

Mini-reviews

Authors from Australia describe how interfering with cell survival is increasingly being chosen as a method of developing a treatment strategy for hormone-resistant prostate cancer. The authors show how several developmental drug candidates have preclinical and clinical activity against cell survival proteins, and that these might be worth considering as possible clinical entities in this condition.

The commonly stated argument that laparoscopy has advanced the cause of donor nephrectomy in renal transplantation is examined by authors from the UK. They present a review of the published reports and show that the evidence base is poor for drawing a conclusion as to whether laparoscopic or open nephrectomy is best.

Interfering with cell-survival signalling as a treatment strategy for prostate cancer

NIALL M. CORCORAN, ANTHONY J. COSTELLO and CHRISTOPHER M. HOVENS

Departments of Urology and Surgery, University of Melbourne, Royal Melbourne Hospital, Parkville, Victoria, Australia

Accepted for publication 7 February 2006

KEYWORDS

prostate, prostatic malignancy, cell survival, apoptosis, signalling

INTRODUCTION

A mounting body of evidence implicates dysregulation of cell-survival signalling pathways in the pathogenesis of hormone-refractory prostate cancer (HRPC), and with the activation of survival mechanisms that counteract chemotherapeutic treatment options. What is not clear as yet is which are the most salient target(s) in these pathways that when inhibited will lead to the most effective clinical outcome. Several developmental drug candidates that have confirmed preclinical and clinical activity against cell-survival proteins are currently worth consideration as possible clinical entities in prostate cancer. We focus on some of these key effectors of cell survival and treatment resistance that are particularly pertinent for prostate malignancies, and review the results of recent trials of new agents that target these proteins.

Bcl-2 FAMILY PROTEINS: GATEKEEPERS OF APOPTOSIS

The Bcl-2 family of proteins have various roles that impinge directly on the balance between

the survival or death of cells. Two key Bcl-2 family proteins are Bcl-2 itself and Bcl_{x_L}. Both of these proteins inhibit apoptosis by blocking the release of cytochrome c from mitochondria. Bcl-2 and Bcl_{x_L} are overexpressed in a very high percentage of prostate cancers, and are thought to contribute significantly to drug resistance. Given the undoubted importance of Bcl-2 family proteins in prostate cancer cell survival, clinical trials using antisense agents that aim to reduce the expression of anti-apoptotic Bcl-2 proteins are underway.

A phase II study of oblimersen sodium (Genasense, G3139), a phosphorothioate antisense oligonucleotide directed to the Bcl-2 mRNA, in combination with docetaxel in 28 patients with HRPC was reported recently [1]. Patients received oblimersen at 7 mg/kg/day continuous i.v. infusion for 7 days, with docetaxel 75 mg/m² as a 1-h infusion i.v. on day 6, every 3 weeks, with an average of six cycles per patient. Overall, 14 of 27 patients had a PSA response, 4 of 12 had an objective response and median survival was 19.8 months. These results are comparable with results of single-agent studies for docetaxel in the treatment of HRPC [2,3]. However, in the study by Tolcher *et al.* there was a thorough analysis of oblimersen and docetaxel pharmacokinetics, which revealed that the steady state plasma oblimersen concentration (C_{ss}) was a significant determinant of PSA response. Of the 28

patients, 15 had an oblimersen C_{5s} of $>5 \mu\text{g}/\text{mL}$, yet 12 of these had a PSA response, whilst of the remaining 12 with an oblimersen C_{5s} of $<5 \mu\text{g}/\text{mL}$, only three had a PSA response. These results intriguingly suggest that attaining a sufficient C_{5s} of oblimersen above the critical $5 \mu\text{g}/\text{mL}$ threshold might lead to a clinical benefit. However, several factors appear likely to compromise the routine clinical attainment of this concentration. Even though all patients were treated with the same $7 \text{ mg}/\text{kg}/\text{day}$ dosing schedule, there was marked variability among patients, as measured by the oblimersen C_{5s} ; 12 of 28 patients were unable to attain the apparent critical threshold of $5 \mu\text{g}/\text{mL}$. Unfortunately, simply raising and/or prolonging the oblimersen dose might necessitate a concomitant dose reduction in docetaxel, as 22 of 28 patients had haematological toxicity requiring a dose reduction in docetaxel to a median of $60 \text{ mg}/\text{m}^2$, 3-weekly, compared to the starting dose of $75 \text{ mg}/\text{m}^2$. Nevertheless, some encouraging signs were reported in this study, underscoring the potential importance of Bcl-2 signalling in prostate cancer. The challenge will now be to overcome the significant dose, delivery and toxicity issues associated with combined Bcl-2 antisense therapies, to develop into clinically useful agents.

