Studies with CWR22 Xenografts in Nude Mice Suggest That ZD1839 May Have a Role in the Treatment of Both Androgen-dependent and Androgen-independent Human Prostate Cancer

Francis M. Sirotnak, Yohung She, Fei Lee, Jing Chen, and Howard I. Scher
Program in Molecular Pharmacology and Therapeutics and Genitourinary Oncology Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT

Purpose: These studies examined the effect of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor ZD1839 (“Iressa”) on CWR22 prostate tumors in nude mice. The effect of ZD1839 was also examined in combination with either bicalutamide (“Casodex”) or cytotoxic agents against a hormone-dependent or -independent variant of CWR22, respectively.

Experimental Design: The xenografts were grown for 4–7 days, then tumor measurements were made and therapy initiated. ZD1839 and bicalutamide were given p.o. on a once-daily, 5-day schedule for 2 successive weeks. Carboplatin and paclitaxel were given every 3–4 days for a total of four doses. Measurements of tumor volume were made twice weekly during treatment and for 2 weeks after treatment. The effect of ZD1839 on EGFR function was assessed by Western blotting of EGFR and its phosphorylated form in CWR22 and variant tumors before and after treatment with this agent.

Results: ZD1839 at its maximum tolerated dose (150 mg/kg) inhibited the growth of androgen-dependent CWR22 by 54%, and the growth of two variants with different degrees of androgen independence and androgen receptor gene expression (CWR22LD1 and CWR22RV1) by 76%. The effects of ZD1839 were similar to those recorded for phosphorylation of EGFR as determined by Western blotting. Coadministration of ZD1839 with a suboptimal dose of bicalutamide was more effective than a higher dose of bicalutamide alone. Coadministration of ZD1839, which required a 2–3-fold attenuation of dose to avoid toxicity, also markedly increased the therapeutic activity of carboplatin and paclitaxel against CWR22RV1, bringing about regression to a degree not seen with either agent alone. Tumor-free mice were seen only with the combination of ZD1839 and paclitaxel.

Conclusions: The results obtained in these related and highly relevant models of human prostate cancer suggest that ZD1839 may have a role in enhancing existing treatments of androgen-dependent and -independent forms of this disease in patients.

INTRODUCTION

EGFR and HER-2/neu receptors, together with their ligands, represent important autocrine regulatory pathways in many tumors (1–4). The blockade of these receptors with antibodies has been shown to have marked antiproliferative actions against these model tumor systems in vitro (5, 6) and in vivo (7–9). Moreover, blockade of these autocrine pathways by the same monoclonal antibodies in combination with cytotoxic agents appears to represent an effective approach to the treatment of neoplasms in patients (10). More recent studies in human tumor xenografts in nude mice (11, 12) have shown that blockade of EGFR function by small-molecule inhibitors at the level of the associated tyrosine kinase could also have profound antiproliferative effects. Earlier studies from this laboratory (13) have shown that one of these inhibitors, ZD1839 (Iressa), markedly potentiates the efficacy against human tumor xenografts of a variety of cytotoxic agents, including antimetabolites, anthracyclines, taxanes, and platinum compounds. This therapeutic enhancement by ZD1839 did not require high levels of EGFR gene expression. For patients with advanced prostate cancer a major obstacle to improving survival is progression of the cancer to androgen-independence; hence, therapies that delay this progression would be beneficial.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by Grants CA0848 and CA56517 from the National Cancer Institute, the Pepsico Foundation, and AstraZeneca.

2 To whom requests for reprints should be addressed, at Laboratory of Molecular Therapeutics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-7952; Fax: (212) 794-3342; E-mail: sirotnaf@mskcc.org.

3 Iressa and Casodex are trademarks of the AstraZeneca group of companies.

Received 11/20/01; revised 8/15/02; accepted 8/27/02.

The abbreviations used are: EGFR, epidermal growth factor receptor; MTD, maximum tolerated dose; AR, androgen receptor; CyclD1, cyclin D1; PSMA, prostate-specific membrane antigen; COX-2, cyclooxygenase-2; 6FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

4 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 11/20/01; revised 8/15/02; accepted 8/27/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by Grants CA0848 and CA56517 from the National Cancer Institute, the Pepsico Foundation, and AstraZeneca.

