventional cytotoxic chemotherapy, this has translated into a survival benefit in breast cancer with a major impact on adjuvant therapy (Piccart-Gebhart *et al*. 2005) as well as in metastatic disease (Slamon *et al*. 2001). Further work has suggested that its efficacy is associated with ERB-B2 receptor density in allowing for antibody-dependent cellular cytotoxicity (ADCC) to take place (Sliwkowski *et al*. 1999). The success in breast cancer has led to its evaluation in other epithelial tumours. In patients with advanced ovarian cancer, the single-agent efficacy of trastuzumab has been assessed in a phase II study (Bookman *et al*. 2003). Of a total of 837 patients who were screened, only 11.4% of these were 2+ or 3+ positive for ERB-B2 by immunohistochemistry. Of the 41 patients who enrolled and whose disease was evaluable, 1 complete response (CR) and 2 partial responses (PR) were observed, giving an overall response rate of 7.3%. It would therefore seem that the low frequency of ERB-B2 protein overexpression in ovarian cancer, coupled to its low single-agent response rate, indicates no significant role for ERB-B2 targeted agents in ovarian cancer. Increasingly, ERB-B2 gene amplification is deemed a much better predictor of response than protein expression in this context.

However, another factor in ERB-B signalling is the process of HER2 dimerisation and this offers another potential target. Even in the absence of HER2 overexpression, dimerisation of HER2 with other members of the EGFR family and the subsequent signalling pathway activation via receptor phosphorylation have been shown to be potent drivers of ovarian tumour cell proliferation (Totpal *et al*. 2003). Moreover, coexpression of HER1 and HER2 is observed more commonly in ovarian cancer than in normal ovarian epithelium (Bast *et al*. 1998) and overexpression of both receptors correlates with a poor prognosis (Berchuck *et al*. 1990). Pertuzumab (rhuMAb 2C4, Omnitarg) is a recombinant, humanised mAb that targets the HER2 dimerisation domain and inhibits ligand-activated dimerisation of HER2. It has been evaluated in a phase II study as a single agent in 123 heavily-pre-treated patients with advanced ovarian cancer (Gordon *et al*. 2006). Clinical activity was observed in 14.5% of patients (including PR, stable disease (SD) >6 months and SD with Ca125 reduction >50%). It was also noted that the prevalence of phosphorylated-HER2 positive (pHER2+) banked ovarian tumour tissue samples within the study was 45%, a much higher figure than the 11.4% of HER2 overexpression described in the aforementioned study involving trastuzumab. In addition, patients with HER2 phosphorylation (pHER2+) demonstrated a longer progression-free survival (PFS) following pertuzumab therapy compared to patients with pHER2- tumours. This could mean that HER2 activation and not overexpression is more important in influencing treatment outcome, indicating that the biologic efficacy of pertuzumab may relate more to disruption of intercellular signalling than ADCC. However, the current data on pHER2 status cannot distinguish between its potential as a predictor of response to pertuzumab as opposed to its utility as a prognostic marker. We note also that pertuzumab has been well tolerated in clinical trials although concerns about the risk of cardiotoxicity were raised in view of its anti-HER2 action. In a review of 167 assessable patients treated with single-agent pertuzumab, left ventricular ejection fraction was reduced to less than 50% of baseline in only 5.4% of cases (Gordon *et al*. 2006). Preclinical data have demonstrated a synergistic interaction between pertuzumab and a range of cytotoxic agents including platinum compounds (Friess *et al*. 2003). On this basis, a randomised phase II trial has recently been completed, in which platinum-sensitive patients (following first relapse) received platinum-based combination chemotherapy with or without pertuzumab. Results of this study should be available in 2007/2008. In addition, a randomised, double-blind, placebo-controlled trial of gemcitabine with or without pertuzumab is being conducted in patients with platinum-resistant ovarian cancer and preliminary safety data indicate that the combination is well tolerated with no unexpected additive toxicity (Glenn *et al*. 2006).

Monoclonal antibodies to EGFR (ERB-B1/HER1)

Epidermal growth factor receptor (EGFR) is overexpressed in up to 70% of ovarian cancers and is associated with advanced disease at presentation, poor prognosis and chemoresistance (Nielson *et al*. 2004). Activation of this receptor could be disrupted by mAbs which bind to the extracellular domain of EGFR. The most advanced anti-EGFR mAb in clinical development is cetuximab (IMC C225, Erbitux). This is a chimeric mAb that has already demonstrated clinical activity in patients with a variety of EGFR-expressing solid tumours, including lung, head and neck and colorectal cancers, in combination with conventional cytotoxic chemotherapeutic agents and radiotherapy. A phase II study evaluating the role of cetuximab in combination with carboplatin and paclitaxel as first line therapy in advanced ovarian cancer demonstrated that the combination was well tolerated with the most common adverse event related to cetuximab being an acneiform rash (Aghajanian *et al*. 2005). *In vitro* models have indicated that the effect of cetuximab on cellular viability is correlated with interference of its down-

stream signalling pathways (AKT- and ERK-phosphoryla-tion) but not EGFR expression (Pan *et al.* 2005).