NF- κ B: A TRANSCRIPTION BOOST FOR CELL SURVIVAL PATHWAYS

NF- κ B family transcription factors are potent inhibitors of apoptotic cell death. Over-activation of the NF- κ B family of transcription factors is associated with several human malignancies, including a series of human prostate cancer cell lines and prostate carcinoma xenografts [4,5]. Notwithstanding this, convincing evidence of enhanced NF- κ B activation in primary prostate cancer tissue is generally lacking, with variable heterogeneous nuclear staining of RelA, indicative of NF- κ B activation [6]. Specific inhibitors of NF- κ B family proteins have yet to progress to the clinic, but preclinical studies indicate that agents that specifically inhibit the proteasome also reduce levels of NF- κ B [7]. Of these agents the most advanced in prostate cancer clinical trials is bortezomib (PS-341, VELCADE), which has recently been approved for treating relapsed and refractory multiple myeloma.

A phase I/II study of bortezomib combined with docetaxel in patients with advanced androgen-independent prostate cancer gives some suggestion of clinical benefit [8]. Two bortezomib dose levels, 1.3 or $1.6 \text{ mg}/\text{m}^2$ combined with docetaxel $40 \text{ mg}/\text{m}^2$, were expanded into two phase II treatment arms given weekly for 2 of 3 weeks in patients with advanced HRPC. At the lower dose, 24% of 25 patients achieved a confirmed PSA decline of more than half and three of 13 patients with measurable disease achieved partial remission, with the remainder achieving stable disease. There was a dose-dependent improvement in these criteria in the groups receiving the higher bortezomib dose, with 36% of 28 patients registering a PSA decline of more than half and 14 of 18 patients with measurable disease achieving partial remission or stable disease. Unfortunately, these data are confounded by the lack of comparison with a docetaxel-alone arm, that would be expected to produce comparable declines in PSA levels, and partial and stable disease effects.

In preclinical studies, both thalidomide and arsenic trioxide have also been shown, amongst other effects, to inhibit NF- κ B activation. Thalidomide also has anti-angiogenic properties, which when coupled with its favourable bioavailability and modest, acute, side-effect profile have spurred several early phase I/II trials as both single and combined therapies in HRPC. A randomized phase II trial of docetaxel plus thalidomide in HRPC was reported recently [9]; 75 patients with chemotherapy-naive metastatic HRPC were randomly assigned to receive either docetaxel $30 \text{ mg}/\text{m}^2$ four times/week \times 3, with a 1-week rest (25 patients) or docetaxel + thalidomide 200 mg daily (50 men).

The combined arm had a greater PSA response (53% vs 37%) and better median progression-free survival (5.9 months vs 3.7 months for docetaxel alone). At 18 months the overall survival was also improved (68% vs 43% for docetaxel alone). When comparing the two arms of this trial the thalidomide combination appeared quite promising, but this enthusiasm is tempered by comparison with previous phase II and III trial data with similar docetaxel administration groups. Weekly docetaxel administration of $30 \text{ mg}/\text{m}^2$ four times/week \times 6, and a 2-week rest, in the pivotal Tax327 phase III trial [3], resulted in PSA responses of 48% and a median survival of 17.4 months. Phase II trial results of weekly