2 To whom requests for reprints should be addressed, at Laboratory of Molecular Therapeutics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-7952; Fax: (212) 794-3342; E-mail: sirotnaf@mskcc.org.

3 Iressa and Casodex are trademarks of the AstraZeneca group of companies.

4 The abbreviations used are: EGFR, epidermal growth factor receptor; MTD, maximum tolerated dose; AR, androgen receptor; CyclD1, cyclin D1; PSMA, prostate-specific membrane antigen; COX-2, cyclooxygenase-2; 6FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.
MATERIALS AND METHODS

The CWR22 xenograft model of human androgen-dependent prostate cancer, obtained through the courtesy of Dr. Thomas Pretlow at Case Western Reserve University, Cleveland, OH, was used for many of the studies described below. In addition, two variants with different degrees of androgen dependence were used: CWR22LD1, which is less androgen-dependent than CWR22, was selected from CWR22 by transplantation in athymic male mice without testosterone supplementation. It grows well in these mice but will not grow in female or castrated male mice. The variant CWR22RV1 (also provided by Dr. Pretlow) is less dependent on androgen and is derived directly from CWR22. This variant will grow well in either female or castrated male mice. Many of the properties of CWR22 and CWR22RV1 have been described in detail in previous publications from the laboratory of Dr. Pretlow (14, 15). The athymic nude mice used for the transplantation of these tumors were NCR/nu athymic nudecs (Sprague Dawley).

Therapy Studies. After tumor growth in mice, a cell suspension in complete RPMI 1640 with 10% FCS was prepared by disrupting the excised tumor and straining the cell suspension through a fine metal grid. The tumor cells, in the form of a pellet after centrifugation at low speed (1000 × g), were mixed with a 50% volume of Matrigel (16) and implanted s.c. in the flank of the animal through a 22-gauge needle. After 4–7 days, tumor measurements were made and therapy initiated. ZD1839 and bicalutamide (Casodex) were given p.o. on a once daily × 5-day schedule for 2 successive weeks. Carboplatin and paclitaxel were given every 3–4 days for a total of four doses. Measurements of tumor volume were made twice weekly during treatment and for 2 weeks after treatment. The results are expressed as mean tumor diameter and increase in tumor volume or, in the case of tumor regression, as decrease in average diameter and tumor volume at the nadir. The data generally represent three to five experiments of 3–4 mice per group, with mice distributed randomly between control and treated groups. The control group was treated with diluent alone. Statistical analysis was carried out using Student’s t test. These studies were carried out in accordance with Principles of Laboratory Animal Care under a protocol approved by the Memorial Sloan-Kettering Cancer Center Animal Care Committee.