As a chimeric antibody, cetuximab is formed by repla-cing the constant region of the original murine mAb with that of a human immunoglobulin. Although this greatly re-duces immunogenicity compared with the original murine mAb, anaphylactic reactions are still relatively common. The formation of human-antimouse antibodies could also account for the loss of efficacy over repeated exposure. A humanised version of cetuximab, matuzumab (EMD 72000), was therefore developed. Following promising efficacy as a single agent in phase I trials, matuzumab was evaluated in a phase II trial with 37 heavily pre-treated patients with ova-rian cancer (median of 7 lines of previous chemotherapies) (Seiden *et al.* 2006). It was well tolerated but no response was observed although 21% of patients maintained SD on the drug for more than 6 months. The combination of matu-zumab (up to 800 mg/week) and paclitaxel (175 mg/m^2 q21 days) was also shown to be well tolerated in a phase I study in patients with non small cell lung cancer (NSCLC) with encouraging efficacy data (22% response rate, 33% SD and 1 patient achieving a durable complete remission with 50% of patients having been pre-treated with chemotherapy) (Kollmannsberger *et al.* 2006). This combination may therefore merit exploration in ovarian cancer.

Possible role of HER3

Another member of the EGFR family, ERB-B3 or HER3, is known to dimerise with HER2. In fact, the HER2/HER3 heterodimer has been shown in experimental models to deliver the most potent and longest lasting signal amongst the possible combinations of the 4 members of the EGFR family (HER1 to HER4) (Freeman 2004). A recent study has highlighted the association between overexpression of HER3 and poorer survival (Tanner *et al.* 2006). In addition, strong expression of HER3 was observed in 53.4% of pati-ents with advanced ovarian cancer. HER3 could therefore represent a potential target for therapy and in fact, mAbs to HER3 have been established; *in vitro* models have shown these to cause internalisation of HER3 receptors with dis-ruption of downstream signalling (van der Horst *et al.* 2005).

Monoclonal antibodies to VEGF

Vascular Endothelial Growth Factor (VEGF) has a major role in ovarian carcinogenesis. It is a key factor in the pro-cess of invasion and metastasis; data from both preclinical models and clinical series point to its major potential in the-rapy. Paley *et al.* (2002) found that an increased level of ex-pression of VEGF was the most important prognostic risk factor for recurrence in patients with early stage ovarian cancer whilst increased intra-tumour microvessel density has been shown to be a negative predictor of survival (Ras-pollini *et al.* 2004). Significantly, mAbs to VEGF in murine models of ovarian cancer have shown efficacy in inducing tumour regression as well as reversing and preventing mal-ignant ascites (Hu *et al.* 2002).

Experimental models have demonstrated that the hap-hazard growth of abnormal, leaky blood and lymph vessels, interstitial fibrosis, and contraction of the interstitial space by stromal fibroblasts within tumours can lead to raised intra-tumoral interstitial fluid pressure, which may impede systemic drug delivery and contribute towards chemoresis-tance (Heldin *et al.* 2004). Combining conventional chemo-therapeutic agents with an angiogenesis inhibitor may therefore enhance intra-tumoral drug penetration by inhibit-ing tumour neo-vascularisation, thereby reducing the intra-tumoral pressure. VEGF may also contribute towards che-moresistance by activation of the P13/AKT pathway. In-deed, inhibitors of PI3 (e.g. LY 294002) have been shown to delay the development of paclitaxel resistance as well as working synergistically with paclitaxel in causing tumour regression (Hu *et al.* 2002). Moreover, paclitaxel is thought

to act not just via stabilisation of microtubules but also via the inhibition of VEGF expression. Thus, VEGF-targeted therapy offers two options – a single agent approach and a chemotherapy combination strategy. Indeed, both may play a role in improving treatment results, and randomised trials are addressing this issue.

The use of bevacizumab, a humanised anti-VEGF mAb, in ovarian cancer patients has been evaluated in 6 published studies thus far, of which 3 are prospective (Burger *et al.* 2005; Garcia *et al.* 2005; Cannistra *et al.* 2006) and 3 are retrospective (Cohn *et al.* 2006; Monk *et al.* 2006; Wright *et al.* 2006). It has been utilised as a single agent (Burger *et al.* 2005; Cannistra *et al.* 2006; Monk *et al.* 2006) and in com-bination with chemotherapeutic agents such as cyclophos-phamide and paclitaxel in a metronomic approach (Garcia *et al.* 2005; Cohn *et al.* 2006), in addition to other chemothe-rapies (Wright *et al.* 2006). Conclusions from these studies are: firstly, that bevacizumab possesses significant single-agent activity in ovarian cancer, demonstrated by the low percentage of patients who progressed on treatment. Al-though the overall response rate is less than 20%, stable dis-ease was maintained in about 60% and median response duration was an impressive 10.5 months. Secondly, the acti-vity of single-agent bevacizumab seems to be similar for platinum-sensitive and resistant patients. Thirdly, treatment is well tolerated; the major concern is the development of serious gastro-intestinal perforations in a minority of cases (Wright *et al.* 2006). Indeed, this led to the discontinuation of one study, although it may be that patient selection in that trial was less rigorous. There is thus an urgent need to iden-tify risk factors for the development of this; as a parallel, in bevacizumab-treated NSCLC patients, features including squamous cell histology, tumour necrosis and cavitation and disease in close proximity to the great vessels were found to be associated with major haemoptysis. A cautious approach is currently being taken in the use of bevacizumab; it is likely that patients with extensive bowel involvement and those who have been heavily pre-treated may be the most likely to develop serious gastro-intestinal perforations. Nevertheless, the encouraging efficacy data, including that seen in combinations with chemotherapy, have led to the commencement of two large randomised trials of first-line therapy; one conducted by the GOG (study 218), the other by the GCIG (ICON7). These examine the use of bevacizu-mab combined with chemotherapy and as a continuation single-agent treatment. Results are anticipated from 2009 onwards.

