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Instructions for Authors

The Instructions for Authors section is in the July 1, 1994, issue. Please refer to the Instructions for Authors for preparation of manuscripts to submit to Cancer.
New Chemotherapeutic Agents for Breast Cancer

Jeffrey S. Abrams, M.D.,* Timothy D. Moore, M.D.,† and Michael Friedman, M.D.‡

The drug discovery programs of the National Cancer Institute and the pharmaceutical industry recently have provided oncologists with a wide array of new chemotherapeutic agents that have considerable potential for breast cancer treatment. Foremost among these new agents are the taxanes, of which paclitaxel and docetaxel, the only members of this class currently in clinical use, have been associated with impressive response rates in patients with metastatic disease. Importantly, they display some evidence clinically of not being cross-resistant with the anthracyclines. Efforts now are being directed toward optimizing dose and schedule in the metastatic setting while integrating these agents into standard adjuvant regimens.

Other agents that have undergone Phase II testing in breast cancer include vinorelbine, edatrexate, and losoxarntrone. It remains to be determined, however, whether these drugs possess substantial advantages over other members of their class. Newer compounds, such as pyrazolocaricine, ICI D169, topoisomerase-I inhibitors, temozolomide, pandomidine, fumagillin (TNP-470), and differentiators like the retinoids, hold substantial promise because of their unique mechanisms of action; however, Phase II testing of these agents is just beginning. Although alternative approaches to treatment, such as gene therapies, monoclonal antibodies, and growth factor inhibitors, are likely to have a positive impact, it is probable that progress will best be made by combining these strategies with chemotherapy. Therefore, continuation of the search for more effective chemotherapeutic agents should remain a high priority.

Key words: chemotherapy, taxol, taxotere, vinorelbine, edatrexate.

A review of the recently updated international meta-analysis of adjuvant therapy for breast cancer indicates that chemotherapy has the potential to save more lives from this disease than from any other malignancy. These pooled results, along with similar findings from National Cancer Institute (NCI)-sponsored cooperative group trials, verify that cytotoxic therapy developed first in the metastatic setting provides a significant survival advantage when applied to earlier stages of disease. Nonetheless, systemic treatment of breast cancer is far from optimal. Cytotoxic or hormonal therapy for metastatic disease is rarely curative, whereas a substantial number of women continue to suffer recurrences and die, despite receiving adjuvant therapy. Clearly, there remains a pressing need to identify active new compounds to supplement those currently in use.

In response to this need, the Division of Cancer Treatment of the NCI and the pharmaceutical industry are presenting oncologists with a plethora of new chemotherapeutic agents, many of which possess unique mechanisms of action. Several of these drugs already have demonstrated significant breast cancer activity, while others show potential in laboratory models. This summary reviews some of the promising new agents for breast cancer patients, including discussions of their mechanisms of action and pharmacokinetics, as well as the initial results of clinical trials of these agents. It concludes with a discussion of strategies that have been adopted to expedite their clinical development.

Paclitaxel

The taxanes are one of the most exciting new classes of chemotherapeutic agents to be developed. The prototype of this novel class, paclitaxel (Taxol, Bristol-Myers Squibb, Princeton, NJ), was approved by the Food and Drug Administration for treatment of refractory ovarian cancer patients in December 1992, but its potential indications are expanding rapidly. Previous reviews documented the difficult formulation of paclitaxel and discuss the problems that complicated its 30-year development. Fortunately, a collaborative effort on the
part of the NCI and the Bristol-Myers Squibb Company has managed to overcome most problems.

Paclitaxel exhibits broad but modest activity in murine and human tumor xenograft models (National Cancer Institute, clinical brochure on Taxol [IND22856, NSC125973], 1989). Interest in this compound was heightened when its unique mechanism of action was discovered by Horwitz and colleagues. These investigators noted that paclitaxel stabilized the microtubules and enhanced their assembly, in contrast to the previously available spindle poisons (colchicine and vincristine) which disrupt such assembly. Microtubules are active in cellular motility and division, intracellular transport, and secretion of protein products. In addition, their stabilization impairs the essential equilibrium between assembly and disassembly required for many dynamic cellular processes. In tumor cells, the histologic correlate of taxane cytotoxicity is represented by the appearance of abnormal microtubule bundles, which accumulate during both interphase and mitosis.