Quantitative Reverse Transcription-PCR Analysis of Gene Expression. Tumor samples were harvested from mice and total RNA prepared with TRIzol reagent (Life Technologies, Inc.) from CWR22 and its variants. First-strand cDNA was prepared using the SUPERSCRIPT system (Life Technologies, Inc.). The quantitation of EGFR, AR, CyclinD1, HER-2/neu, PSMA, and COX-2 gene expression was carried out with the aid of an ABI Prism 7700 Sequence Detection System (TAQMAN; PE Systems, Foster City, CA). The methodology has been described previously in detail (17, 18). The specific cDNAs of interest and the β-actin reference cDNA were amplified separately with the TAQMAN, using an oligonucleotide probe with a 5’fluorescent reporter dye (6FAM) and a 3’quencher dye.
(TAMRA). The primer and probe sets were designed using primer express software (Applied Biosystems). The sequences were as follows: (a) EGFR, forward primer: 5'/H11032 GCGTCTCTTGCCGGAATGT 3'/H11032; reverse primer: 5'/H11032 CTTCGGCTCACCCTCCAGAAG 3'/H11032; and probe: 6FAM-TGCACT-TGTCCACGCATTCCCTG-TAMRA; (b) AR, forward primer: 5'/H11032 TGTGGAAGCTGCAAGGTCTTC 3'/H11032; reverse primer: 5'/H11032 CGGAATTTATCAATAGTGCAATCATT 3'/H11032; and probe: 6FAM-CTTCTGTTTCCCTTCAGCGGCTCTTTTG-TAMRA; (c) CyclD1, forward primer: 5'/H11032 CGTCCATGCGGAAGATCGT 3'/H11032; reverse primer: 5'/H11032 CATGGCCAGCGGGAAGA 3'/H11032; and probe: 6FAM-TCTGTTCCTCGCAGACCTCCAGCA-TAMRA; (d) HER-2/neu, forward primer: 5'/H11032 AAGCCTCACAGAGATCTTGAAAGG 3'/H11032; reverse primer: 5'/H11032 TCCACAAAATCGTGTCCTGGTA 3'/H11032; and probe: 6FAM-TTGATCCAGCGGAACCCCCAGC-TAMRA; (e) PSMA, forward primer: 5'/H11032 CCAGCGTGG-AAATATCCTAAATCT 3'/H11032; reverse primer: 5'/H11032 GCATATTCATT-TGCTGGGTAACC 3'/H11032; and probe: 6FAM-AATGGTGCAG-GAGACCCTCTCACACC-TAMRA; and (f) COX-2, forward primer: 5'/H11032 GAATCATTCACCAGGCAAATTG 3'/H11032; reverse primer: 5'/H11032 CTTTCTGTACTGCGGGTGGAA 3'/H11032; and probe: 6FAM-TTCCTACCACCAGCAACCCTGCCA-TAMRA.

Relative quantitation was done using the comparative C_t (threshold cycle) method. The C_t is the fractional cycle number at which the amplified target reaches a fixed threshold. The amount of target gene normalized to an endogenous reference (/H9252-actin) is given by \(2^{-\text{Ct}(\text{target gene}) - \text{Ct}(\text{reference gene})}\) where \(\text{Ct}(\text{target gene})\) is C_t. However, this relationship is based on the assumption that the efficiency of target and reference amplification is approximately equal. A validation experiment compared serial dilutions of target genes and /H9252-actin. The absolute value of slope of log input amount versus \(\text{Ct}\) should be ~0.1, which would indicate that the efficiencies of target and reference amplifications are equal. In our experiments it varied between 0.021 and 0.051. For comparison, we also calculated the efficiencies of different runs. Different dilutions of a control cDNA were run in triplicate amplifying /H9252-actin to generate a standard curve. The slope of the curve was used to calculate the run efficiency using the relationship \(E = (10^{-\text{S}} - 1)\), where E is the efficiency and S is the slope. In our hands the efficiencies varied from 0.966 to 0.986 (correlation coefficient >0.998).

**Western Blot Analysis.** These were performed by a standard procedure (19) using aliquots of cell lysates (60 μg), which were separated on 10% SDS polyacrylamide gels and transferred on to nitrocellulose filters. Protein concentration was

| Table 2 Relative gene expression at the level of mRNA in CWR22 and its variants |
|---------------------------|---------------------------|---------------------------|---------------------------|
| Gene          | Tumor, mean (SD) | P     |   |
| (a) CWR22  | (b) CWR22LD1 | (c) CWR22RV1 | I     | II    | III   |
| AR        | 4.77 (1.19) | 6.52 (1.32) | 74.74 (16) | 0.0092 | <0.0001 | <0.0001 |
| CyclD1   | 2.43 (1.50) | 2.69 (2.03) | 267.42 (91.55) | 0.7780 | <0.0001 | <0.0001 |
| EGFR     | 10.52 (3.65) | 7.88 (2.64) | 102.09 (28.24) | 0.0974 | <0.0001 | <0.0001 |
| HER-2    | 7.01 (3.54) | 8.29 (5.39) | 8.81 (3.64) | 0.5694 | 0.3037 | 0.8193 |
| PSMA     | 104.17 (29.94) | 101.3 (25.44) | 77.10 (5.16) | 0.8349 | 0.0268 | 0.0313 |
| COX-2    | 0.23 (0.12) | 0.15 (0.035) | 6.13 (4.12) | 0.1039 | 0.0237 | 0.0228 |