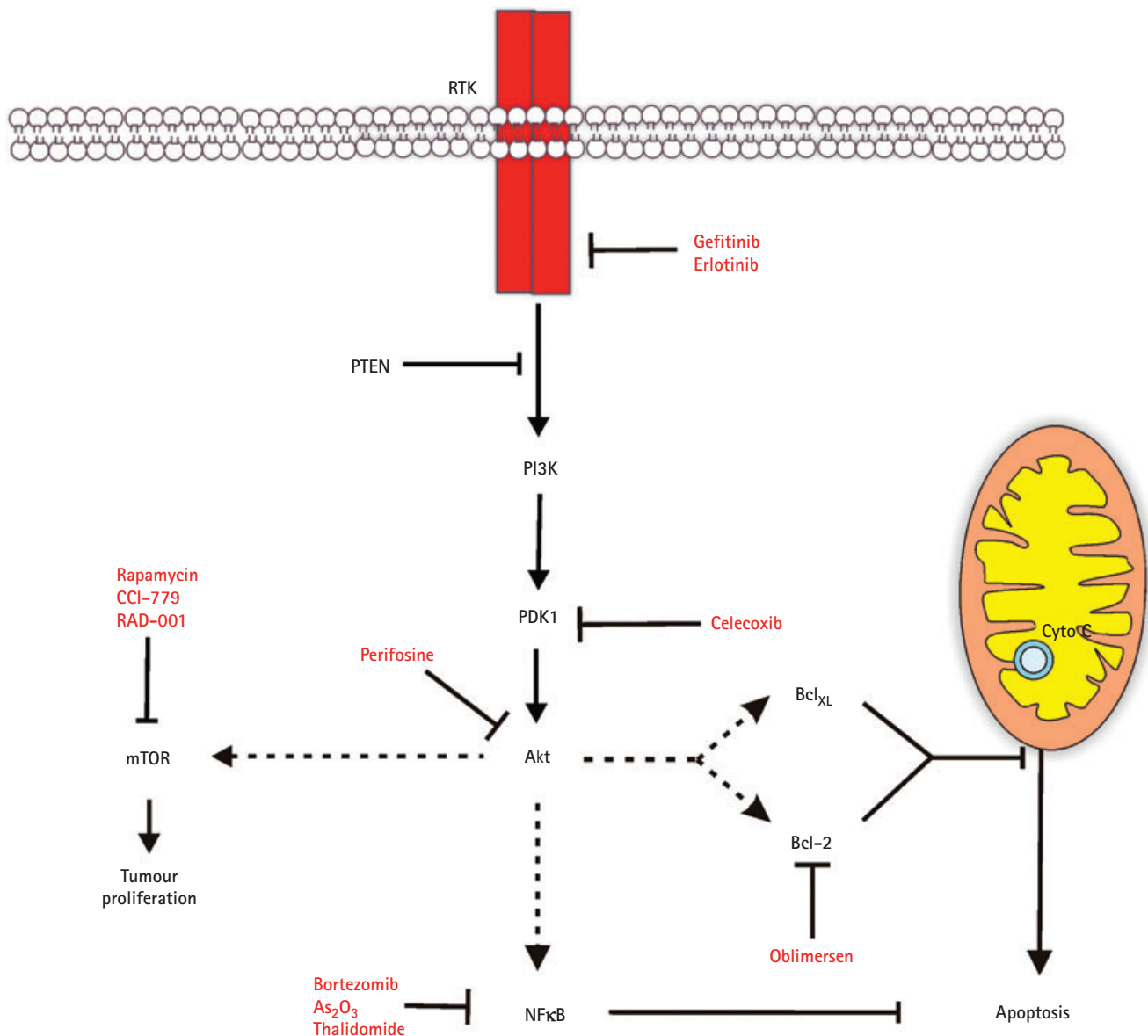
docetaxel regimens at $35\text{--}40 \text{ mg}/\text{m}^2$ four times/week \times 6 and a 2-week rest resulted in PSA declines of 41–64% [10–13]. Even though the docetaxel treatment in the study by Dahut *et al.* was for a shorter cycle than previous studies (3-weekly, 1-week rest, vs 6-weekly, 2-week rest) the cumulative docetaxel dose over a 2-month period was the same.