Monoclonal antibodies to α–folate receptor

The α–folate receptor (FRA) represents an attractive target as it is over-expressed in the majority of ovarian cancers but is largely absent from normal tissues. Consequently, a humanised mAb to the FRA, MORAb-003, has been dev-eloped. Binding of MORAb-003 to FRA can prevent phos-phorylation of substrates specific for the Lyn kinase, sup-press proliferation of cells over-expressing FRA and med-iate FRA-positive tumour cell killing via antibody-depen-dent cellular and complement-dependent mechanisms. MORAb-003 can suppress *in vivo* growth of FRA-expres-sing tumours in rodent xenograft models. A phase I study in patients with platinum-resistant ovarian cancer has opened and is in the accrual phase (Konner *et al.* 2006).

Although not a mAb, the targeting of the FRA is espe-cially relevant for a novel thymidylate synthase inhibitor, BGC 945, which is highly selective for the FRA and is in its late pre-clinical stages of development. This agent poten-tially avoids the toxicities associated with the existing thy-midylate synthase inhibitors due to their non-specific uptake by normal tissues (Gibbs *et al.* 2005). Phase I studies of this interesting agent are anticipated to begin in the near future.

Immunomodulators

Three main approaches have been taken and they are illus-

trated by the following examples.

Increasing immunogenicity of tumour cells

The first approach aims to increase the immunogenicity of Ca125-expressing cells. This is mediated by oregovomab, a xenotypic IgG$_{1\kappa}$ of murine origin with a high affinity for Ca125 (both on the cell surface and within the circulation). In an initial trial in which patients received oregovomab following induction therapy, the induction of an immune response significantly correlated with outcome measures (Gordon et al. 2004). However, a subsequent randomised phase II study with PFS and OS as endpoints failed to demonstrate a significant benefit (Berek et al. 2004). Nevertheless, it is possible that a subset of patients with favourable prognostic factors may gain benefit from this therapy and a confirmatory phase III study is ongoing. Biologically, this mAb is shown to work via hypersensitivity type II and III-like mechanisms with formation of immune complexes leading to cellular immunity, antigen clearance and cell kill (Evans et al. 2003). Tolerability has not been a problem and the concern lies mainly in the likely presence of immunosuppressive pathways that may hamper its efficacy.

Binding to regulators of immune system

The second approach aims to impact key signalling molecules in the regulation of the immune system. Examples include MDX-010 and CP-675 206, mAbs that bind to CTLA-4; the latter is a ligand that competes with CD28 for the co-stimulatory molecules, B7.1 and B7.2, on antigen presenting cells and mediates inhibition of T-cell activation. MDX-010 has shown clinical activity in patients with advanced ovarian cancer (Hodi et al. 2004) but non-specificity and autoimmune toxicities may preclude this class of drug from gaining approval for widespread use. Developing bispecific mAbs (e.g. recognising an immune modulator as well as a tumour cell specific antigen) may represent a way forward in overcoming non-specificity. Interestingly, other studies evaluating the role of an anti-CTLA-4 concept have shown that responses usually occur late (several weeks to few months) and this may preclude the recruitment of patients with aggressive tumours who have other therapeutic options.

Another example is the use of anti-tumour necrosis factor-α (TNFα) therapies including etanercept (soluble p75 TNF-receptor) and infliximab (mAb to TNFα). These have been licensed for use in certain autoimmune conditions including inflammatory arthropathies and Crohn's disease. TNF is known to be a transforming agent for carcinogen-treated fibroblasts (Komori et al. 1993). Moreover, anti-TNFα therapies have been shown to prevent leukocyte infiltration, reduce chemokine and matrix metalloproteases, as well as inhibit angiogenesis, all of which are important targets for ovarian cancer. A phase I/II study involving etanercept has been carried out in 30 patients with advanced ovarian cancer; at a dose of 25 mg/kg given twice or thrice weekly, it was well-tolerated and 6 patients had SD for 22 to 24 weeks (Madhusudan et al. 2005). Another study is currently examining the role of infliximab in ovarian cancer patients with ascites. Tumour biology forms part of the focus of this study and when administered intravenously, infliximab was found to distribute into the ascites at levels correlating with biological activity in vitro. There were also corresponding changes to gene expression profile of ascitic cells, alongside reduction in the levels of TNFα in ascites and plasma (Charles et al. 2006). Further results from this study are awaited.

Yet another example is the use of immunoconjugates to deliver cytotoxic agents such that the surface antigen-immunoconjugate complex is internalised upon binding. Denileukin diftitox (DAB389IL2, Ontak) is a fusion toxin consisting of interleukin-2 (IL-2) genetically fused to diphtheria toxin and has been approved to treat cutaneous T cell lymphoma, as well as having shown to produce response rates of 33% and 25% in chronic lymphocytic leukaemia and non-Hodgkin lymphoma, respectively (Pastan et al. 2006). Recent studies demonstrated that denileukin diftitox depletes human regulatory T cells and is associated with improved measures of T cell immunity and clinical improvements in ovarian cancer (Barnett et al. 2005). A follow-up phase II trial is ongoing to assess its efficacy in the treatment of relapsed ovarian cancer (Barnett et al. 2006).

Molecular mimicry

The third approach takes the form of molecular mimicry and is exemplified by an anti-idiotype mAb, ACA-125. The specificity of this mAb is for the antigen-binding region (Fab) of OC-125, another mAb that binds Ca125. Thus, the Fab of ACA-125 mimics the structure of a dominant epitope in the Ca125 extracellular repeat sequence. Proof-of-concept studies have been carried out in patients with ovarian cancer successfully demonstrating the elicitation of immune responses specific to Ca125 post-treatment (Reinartz et al. 2004).