* I = a vs. b; II = a vs. c; III = b vs. c.
Table 3  The antitumor effect of ZD1839 and bicalutamide given alone or in combination against CWR22LD1 in NCR/nu mice

Measurements were made 1 day after treatment or, in the case of regression, recorded at the nadir after treatment. Mice were treated daily for 5 days in 2 successive weeks. Four experiments of 3–4 mice per group.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose, mg/kg</th>
<th>Body weight change, %</th>
<th>Mean (SE) tumor diameter, mm</th>
<th>Change in tumor volume, mm³</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High tumor burden</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>+1</td>
<td>9.3 (1.5)</td>
<td>+368</td>
<td>4</td>
</tr>
<tr>
<td>ZD1839</td>
<td>150</td>
<td>–4</td>
<td>7.2 (1.3)</td>
<td>+141</td>
<td>4</td>
</tr>
<tr>
<td>Bicalutamide</td>
<td>25</td>
<td>+1</td>
<td>8.1 (1.0)</td>
<td>+225</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>–1</td>
<td>7.8 (1.4)</td>
<td>+195</td>
<td>4</td>
</tr>
<tr>
<td>ZD1839 + bicalutamide</td>
<td>150 + 25</td>
<td>0</td>
<td>5.8 (1.1)</td>
<td>+39</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>150 + 50</td>
<td>–1</td>
<td>5.5 (0.8)</td>
<td>+24</td>
<td>4</td>
</tr>
<tr>
<td><strong>Low tumor burden</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>+2</td>
<td>6.2 (1.3)</td>
<td>+121</td>
<td>9</td>
</tr>
<tr>
<td>ZD1839</td>
<td>150</td>
<td>–3</td>
<td>4.3 (0.5)</td>
<td>+29</td>
<td>9</td>
</tr>
<tr>
<td>Bicalutamide</td>
<td>50</td>
<td>+1</td>
<td>3.9 (0.4)</td>
<td>+19</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
<td>4.0 (0.6)</td>
<td>+20</td>
<td>6</td>
</tr>
<tr>
<td>ZD1839 + bicalutamide</td>
<td>150 + 50</td>
<td>–3</td>
<td>2.2 (0.5)</td>
<td>−12 (−71%)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>150 + 100</td>
<td>–2</td>
<td>2.3 (0.6)</td>
<td>−10 (−59%)</td>
<td>6</td>
</tr>
</tbody>
</table>

*Initial tumor diameter was 5.1 (1.3) mm (65 mm³).

*Initial tumor diameter was 3.2 (0.6) mm (17 mm³).

Results

Testosterone Requirements for Growth of CWR22 and Its Variants. Male NCR/nu mice have testosterone levels in blood of 13 ± 3 ng/dl (Table 1), insufficient to support growth of parental CWR22. For growth of this tumor, testosterone levels were brought to 1330 ± 200 ng/dl by testosterone supplementation using a slow-release pellet implanted s.c. in the mice. CWR22LD1 will grow in male mice without testosterone supplementation but will not grow in female or castrated male mice, which have testosterone blood levels of <2 ng/ml (Table 1). CWR22RV1 will grow in female, male, and castrated male mice. CWR22LD1 and CWR22RV1 were derived by selection after transplantation of a large tumor burden into male or castrated male mice, respectively. A period of several months was required for these variants to emerge and grow well under these conditions.