TARGETED INHIBITION OF RECEPTOR TYROSINE KINASES (RTKs)

Overactivity of the kinase signalling function of RTKs has been implicated in the cause of several human cancers. Increased levels of expression of the epidermal growth factor receptor (EGFR) and a truncated constitutively active mutant, EGFRvIII, have also been detected in human prostate cancer specimens [14]. A case could therefore be made to trial TK inhibitors that have been developed against the EGFR, i.e. gefitinib (Iressa) or erlotinib (Tarceva), or the humanized monoclonal antibodies such as cetuximab (Erbix, C225) in prostate tumours.

Gefitinib has been conditionally approved as a third-line monotherapy treatment for nonsmall cell lung cancer (NSCLC) after successful phase II trials for stage III and IV NSCLC. However, only a minority of patients with NSCLC achieved objective tumour responses with gefitinib monotherapy. A compelling molecular rationale for this effect was provided by two studies showing that nearly all of the responsive patients harboured somatic mutations within the EGFR kinase domain, whereas tumours bearing the wild-type receptor were essentially unresponsive to this inhibitor [15]. The same mutants are also highly susceptible to the distinct EGFR TK inhibitor erlotinib. Despite the impressive correlation of the mutants with partial and complete objective responses to gefitinib in NSCLC, up to 30% of patients reported stabilization of disease [16], a response rate that is not readily accounted for by the presence of activating EGFR mutants or indeed of EGFR expression levels [17]. These mutants have also only been identified in NSCLC and are essentially absent in other solid tumours [18]. The recent publication of a disappointing randomized phase II study of two doses of oral gefitinib $250 \text{ mg}/\text{day}$ (19 men) or $500 \text{ mg}/\text{day}$ (21 men) in patients with minimally symptomatic HRPC underlines the problem of predicting solid tumour responsiveness to RTK inhibitors [19].

FIG. 1. A schematic diagram of the major cell-survival/treatment-resistance pathways. The PTEN/PI3K/Akt pathway is activated by RTKs at the cell membrane or through loss of PTEN, leading to constitutive activation of Akt and the anti-apoptotic proteins Bcl_{xL}, Bcl-2 and NF-κB, as well as mTOR. Trial drugs that inhibit some of the effectors of these pathways are depicted in red.



None of the 40 patients had a PSA or objective measurable response; 14% had stable PSA levels and 14% had stable disease (duration 2.5–16.8 months). From these results it would appear that EGFR inhibitors such as gefitinib have minimal prospects as monotherapy in HRPC.

INHIBITION OF PDK-1 AND Akt

Loss of PTEN is a prevalent event in several human malignancies, in particular prostate

cancer. PTEN hypofunction leads to the concomitant up-regulation of signalling activity of the PDK-1 and Akt kinases. As such, inhibition of key signal-transducing molecules such as PDK-1, and in particular the Akt kinases, would appear to be an excellent strategy to circumvent the consequences of loss of PTEN. In this context it is interesting that the NSAID cyclooxygenase-2 (COX-2) inhibitor, celecoxib (celebrex) has PDK1 inhibitory activity [20].

Several lines of evidence suggest that COX-2 might be a valid target for chemoprevention and treatment strategies for human cancers. Overexpression of COX-2 has been correlated with advanced disease and poor prognosis in bladder, breast and colon cancer. In contrast, the link between COX-2 overexpression and prostate cancer progression is inconclusive. No correlation has been found with COX-2 expression levels in prostate cancer vs benign prostatic tissue, nor with Gleason score or staging [21]. However, various studies indicate

that NSAIDs might have a preventive effect in prostate cancer [22,23], suggesting that the inhibitory activity on other than COX-2 of these agents, possibly via inhibition of PDK-1 and thence Akt, may underscore their clinical activity in preventing prostate cancer.