Carrier of radioisotopes or other drugs

Antibody-directed enzyme pro-drug therapy (ADEPT) and gene-directed enzyme pro-drug therapy (GDEPT) aim to increase tumour-cell-specific drug exposure to overcome drug resistance with minimal toxicity to normal tissues, and are in development. However, these strategies are currently limited by their ability to administer sufficient quantities of drugs systemically, for them to be repeated over a period of time, and/or the ability to achieve tumour specificity.

Radiotherapy can be targeted by conjugating radioisotopes to mAbs. Yttrium-90 (Y90) labelled anti-MUC1 and anti-HMFG1 mAbs (administered intraperitoneally) have been developed. However, a randomised phase III trial of the latter and a phase II trial of the former have not demonstrated any benefit (Seiden et al. 2004). Other radio-labelled mAbs are in early clinical trials, including conjugates of Y90 with mAb against the Lewis Y antigen and glycoprotein 72. It may be possible to increase the efficacy of future radio-labelled mAbs by increasing their ADCC capability. Alternatively, the type of radioisotope and dose delivered could be altered.

SMALL MOLECULE DRUGS

Targeting members of the EGFR family

As opposed to blocking receptor activation via binding to the extracellular domain of EGFR, small molecule TK inhibitors, such as gefitinib (ZD1839, Iressa) or erlotinib (OCI-774, Tarceva), selectively and competitively inhibit receptor TK activity by blocking the adenosine tri-phosphate binding site within the TK domain. These agents have been approved for use in NSCLC and significantly, a recent prospective phase II study of first-line single-agent erlotinib in NSCLC with EGFR mutation demonstrated a response rate of 90% (Paz-Ares et al. 2006).

Erlotinib as a single agent has been evaluated in a phase II study in ovarian cancer (Gordon et al. 2005). In a cohort of 34 patients with HER1+ platinum-refractory disease, PR was observed in 6% of these patients with another 44% demonstrating disease stabilisation. This compound is generally well tolerated and common toxicities include rash (68%) and diarrhoea (38%), in keeping with published data.

However, in a GOG phase II study of single agent getifinib in 30 ovarian cancer patients with progressive disease (Schilder et al. 2005), only 1 PR was seen and only 4 patients had a progression free interval greater than six months. Interestingly, skin rash of grade 3 or higher was associated with a better clinical outcome, consistent with findings from other studies involving gefitinib.

Data from EGFR mutation analysis of tumours from NSCLC patients treated with either agent indicates that

response to both agents is restricted to tumours with in-frame deletions of exon 19 (Lynch *et al*. 2004); this has clear relevance as a biomarker for selection of patients most likely to benefit from these therapies. Samples from patients with recurrent ovarian cancer treated with gefitinib in the GOG trial have similarly been analysed for these mutations (Schilder *et al*. 2005); significantly, a tumour sample from the 1 patient who exhibited response to single-agent gefitinib had a mutation in the catalytic domain of EGFR. Moreover, markers of efficacy may not be confined to the molecular level; epidemiological data from the NSCLC studies has also pointed to the following as predictors of response to treatment with anti-EGFR TK inhibitors, namely: female sex, non-smoker, far-eastern ethnicity and adenocarcinoma histology.

Preclinical models have explored the biology of combining these agents with conventional cytotoxics with *in vitro* work suggesting an association between acquired resistance to cytotoxic chemotherapeutic agents, increased sensitivity to EGFR inhibition and increased EGFR expression (Dai *et al*. 2005). Targeted inhibition of EGFR signalling pathways has also been shown to augment taxane-induced cell death (Qiu *et al*. 2005). Thus, clinical studies are ongoing to investigate the feasibility and efficacy of this concept. Erlotinib in combination with carboplatin and docetaxel was evaluated in a phase I/IIa study of 53 patients as first-line therapy (Agarwal *et al*. 2004). The combination was associated with increased gastro-intestinal and skin toxicities such that during chemotherapy, the maximum tolerated dose of erlotinib was only 75 mg. However, as a single agent following the completion of chemotherapy, the dose of 150 mg was well tolerated. A randomised trial in ovarian cancer patients involving erlotinib as maintenance therapy following initial treatment with platinum-based treatment is ongoing within the EORTC. Another combination that has been evaluated is that of weekly topotecan and gefitinib; this has been shown in a recent phase I study to be well tolerated (Slomovitz *et al*. 2006).

As modulators of specific signalling pathways with high therapeutic ratios, combinations of targeted agents (concurrently or sequentially) could potentially cause less toxicity compared with conventional chemotherapy. For instance, the combination of erlotinib and bevacizumab in recurrent NSCLC has demonstrated tolerability and activity in early clinical studies (Herbst *et al*. 2005). Also, there are pre-clinical data showing synergism when a HER1/EGFR TK inhibitor and an anti-HER1/EGFR mAb are combined. Gefitinib in combination with cetuximab had a greater than additive effect on cell proliferation, apoptosis and HER1/EGFR-dependent signalling, compared with either agent alone (Matar *et al*. 2004).

Interfering with the cooperation that exists between receptors could represent another strategy to improve the efficacy of ErbB-targeted therapies. Two agents that inhibit multiple ErbB receptors that have been evaluated in clinical trials include canertinib (CI-1033) and lapatinib (GW-572016, Tykerb).