Levels of Expression of EGFR, AR, and Some Other Proliferation-related Genes in CWR22 and Its Variants. Using real-time quantitative reverse transcription-PCR we characterized CWR22 and its variant according to the expression of EGFR and AR genes, and several other genes known to alter with change in proliferation potential in human tumor cells. In addition, expression of AR and EGFR in CWR22 and its variants was also determined at the level of encoded proteins by Western blotting (Fig. 1). In the former case, this was carried out to verify that the modest but significant increase in the level of expression of AR in CWR22LD1 compared with CWR22 actually occurred at the level of the protein. In the latter case, we showed that expression of EGFR at the protein level (Fig. 1) was consistent with the large difference in expression of this gene in CWR22RV1 compared with both CWR22 and CWR22LD1. The additional data in Fig. 1 documents the effect of ZD1839 on the level of phosphorylated EGFR obtained from CWR22, CWR22LD1, and CWR22RV1 tumors in treated mice. In each case, a decrease in p-EGFR was observed, with the relative level of p-EGFR in treated and control mice being similar for each tumor. We found that levels of EGFR gene expression were increased 10–13-fold in CWR22RV1 compared with either CWR22 or CWR22LD1 (Table 2). Levels of AR gene expression in CWR22LD1 and CWR22RV1 were 1.4- and 16-fold higher, respectively, than in CWR22. In contrast, levels of HER-2 and PSMA gene expression were similar in all three of the tumor types, whereas the level of CycD1 and COX-2 gene expression in CWR22RV1 was 100–110-fold and 27–40-fold higher, respectively, than in the other two tumors.

Antiproliferative Effects of ZD1839 against CWR22 and Its Variants. Three to 4 days after implantation of CWR22 and its variants, mice were treated with ZD1839 at its MTD. Our prior studies showed that 150 mg/kg was the MTD on the schedule that was used here (once daily on 5 days for 2 consecutive weeks; Ref. 13). At this dose no lethal
toxicity was observed, and the average weight loss of treated animals was only 2–3%. With each tumor, appreciable suppression of tumor growth was observed during week 2 of treatment (Fig. 2); at the end of treatment this was 54% against CWR22, and 76% against CWR22LD1 and CWR22RV1 (p < 0.05). This same increase in growth inhibition of CWR22LD1 and CWR22RV1 was observed although the level of expression of the EGFR gene was 10–12-fold higher in CWR22RV1 than in CWR22LD1 (Table 2).

Studies of ZD1839 and Bicalutamide, Alone and Combined, against CWR22LD1. The testosterone supplementation available for use in mice to grow CWR22 raises the level of testosterone in blood 2–3-fold higher (1330 ± 200 ng/dl) than that found (400–800 ng/dl) in human blood (Table 1). For this reason it did not appear feasible to attempt to demonstrate an effect of bicalutamide against this tumor in mice. In fact, we found (data not shown) that bicalutamide given p.o. at doses up to 100 mg/kg had no effect on the growth of the CWR22 tumors in mice. This is four times the dose given to patients on a per m² basis (25 mg/m²). Instead, we used CWR22LD1 in un-supplemented male mice for these studies. Fig. 3 shows that the androgen dependence of this variant in male mice was sufficient to demonstrate an antiproliferative effect of bicalutamide using the same schedule of administration as that used for ZD1839 (p.o., once daily for 5 for 2 successive weeks). In this case a maximum antiproliferative effect (80–90% inhibition) was obtained in the range of 50–100 mg/kg bicalutamide. Interestingly, treatment of mice with a higher dose of bicalutamide resulted in less suppression of growth of this tumor than that observed at 50–100 mg/kg. The data in Table 3 and Fig. 4, A and B, show that ZD1839 could be given at its single-agent MTD with bicalutamide at 50–100 mg/kg without any increase in toxicity. Also, coadministration of ZD1839 and bicalutamide was substantially more antiproliferative against CWR22LD1 than either ZD1839 or bicalutamide alone. In the experiment with the higher tumor burden (Table 3, Exp. 1; Fig. 4A) and a dose of bicalutamide in the range of 25–50 mg/kg, which was only modestly antiproliferative alone, ZD1839 brought about a 6-fold enhancement (p < 0.05) in activity. Against a lower tumor burden (Table 3, Exp. 2; Fig. 4B), in which bicalutamide by itself was more antiproliferative, bicalutamide given with ZD1839 resulted in 45–70% regression (p < 0.001). Interestingly, the same dose of ZD1839 with only 50 mg/kg bicalutamide was more efficacious than bicalutamide alone at 100 mg/kg. Thus, it would seem that ZD1839 has a substantial sparing effect when combined with bicalutamide.