Preliminary results of two phase II studies using high-dose (400 mg oral twice daily) celecoxib combined with chemotherapeutic regimens have been reported. In one study of a combination with epirubicin and estramustine in 14 patients, with 10 evaluable for response, six had a PSA decline by more than half, one had a response on bone scan, and one on CT [24]. Even though there was some evidence, as shown by PSA levels and the radiographic response, of a clinical response, the contribution of celecoxib to this effect cannot be determined from this trial. Somewhat more promising results were reported with a weekly docetaxel, estramustine and daily celecoxib (400 mg oral twice daily) regimen [25]. Of 33 patients treated, 25 were evaluated for response, 11 of whom had measurable disease; one had a complete response, two a partial response and four stable disease, with an overall response rate of 27%. Encouragingly, 70% of patients had a PSA decline by more than half. However, grade 3–4 toxicity and one toxic death due to gastric perforation associated with celecoxib, in combination with the greater risk of severe cardiovascular events [26], might limit future testing of this combination.

Given that signalling at the level of the Akt kinases appears to be a key amplification point in the PTEN/PI3K/Akt pathway, effective inhibition of these kinases would be expected to have profound effects on prostate cancer development and progression. However, prospective inhibitors of these kinases appears to be still in the preclinical stages of development [27]. The oral alkylphospholipid, perifosine, decreases Akt activation by inhibiting Akt recruitment to the membrane. A phase II trial using daily perifosine as a single agent in 19 patients with progressive, metastatic HRPc was reported recently [28]. Of the 19 patients evaluable for response, none had a PSA decline by more than half, there were no radiographic responses and the median time to progression was only 4 weeks. Despite these poor results from monotherapy, the true clinical effectiveness of Akt inhibitors would be expected to reside as combined treatments with conventional cytotoxic

agents, and it is hoped that combination trials with Akt inhibitors will persist.

INHIBITION OF mTOR

Several lines of evidence have focused attention on the key role of Akt in modulating the function of a cellular nutrient-sensing mechanism, the 'mammalian target of rapamycin' (mTOR) pathway. Aberrant control of this pathway leads to increased cell and organ size and hyperproliferation. The mTOR kinase appears to be the main signal-integrating point in this pathway, receiving inputs via nutrient and energy levels and via the PTEN/PI3K pathway through the action of Akt. Inhibition of mTOR through the natural antibiotic rapamycin, originally developed as an immunosuppressant, has now been proposed for a therapeutic role in tumours such as prostate malignancies, where PTEN loss is highly penetrant [29]. Rapamycin and its esterified derivatives, CCI-779 and RAD-001, have shown promise in preclinical tests, and some encouraging results with CCI-779 in phase I/II trials in RCC and metastatic breast cancer have been reported [30,31].

However, experimental resistance to rapamycin and its esterified derivatives has already been shown through the anti-apoptotic effects of Bcl-2 in a mouse model of prostate intraepithelial neoplasia [32]. As high levels of Bcl-2 family proteins are prevalent in HRPc, and inhibition at the level of mTOR is unlikely to inhibit the Akt driven boost in the levels and function of the Bcl-2 proteins, clinical resistance to mTOR inhibitors in HRPc might be expected to inevitably ensue.

Clinical trials with mTOR inhibitors are currently underway in advanced prostate cancer [33]. However, it is likely that these agents will need to be combined with either chemotherapeutic or newer anti-Bcl-2 agents in development before the potential promise of this class of inhibitors can be properly assessed.

CONCLUSIONS

Overactivity of cell-survival signalling pathways appears to be a critical molecular switch in the progression of prostate cancer to androgen independence. Advances in our understanding of cellular survival

mechanisms, coupled with the development and trialling of several inhibitors of survival/treatment resistance signalling cascades, allow cautious optimism that agents able to some degree to circumvent the clinical progression of prostate cancer will become available in the not too distant future.