Canertinib is an irreversible pan-ERB-B inhibitor. When evaluated in phase I studies using a weekly schedule, canertinib was associated with thrombocytopenia and hypersensitivity reactions (Allen *et al*. 2003). More frequent administration schedules were subsequently explored and using these, canertinib was better tolerated with evidence of target modulation (Rowinsky *et al*. 2003). Disappointingly, in a phase II study involving 105 patients with platinum resistant disease, no objective response was observed (Campos *et al*. 2004).

Lapatinib is a quinazoline derivative that functions as a reversible, dual ERB-B TK inhibitor (HER1 and HER2). It is well tolerated taken once daily when evaluated in a phase I study (Burris *et al*. 2003). Importantly, lapatinib has shown clinical activity in malignancies refractory to agents that target individual ERB-B receptors. Also, lapatinib has been shown to inhibit the growth of trastuzumab-conditioned HER-2-positive breast cancer cells at concentrations

similar to those required for trastuzumab-naive cells (Konecny *et al*. 2003). Randomised trials in HER2+ breast cancer have confirmed the activity of lapatinib in combination with chemotherapy (Moy and Goss 2006) and its registration in this indication is anticipated. Currently, a phase II study involving lapatinib in patients with ovarian cancer is ongoing within the GOG and randomised trials are being considered. Unlike trastuzumab, lapatinib also inhibits phosphorylation of p95$^{\text{HER-2}}$, a fragment of the HER-2 receptor produced by proteolytic cleavage of the extracellular domain (Xia *et al*. 2004). The roles of p95 and other truncated receptors in signal transduction remain to be clarified.

Targeting other mediators of signal transduction pathways

Following recognition of extracellular signals at the cell surface, linked cytoplasmic and nuclear biochemical cascades are activated. These elaborate networks of intracellular signals are brought about by changes in enzymatic activity, protein phosphorylation and localisation, as well as the formation of protein-protein complexes. The Ras/Raf/MAPK, PI3K/Akt, PKC and PKA are integral components of important signal transduction pathways; when dysregulated, these pathways could contribute to malignant transformation. Indeed, components of these pathways, including k-ras, b-raf, akt and the p110-α subunit, have been shown to be oncogenes in several malignancies and represent potential therapeutic targets. Moreover, these pathways are critical in the process of apoptosis signalling which determine the sensitivity of cancer cells to cytotoxic agents.

The PI3/Akt pathway and related signals

The PI3K/Akt pathway is a particularly attractive target in ovarian cancer. The genes coding for the p110-α subunit of PI3K as well as AKT2, a protein downstream of PI3KCA, are amplified in a significant proportion of cases of the disease (Bellacosa *et al*. 1995; Campbell *et al*. 2004). The first generation PI3K inhibitors, such as LY294002, while effective *in vitro*, did not progress to clinical trials due to poor pharmacological profiles. However, novel PI3K and PKB inhibitors are in pre-clinical development and clinical trials are anticipated in the near future.

Lysophosphatidic acid, a growth factor found in ovarian cancer ascites, has been shown to promote cell survival by activating the PI3K/AKT pathway and thus represent a further valid therapeutic target. In addition, amplification of AKT has been observed in undifferentiated ovarian cancer and may lead to resistance to various drugs, including taxanes, due to apoptotic failure (Bellacosa *et al*. 1995). In this context, another potentially important molecular target is the HSP90 molecular chaperone, which serves to stabilize a number of mutated and overexpressed signaling proteins that promote cell survival and proliferation. Hence, HSP-90 inhibition appears an attractive target for modulating dug resistance. The HSP-90 inhibitor, 17-allyamino, 17-demethoxygeldanamycin (17-AAG), has been shown to sensitise ovarian tumour cells with constitutively active AKT to paclitaxel (Sain *et al*. 2006) and may also have additive or synergistic effects in combination with cisplatin, doxorubicin and paclitaxel (Nguyen *et al*. 2001).

Mammalian target of rapamycin (MTOR) lies downstream of PI3K, and cancer-related changes in mTOR kinase substrates and their associated proteins have been reported in ovarian cancer (Dancy 2006). MTOR inhibitors such as rapamycin analogues (temsirolimus (CCI 779) and everolimus (RAD 001)) are now in clinical trials both as single agents and in combination with standard chemotherapeutic agents. Encouraging results have emerged from phase I and II studies evaluating the use of temsirolimus in patients with endometrial, breast and renal cell cancer. Interestingly, the activity of temsirolimus in endometrial cancer was not correlated with loss of PTEN, indicating the complexity of the pathways involved (Oza *et al*. 2006). Evero-

limus has been evaluated in a phase I/II study with good tolerability and possible efficacy in myelodysplastic syndrome. However, as yet, no Phase II trials in ovarian cancer have been reported for these agents.

Src pathway

The non–receptor protein tyrosine, Src, is a 60-kDa protein that is the archetypal member of a nine-gene family, including Src, Yes, Fyn, Lyn, Lck, Hck, Fgr, Blk, and Yrk, that plays a critical role in myriad cellular functions. *In vitro* and *in vivo* models have provided strong support for the use of Src inhibitors in cancer therapy. Firstly, in models of epithelial cancer, Src activation promotes a more migratory and invasive phenotype (Avizienyte and Frame 2005). Secondly, Src regulates critical angiogenic factors that promote tumour progression (Mukhopadhyay *et al.* 1995). In fact, Src activation is required for hypoxia-mediated expression of VEGF. Currently, three inhibitors of Src, dasatinib (BMS354825), AZD-0530 and SKI-606, have reached phase I studies. These small molecule inhibitors are directed at the ATP-binding site of Src family kinases and thus are also inhibitors of kinases with closely related structure, such as Abl. It is too early to comment on the effectiveness of any of the Src inhibitors, but dasatinib and AZD-0530 seem well tolerated in phase I studies (Thomas and Brugge 1997; Frame 2004). Significantly, taxol-resistant ovarian tumour cells have been associated with increased Src activation, and Src inhibitors sensitise these cells to taxol (Chen *et al.* 2005; George *et al.* 2005), pointing towards the rational combination of these to be tested in clinical trials.