Studies of ZD1839 with Cytotoxic Therapy against CWR22RV1. We also evaluated the combined effects of ZD1839 with carboplatin or paclitaxel against the androgen-independent variant, CWR22RV1. Both cytotoxics are active agents in the treatment of this form of human prostate cancer in patients. Although we have data from similar studies with other prostate tumors (PC-3 and TSU-PR1), the androgen-independent CWR22RV1 is of much greater relevance to the androgen-independent form of this neoplasm in patients (14, 15). These earlier studies (13) showed that a 2–3-fold attenuation of ZD1839 dosage when given with cytotoxic therapy was necessary to avoid excessive toxicity in mice. Therefore, in the present experiments we treated tumor-bearing mice with 65 mg/kg of ZD1839 p.o. on a once-daily schedule on 5 days for 2 consecutive weeks along with either carboplatin (50 mg/kg) or paclitaxel (25 mg/kg) given i.p. twice weekly for a total of four doses. The results in each case were compared with those obtained with the MTD of ZD1839, carboplatin, or paclitaxel, given alone on their respective schedules (Table 4; Fig. 5, A and B). These results show that in the case of each cytotoxic agent there was substantial enhancement of therapeutic activity when given in combination with ZD1839. With carboplatin the combined effect of both agents resulted in some regression of the tumor (Table 4; Fig. 5A), whereas, alone, carboplatin (p < 0.01) and ZD1839 (p < 0.01) were only modestly growth inhibitory at their respective MTDs. With paclitaxel, although a much larger tumor burden was allowed to develop before initiation of treatment, the combined effect of both agents resulted in marked regression of tumor (Table 4; Fig. 5B), with tumor-free mice obtained shortly after cessation of treatment. In contrast, paclitaxel alone was only growth inhibitory, albeit to a substantial
extent (paclitaxel versus ZD1839 + paclitaxel; \( P < 0.01 \)). Although the data as presented do not allow discrimination between additive and synergistic effects of either pair of combined agents, the results clearly suggest that these agents in combination interact very favorably to increase efficacy to a level not obtainable by each agent alone.

**DISCUSSION**

Differences in hormonal dependence for growth is a major characteristic of early and advanced stages of prostate cancer in patients. In the studies described here we sought to ascertain the role of the EGFR-tyrosine kinase inhibitor ZD1839 in the treatment of human androgen-dependent and -independent prostate cancer using highly relevant *in vivo* models. Our results suggest that ZD1839 may have such a role in each tumor type both as a single agent and in combination with existing therapies. The CWR22 tumor used in these studies represents the first human tumor model that both is strongly androgen dependent and could be reproducibly grown as a xenograft in nude mice with the aid of androgen supplementation (15). We also used one of the androgen-independent variants derived from CWR22 for studies of ZD1839 in combination with the cytotoxic agents used currently to treat this form of the disease in patients. Against this tumor, ZD1839 could be coadministered at a tolerated dose that markedly enhanced the therapeutic activity of carboplatin and paclitaxel. These results expand on our earlier results obtained with TSU-PR1 and PC-3 human prostate tumors (13). CWR22 has greater relevance (15) to human prostate cancer in patients, having different but more clinically relevant AR status from the other two tumors. For studies with ZD1839 and bicalutamide, it was necessary to derive a variant of CWR22 (CWR22LD1) that was somewhat less androgen-dependent than CWR22 and able to grow in nude mice without androgen supplementation. There are no genetic differences between the AR gene of the CWR22LD1 variant and CWR22 tumor cells,5 and the level of

---

5 F. M. Sirotnak, unpublished observations.

**Table 4** The antitumor effects of ZD1839 and carboplatin, or ZD1839 and paclitaxel, when given alone or in combination against CWR22RV1 in mice