ACKNOWLEDGEMENTS

We thank Scott Van Appledorn for critical comments and Daniel Martin for graphics. Supported by the Melbourne Urology Trust, a generous gift from the Cybec Foundation, the NHMRC, the Sir Benjamin Rank Fellowship and the Friends of the Neuroscience foundation.

CONFLICT OF INTEREST

A. Costello is a medical adviser to Novartis Australia. Sources of funding: NHMRC, Melbourne Urology Trust, Cybec Foundation.

REFERENCES

- 1 Tolcher AW, Chi K, Kuhn J *et al*. A phase II, pharmacokinetic, and biological correlative study of oblimersen sodium and docetaxel in patients with hormone-refractory prostate cancer. *Clin Cancer Res* 2005; **11**: 3854–61
- 2 Petrylak DP, Tangen CM, Hussain MH *et al*. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med* 2004; **351**: 1513–20
- 3 Tannock IF, de Wit R, Berry WR *et al*; TAX 327 Investigators. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 2004; **351**: 1502–12
- 4 Palayoor ST, Youmell MY, Calderwood SK, Coleman CN, Price BD. Constitutive activation of I κ B kinase alpha and NF- κ B in prostate cancer cells is inhibited by ibuprofen. *Oncogene* 1999; **18**: 7389–94
- 5 Gasparian AV, Yao YJ, Kowalczyk D *et al*. The role of IKK in constitutive activation of NF- κ B transcription factor in prostate carcinoma cells. *J Cell Sci* 2002; **115**: 141–51
- 6 Lessard L, Mes-Masson AM, Lamarre L, Wall L, Lattouf JB, Saad F. NF- κ B nuclear localization and its prognostic significance in prostate cancer. *BJU Int* 2003; **91**: 417–20