Ubiquitin-proteasome pathway

The ubiquitin-proteasome pathway is a highly conserved intracellular pathway for the degradation of proteins and plays a significant role in neoplastic growth and metastasis. The ordered and temporally regulated degradation of numerous key proteins, such as cyclins, cyclin-dependent kinase inhibitors and tumour suppressors, is required for cell cycle progression and mitosis (Goldberg *et al.* 1995). For instance, proteasome is required for the activation of nuclear factor-kappa B (NF-κB) by degradation of its inhibitory protein, I-κB (Palombella *et al.* 1994). NF-κB is a transcription factor that upregulates a number of proteins involved in cancer progression, including several pro-angiogenic factors and anti-apoptotic factors. An inhibitor of proteasome, bortezomib, has been approved for use in multiple myeloma and much work is now focussed on its potential use in other haematological malignancies. However, data from pre-clinical models suggest bortezomib may also be useful in the treatment of solid tumours, including ovarian cancer. Firstly, bortezomib has been shown to induce apoptosis at least as effectively in spheroidal cell cultures in ovarian cancer cell lines as in monolayer cultures when used as a single agent and in combination with other standard chemotherapeutic agents (Nagourney *et al.* 2006). Secondly, chemoresistance in solid tumours has been associated with NF-κB activation (Cusack 2003). For example, platinum-based agents have been shown to rapidly induce chemoresistance by activating NF-κB (Yan *et al.* 1999). Blocking NF-kB activation with bortezomib therefore represents a potential target for overcoming the apoptosis failure that develops during ovarian cancer treatment. A phase I study of bortezomib in combination with carboplatin in 15 patients with recurrent ovarian cancer has been performed with manageable toxicities at the recommended dose level and further studies are awaited (Aghajanian *et al.* 2005). These may involve combinations with other agents; recent positive data in multiple myeloma in which bortezomib was combined with pegylated liposomal doxorubicin (a single agent with significant activity in ovarian cancer) may be relevant in this context (Orlowski *et al.* 2005).

Protein kinase C

Protein kinase C (PKC) isoforms play important roles in intracellular transduction of signals for growth, proliferation and apoptosis, and consequently have been variously implicated in oncogenesis. PKC-α inhibition has also been implicated in reversal of multi-drug resistance, via regulation of PGP-phosphorylation and enhancement of platinum sensitivity in ovarian cancer cell lines. Downregulation of PKC has been shown to occur after the prolonged binding of bryostatin (despite its activity as an agonist); this is a macrocytic lactone which has been isolated from the marine invertebrate, bryozoan. A total of 72 patients with ovarian cancer have been treated with single-agent bryostatin in two phase II studies (Armstrong *et al.* 2003; Clamp *et al.* 2003) with another 8 patients receiving the combination of cisplatin and bryostatin in a separate phase II study (Morgan *et al.* 2005). Myalgia was the most frequent and severe toxicity. In the single agent cohort, only 1 partial response with 19 cases of stable disease was observed, and bryostatin thus appears to be inactive in ovarian cancer. This could be due to the relative non-specificity of bryostatin for the different isozymes of PKC. However, ISIS 3521, an anti-sense oligonucleotide specific for the PKC-α isoform, given as a 21 day continuous infusion, has also been found to be inactive in a phase II study in ovarian cancer (Advani *et al.* 2004). Interestingly, while expression levels of both PKC-í and PKC-α have been shown to be elevated in invasive tumours relative to benign ones, only PKC-í expression was negatively implicated as a poor prognostic factor (Weichert *et al.* 2003). This may point to further specific and more targeted approaches to PKC inhibition.

VEGF, multi-TK inhibitors and anti-angiogenic factors

There are several TK inhibitors of VEGF (with varying degrees of specificities to the VEGF target) currently in different stages of clinical development. Large, randomised phase III studies have demonstrated the efficacy of single-agent multi-TK inhibitors, sorafenib (BAY 43-9006) and sunitinib (SU 11248), in patients with renal cell carcinoma and gastrointestinal stromal tumours who have failed standard therapies, respectively (Demetri *et al.* 2005; Escudier *et al.* 2005). ZD6474 in combination with conventional chemotherapy has also shown encouraging efficacy data in phase II studies in patients with advanced stage NSCLC (Herbst *et al.* 2005; Johnson *et al.* 2005). Other agents, including AZD2171, BIBF1120 and AEE788, are currently undergoing phase I/II evaluations and appear promising with manageable toxicities (common being hypertension, fatigue, anorexia, nausea and vomiting) (Drevs *et al.* 2005; Lee *et al.* 2005; Martinelli *et al.* 2005). Interestingly, there has not yet been any published trial of a VEGF receptor targeted TK inhibitor evaluated as a single-agent specifically in patients with ovarian cancer.