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose, mg/kg</th>
<th>% Body weight change</th>
<th>Mean (SE) tumor diameter, mm</th>
<th>Change in tumor volume, mm(^3)</th>
<th>CR(^a), n/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboplatin(b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>+2</td>
<td>9.3 (1.2)</td>
<td>+380</td>
<td>0/12</td>
</tr>
<tr>
<td>ZD1839</td>
<td>150</td>
<td>–2</td>
<td>6.3 (1.0)</td>
<td>+85</td>
<td>0/9</td>
</tr>
<tr>
<td>Carboplatin + carboplatin</td>
<td>50</td>
<td>–3</td>
<td>6.1 (1.0)</td>
<td>+74</td>
<td>0/9</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td></td>
<td>–3</td>
<td>3.8 (0.7)</td>
<td>–13 (–29%)</td>
<td>0/9</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>+1</td>
<td>14.2 (1.0)</td>
<td>+1388</td>
<td>0/9</td>
</tr>
<tr>
<td>ZD1839</td>
<td>150</td>
<td>–2</td>
<td>12.3 (2.0)</td>
<td>+936</td>
<td>0/9</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>25</td>
<td>–3</td>
<td>7.0 (2.0)</td>
<td>+87</td>
<td>0/9</td>
</tr>
<tr>
<td>ZD1839 + paclitaxel</td>
<td>65 + 25</td>
<td>–3</td>
<td>2.9 (1.0)</td>
<td>–82 (–86%)</td>
<td>2/9</td>
</tr>
</tbody>
</table>

\(a\) CR, complete regression.

\(b\) Initial tumor diameter was 4.4 (0.6) mm (45 mm\(^3\)).

\(c\) Initial tumor diameter was 5.6 mm (0.6) mm (95 mm\(^3\)).

---

**Fig. 5** The antitumor effects of carboplatin (A) and paclitaxel (B) against CWR22RV1 in NCR/nu mice with and without coadministration of ZD1839. The mice were treated with 150 mg/kg ZD1839, 50 mg/kg carboplatin, 25 mg/kg paclitaxel, or 65 mg/kg ZD1839 plus 50 mg/kg carboplatin or 25 mg/kg paclitaxel combined. Additional details are given in the text. SE for four experiments ±28%.
expression of this gene was only slightly higher in this variant than in CWR22. Moreover, despite the decreased androgen dependence, the growth of this tumor was significantly suppressed after treatment with this antiandrogen. Coadministered ZD1839 substantially enhanced the therapeutic activity of bicalutamide and also allowed a substantial dose reduction of this antiandrogen that in combination was more efficacious than a higher dose of bicalutamide alone.

Equally important, the combined use of ZD1839 with bicalutamide in patients might significantly forestall the development of hormonal independence as has been demonstrated recently in breast cancer models (20, 21). It should also be pointed out that the degree of antiproliferative action of bicalutamide in these experiments against this experimental tumor model depended on the tumor burden that existed when treatment was initiated. Mice with higher tumor burdens were less responsive overall. The tumor burden in these studies is probably not representative of the clinical situation, because it is most likely beyond the micrometastatic disease now usually targeted in patients by antiandrogen therapy.

The CWR22LD1 tumor derived in these studies, which exhibited less androgen dependence than CWR22, had a molecular profile somewhat different from either CWR22 or the androgen-independent CWR22RV1. Expression of the AR, CyclD1, EGFR, and COX-2 gene was similar to CWR22, and expression of the AR and COX-2 was greater than in CWR22RV1. Thus, expression of EGFR, CyclD1, and COX-2 remained unchanged during early onset of androgen independence, whereas expression of AR was somewhat increased. In contrast, HER-2/neu and PSMA expression did not change with each stage toward androgen independence of this tumor. The biological significance of these alterations has yet to be determined but is of interest because CWR22LD1 could be considered a model for an early, intermediate stage in the progression toward androgen independence. It was also of interest to note in this context that the antiproliferative action of ZD1839 against these tumors was greater in the case of tumors with some degree of androgen independence but did not appear to correlate with the relative level of EGFR expression. Additional work will be required before the significance of this finding is known.

REFERENCES

Prostate Cancer
Androgen-dependent and Androgen-independent Human Prostate Cancer

Francis M. Sirotnak, Yohung She, Fei Lee, et al.

*Clin Cancer Res* 2002;8:3870-3876.

**Updated version**
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/8/12/3870

**Cited articles**
This article cites 20 articles, 10 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/8/12/3870.full.html#ref-list-1

**Citing articles**
This article has been cited by 18 HighWire-hosted articles. Access the articles at:
/content/8/12/3870.full.html#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.