- 7 An J, Fisher M, Rettig MB. VHL expression in renal cell carcinoma sensitizes to bortezomib (PS-341) through an NF-kappaB-dependent mechanism. *Oncogene* 2005; **24**: 1563–70
- 8 Price N, Dreicer R. Phase I/II trial of bortezomib plus docetaxel in patients with advanced androgen-independent prostate cancer. *Clin Prostate Cancer* 2004; **3**: 141–3
- 9 Dahut WL, Gulley JL, Arlen PM *et al.* Randomized phase II trial of docetaxel plus thalidomide in androgen-independent prostate cancer. *J Clin Oncol* 2004; **22**: 2532–9
- 10 Beer TM, Pierce WC, Lowe BA, Henner WD. Phase II study of weekly docetaxel in symptomatic androgen-independent prostate cancer. *Ann Oncol* 2001; **12**: 1273–9
- 11 Berry W, Dakhil S, Gregurich MA, Asmar L. Phase II trial of single-agent weekly docetaxel in hormone-refractory, symptomatic, metastatic carcinoma of the prostate. *Semin Oncol* 2001; **28** (Suppl. 4): 8–15
- 12 Ferrero JM, Foa C, Thezenas S *et al.* A weekly schedule of docetaxel for metastatic hormone-refractory prostate cancer. *Oncology* 2004; **66**: 281–7
- 13 Gravis G, Bladou F, Salem N *et al.* Weekly administration of docetaxel for symptomatic metastatic hormone-refractory prostate carcinoma. *Cancer* 2003; **98**: 1627–34
- 14 Olapade-Olaopa EO, Moscatello DK, MacKay EH *et al.* Evidence for the differential expression of a variant EGF receptor protein in human prostate cancer. *Br J Cancer* 2000; **82**: 186–94
- 15 Lynch TJ, Bell DW, Sordella R *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004; **350**: 2129–39
- 16 Kris MG, Natale RB, Herbst RS *et al.* Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003; **290**: 2149–58
- 17 Cappuzzo F, Gregorc V, Rossi E *et al.* Gefitinib in pretreated non-small-cell lung cancer (NSCLC): analysis of efficacy and correlation with HER2 and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. *J Clin Oncol* 2003; **21**: 2658–63
- 18 Barber TD, Vogelstein B, Kinzler KW, Velculescu VE. Somatic mutations of EGFR in colorectal cancers and glioblastomas. *N Engl J Med* 2004; **351**: 2883
- 19 Canil CM, Moore MJ, Winquist E *et al.* Randomized phase II study of two doses of gefitinib in hormone-refractory prostate cancer: a trial of the National Cancer Institute of Canada–Clinical Trials Group. *J Clin Oncol* 2005; **23**: 455–60
- 20 Kulp SK, Yang YT, Hung CC *et al.* 3-phosphoinositide-dependent protein kinase-1/Akt signaling represents a major cyclooxygenase-2-independent target for celecoxib in prostate cancer cells. *Cancer Res* 2004; **64**: 1444–51
- 21 Zha S, Gage WR, Sauvageot J *et al.* Cyclooxygenase-2 is up-regulated in proliferative inflammatory atrophy of the prostate, but not in prostate carcinoma. *Cancer Res* 2001; **61**: 8617–23
- 22 Habel LA, Zhao W, Stanford JL. Daily aspirin use and prostate cancer risk in a large, multiracial cohort in the US. *Cancer Causes Control* 2002; **13**: 427–34
- 23 Roberts RO, Jacobson DJ, Girman CJ, Rhodes T, Lieber MM, Jacobsen SJ. A population-based study of daily nonsteroidal anti-inflammatory drug use and prostate cancer. *Mayo Clin Proc* 2002; **77**: 219–25
- 24 Zhong BK, Hwang S, Cogswell J *et al.* Second line treatment of hormone resistant prostate cancer. A phase II trial of epirubicin, estramustine phosphate and celecoxib. *ASCO Annual Meeting, Florida* 2005: A4754
- 25 Kasimis BJ, Hwang S, Chang VT *et al.* High dose celecoxib and docetaxel in patients with hormone resistant prostate cancer. results of an ongoing phase II trial. In *ASCO Annual Meeting, Florida* 2005: A4704
- 26 Fitzgerald GA. Coxibs and cardiovascular disease. *N Engl J Med* 2004; **351**: 1709–11
- 27 Yang L, Dan HC, Sun M *et al.* Akt/protein kinase B signaling inhibitor-2, a selective small molecule inhibitor of Akt signaling with antitumor activity in cancer cells overexpressing Akt. *Cancer Res* 2004; **64**: 4394–9
- 28 Posadas EM, Gulley J, Arlen PM *et al.* A phase II study of perifosine in androgen independent prostate cancer. *Cancer Biol Ther* 2005; **4**: 1133–7
- 29 Neshat MS, Mellinghoff IK, Tran C *et al.* Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc Natl Acad Sci USA* 2001; **98**: 10314–9
- 30 Raymond E, Alexandre J, Faivre S *et al.* Safety and pharmacokinetics of escalated doses of weekly intravenous infusion of CCI-779, a novel mTOR inhibitor, in patients with cancer. *J Clin Oncol* 2004; **22**: 2336–47
- 31 Atkins MB, Hidalgo M, Stadler WM *et al.* Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J Clin Oncol* 2004; **22**: 909–18
- 32 Majumder PK, Febbo PG, Bikoff R *et al.* mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat Med* 2004; **10**: 594–601
- 33 Huang S, Houghton PJ. Inhibitors of mammalian target of rapamycin as novel antitumor agents: from bench to clinic. *Curr Opin Invest Drugs* 2002; **3**: 295–304

Correspondence: Niall Corcoran, Department of Urology, 5th Floor Clinical Sciences Building, University of Melbourne, Royal Melbourne Hospital, Parkville, 3050, VIC, Australia.
e-mail: niall.corcoran@mh.org.au

Abbreviations: HRPC, hormone-refractory prostate cancer; C_{ss} , steady state concentration; RTK, receptor tyrosine kinase; EGFR, epidermal growth factor receptor; NSCLC, nonsmall cell lung cancer; COX-2, cyclooxygenase-2; mTOR, mammalian target of rapamycin.