Sorafenib has inhibitory activity against a multitude of targets including c-raf, b-raf, VEGR 1, 2 and 3, PDGFR-β, c-kit and flt-3, and has clinical activity in ovarian cancer. For instance, in a phase I study using single-agent sorafenib at a dose of 200 mg b.d. on a 4 week on / 1 week off schedule, 4 out of the 10 patients with ovarian cancer within the study had disease stabilisation and stayed on the drug for more than 30 weeks (Moore *et al.* 2005). Its use in combination with other chemotherapies in the treatment of ovarian cancer is being explored currently in early clinical trials. A recent phase II study of sorafenib in combination with gemcitabine in recurrent ovarian cancer has been examined and the combination appears to be well tolerated with sorafenib administered at 400 mg orally twice daily and gemcitabine 1000 mg intravenously once weekly (Welch *et al.* 2006).

AZD2171 is a more specific targeted agent than sorafenib and inhibits the kinase activity of VEGFR2. Phase I studies have confirmed the feasibility of combining this drug with the carboplatin/paclitaxel doublet (Laurie *et al.*

2006), and without the need for phase II trials the drug is proceeding directly into a randomised phase III trial in relapsed ovarian cancer in combination with platinum-based chemotherapy. This large inter-group trial (ICON-6) will explore its potential, both with chemotherapy and as a continuation single-agent treatment.

BIBF1120 is another example of a small molecule VEGF receptor targeted TK inhibitor. Notably, it also has activity against PDGF and FGF receptor kinases as well as members of the src family of tyrosine kinases (Lee *et al*. 2005). It is undergoing evaluation in ovarian cancer in a novel trial design, in which relapsed patients at high risk of early further relapses are randomised either to receive BIBF1120 or placebo. Results are anticipated from 2008 onwards.

Imatinib is another small molecular agent with activity directed against multiple targets including bcr-abl, PDGFR and c-kit. It has gained FDA approval for use in the treatment of CML and GIST. Interestingly, ovarian cancer cells express high levels of PDGFRα and PDGF (Henriksen *et al*. 1993). *In vitro* models show that imatinib can inhibit PDGFRα phosphorylation, leading to growth inhibition (Matei *et al*. 2004). Despite good tolerability, imatinib did not produce clinical responses in a phase II study evaluating its use as a single agent in recurrent ovarian cancer (16 patients were assessed for toxicity and 12 for efficacy) although 33% of patients had stable disease ranging from 3.8 to 8+ months (Coleman *et al*. 2006). Of note, there was no correlation observed between clinical activity and target expression (of c-abl, c-kit and PDGFRβ).

It is thought that imatinib could also impact on the delivery of anti-cancer therapy by reducing intratumour interstitial pressure. Indeed, in another study, the intra-tumour level of the novel cytotoxic agent, epothilone, was considerably increased compared to normal tissues when epothilone was coadministered with imatinib (Pietras *et al*. 2003). The combination of imatinib and other chemotherapeutic agents (such as doxetacel) is therefore currently being evaluated in early clinical trials.

As an inhibitor of non-voltage gated calcium channels and calcium channel-mediated downstream signalling pathways, carboxyamidotriazole (CAI) has been shown to have inhibitory effects on angiogenesis, tumour growth, invasion and metastasis in experimental models. In a phase II study, CAI demonstrated a favourable toxicity profile with clinical activity in 14 (13 SD and 1 PR) out of the 36 recruited patients with relapsed ovarian cancer, suggesting a potential role for CAI as maintenance therapy (Hussain *et al*. 2003).

DMXAA (AS1404) is a small-molecule vascular disrupting agent, which in animal models shows synergistic effects with cytotoxics, including taxanes and platinum agents. Agents targeting tumour vasculature offer a complementary approach to anti-angiogenesis therapy. Indeed, the potential combination with a VEGF receptor-targeted treatment is highlighted by recent published data. A phase II study is currently evaluating DMXAA in combination with carboplatin and paclitaxel in recurrent platinum-sensitive ovarian cancer patients and preliminary safety data are encouraging (Gabra 2006). Results of efficacy assessments are awaited.

The notion of combining anti-angiogenic treatments may be of particular relevance in ovarian cancer. Recent clinical experience is instructive. A phase I trial is combining sorafenib with bevacizumab and preliminary data indicate a high level of activity in relapsed ovarian cancer but with a higher than anticipated level of toxicity (Azad *et al*. 2006). Clearly this approach will need further cautious exploration.

DNA repair

Cells with deficiencies in homologous recombination (HR) pathway genes, including BRCA 1 and 2 and FANC, are unable to repair DNA cross-links and DNA double-strand breaks by error-free HR, thus resulting in genomic insta-

bility and cancer predisposition (Tutt and Ashworth 2002). Hence, HR deficient cells are particularly platinum-sensitive as they are unable to repair DNA-damage caused by inter and intra-strand adducts, whereas platinum-resistance may occur, at least in part, through the function of intact HR DNA repair pathway genes, particularly FANC and BRCA (Olopade *et al*. 2003). Recently, deficiencies in HR pathway genes have also been found to confer extreme sensitivity of ovarian cells (with non-functioning BRCA 1/2) to inhibition of poly(ADP-ribose) polymerase (PARP), an enzyme involved in base excision repair, which is a key pathway in the repair of DNA single strand breaks (Farmer *et al*. 2005). BRCA 1/2 mutations are an uncommon feature in ovarian cancer, although the frequency may have been underestimated. They may be found in up to 15% of cases; moreover the lack of function of these genes may be a much more common feature of sporadic ovarian cancer through mechanisms such as gene hypermethylation (Esteller *et al*. 2000). One study has indicated that up to 32% of cases of high grade serous papillary ovarian cancer may have this feature (Press *et al*. 2006), thus considerably increasing the potential for a PARP inhibition approach to treatment. Our unit is currently running a Phase I study of a PARP inhibitor (KU 59436) and we have observed significant clinical efficacy in patients with BRCA-associated ovarian cancer, together with a favourable toxicity profile when the drug is administered on a continuous oral schedule (Yap *et al*. 2007, Proceedings of ASCO, in process).

Endothelins

The effects of endothelin-A receptor (ET$_A$-R) signalling are wide ranging and involve both tumour cells as well as their surrounding stroma, including the vasculature (Bagnato *et al*. 2005). Pre-clinical work has linked ET$_A$-R overexpression with tumour progression in ovarian cancer. Moreover, the ET$_A$-R antagonist, atrasantan (ABT-627), inhibits cell proliferation and growth of ovarian carcinoma xenografts and displays synergistic effects when combined with taxanes (del Bufalo *et al*. 2002). Thus, ET$_A$-R could be useful as a potential target for anti-cancer therapy.

CONCLUSIONS

In the concluding section, we make brief comments on future trends in molecular targeted therapy as well as major challenges ahead.

Gene therapy using viral vectors

The progress of gene therapy in cancer therapeutics has slowed overall after the failure of clinical trials to demonstrate any significant clinical benefit as well as concerns about its safety. The limitation of its clinical utility is likely to be due to a combination of poor transfection efficacy of vectors, host immune responses, the potential lack of clinical significance of the molecular target and the fear that viral vectors, being the most potent vector recognised thus far, may regain their pathogenicity post-administration. Nevertheless, the fact that ovarian cancer is predominantly confined to the peritoneal cavity for much of its natural history continue to attract the notion that intraperitoneal treatment with more potent gene-directed approaches may be successful.

Initial studies involved the intraperitoneal administration of adenoviruses. A phase I study with ONYX-015, an attenuated adenovirus designed to replicate selectively in cells with non-functioning p53, demonstrated the feasibility of this approach but also the difficulty in achieving satisfactory level of viral replication in the presence of bulky disease (Vasey *et al*. 2002). Moreover, antitumour activity was modest. Subsequent research is focusing on ways to improve tumour cell uptake of these intraperitoneally administered vectors.

Other published gene therapeutic approaches in ovarian

cancer have targeted erbB2 expression. The first strategy involves the liposomal-mediated gene transfer of the adenoviral protein, E1A. This has been shown to downregulate erbB2 expression with reduced tumour proliferation *in vitro* and *in vivo*. Despite phase I studies showing good tolerability when administered intraperitoneally, no objective clinical response was observed (Hortogagyi *et al.* 2001). The second strategy involves the expression of a single-chain recombinant antibody to erbB2 using the adenoviral vector, Ad21. Although this antibody is of low molecular weight (with likely high intra-tumoral penetrability), its short half-life, lack of Fc domain and inability to induce ADCC may contribute to reduce its antitumor efficacy. Indeed, no objective response was evident when this was tested in a phase I trial (Alvarez *et al.* 2000). More recent work has highlighted the feasibility of an *in vivo* full-length antibody gene delivery system (Jiang *et al.* 2006). Recombinant anti-HER-2–expressing adenovirus (Ad5-TAb) *in vivo* produced a full-length antibody with binding specificity similar to that of trastuzumab and at high, sustained serum concentrations after a single administration with significant tumour shrinkage in murine models. Clinical trials utilising this approach are eagerly awaited.

Combinations of therapies

Unlike CML where a single molecular event is the main driver of malignancy, multiple steps are involved in ovarian cancer formation and progression (Aunoble *et al.* 2000; Havrilesky and Berchuck 2001). There is thus a need for strategies to integrate molecular targeted agents with conventional therapies and to explore rational combinations with other targeted approaches. These include other anti-receptor therapies, receptor-downstream signalling transduction inhibitors, and targeted approaches interfering with other essential drivers of cancer, such as angiogenesis.

Clinical settings where these may be used

The acceptable toxicity profile of several molecular targeted agents opens the possibility of using these agents for long-term, chronic "maintenance" therapy. However, there is currently no convincing evidence to support this approach in ovarian cancer. Standard cytotoxics, particularly paclitaxel, are being evaluated in this context. However, molecular targeted agents may offer a more rational alternative. Hence, in addition to their potential role in first-line treatment and in relapsed disease in overcoming chemoresistance in combination with carboplatin (with or without paclitaxel), these agents may also prove useful as a "chronic" single-agent maintenance treatment.

Patient selection for therapy

Ovarian cancer constitutes a heterogeneous disease. For instance, different histological sub-types of ovarian cancer are associated with different cell surface markers, growth behaviour and prognosis. The heterogeneity of ovarian tumour biology is also reflected in the variable outcomes to treatments and a challenge we currently face is the discovery of effective biomarkers to guide patient selection and choice of treatment. We predict that future treatments of ovarian cancer will be tailored according to the biology of each patient at the level of gene expression profiling and molecular detail beyond the categories we currently employ.

Innovation in the face of changing economic climates

One of the major challenges facing all of those involved in drug development in ovarian (and other) cancer is the escalating cost involved. The move towards combinations of new molecular targeted agents can only exacerbate this problem. The cost of health care in general continue to rise inexorably, and the issue of drug costs will therefore have

to be faced. One solution is to develop more robust molecular diagnostics to select those patients who will benefit from treatment, as described above. In this way, costs of ineffective treatment for large numbers of patients can be saved.

Moreover, our current clinical trial infrastructure is rather inadequate to evaluate the aforementioned agents and combinations. Thus, innovative trial designs are urgently needed and careful considerations will need to be made as we continue to move forward, especially over the next few years.

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