Phase I trials with paclitaxel began in 1984, and numerous studies have been published.1-4 The development of paclitaxel initially was hindered by the occurrence of hypersensitivity reactions (HSRs).4,5 A prolongation of the duration of administration to 24 hours combined with the institution of a premedication regimen consisting of hydrocortisone, antihistamines, and H2 blockers greatly reduced the incidence of HSRs to approximately 2% (Bristol-Myers Squibb Co., unpublished data, 1992). It remains unclear whether the phenomenon is mediated primarily by paclitaxel itself or the cremophor vehicle (polyoxyethylated castor oil).

With effective prophylaxis of the HSRs, neutropenia became the dose-limiting toxicity (DLT). Fortunately, paclitaxel is relatively platelet sparing. Attempts to overcome neutropenia have led to the use of human granulocyte colony stimulating factor (G-CSF). Inclusion of G-CSF has permitted paclitaxel dose escalations up to 250 mg/m².14 Above this level, a dose-limiting peripheral neuropathy supervenes.

Acute toxicities associated with paclitaxel and their relative frequencies are shown in Table 1 (Bristol-Myers Squibb Co., unpublished data, 1992). Because of the HSRs, cardiac monitoring was mandatory in the early trials with this drug. Asymptomatic bradycardia was the most frequent finding, but symptomatic supraventricular and ventricular tachycardias were seen occasionally. Analysis of this data suggest that cardiac monitoring is unnecessary in people without a history of cardiac ischemia. However, NCI-sponsored trials have excluded patients with a history of ischemia or conduction disturbance, so treatment recommendations for this cohort remain uncertain. The Eastern Cooperative Oncology Group currently is conducting a trial specifically designed to test paclitaxel in such patients that should provide treatment guidelines in the future.

Currently, the optimal duration of taxol administration is being investigated (Table 2).15-17 To administer paclitaxel in an outpatient setting, a 3-hour infusion is used. Conversely, other investigators now are prolonging the infusion schedule to 72 hours,16 96 hours,17

| Table 1. Incidence of Paclitaxel Toxicity in Single-Agent Studies14 |
|-----------|------------------|
| Study     | Incidence (%)    |
| Bone marrow |                  |
| Neutropenia <2,000/mm³  | 92            |
| <500/mm³       | 67            |
| Leukopenia <4,000/mm³  | 93            |
| <1,000/mm³     | 26            |
| Thrombocytopenia <100,000/mm³ | 27 |
| <50,000/mm³   | 10            |
| Anemia <11 g/dl     | 90            |
| <8 g/dl        | 24            |
| Infusions      | 35            |
| Bleeding       | 19            |
| Packed cell transfusions | 34 |
| Platelet transfusions | 3  |
| Hypersensitivity reactions* |        |
| Any            | 41            |
| Severe         | 2             |
| Cardiovascular |               |
| Bradycardia during infusion | 10 |
| Hypotension during infusion | 23 |
| Severe cardiovascular events | 1  |
| Abnormal EKG   |               |
| All patients (n = 402) | 30 |
| Patients with normal baseline (n = 236) | 19 |
| Peripheral neuropathy |           |
| Any symptoms   | 62            |
| Severe symptoms | 4             |
| Myalgia/arthritis |           |
| Any symptoms   | 55            |
| Severe symptoms | 4             |
| Gastrointestinal |               |
| Nausea and vomiting | 59 |
| Diarrhea       | 43            |
| Mucositis      | 39            |
| Alopecia       | 82            |
| Hepatic patients with normal baseline and toxicity data |          |
| Bilirubin elevations (n = 270) | 8 |
| Alkaline phosphatase elevations (n = 293) | 23 |
| AST (SGOT) elevations (n = 287) | 16 |

* All patients received premedication regimen.
and, most recently, 14 days (J. O'Shaughnessey, M.D., personal communication, August 1993).20 This latter approach may allow paclitaxel to effect more of the slowly cycling cells commonly found in solid tumors. Furthermore, prolonged drug exposure has been shown in vitro to be capable of overcoming paclitaxel resistance.19

Incomplete information is available on mechanisms of paclitaxel clearance from the body. It is known that greater than 95% of the circulating drug is extensively metabolized by the liver. In fact, Wilson et al.21 have shown that patients with coexisting liver dysfunction have a significantly reduced total body clearance of paclitaxel and suffer severe toxicity requiring dose reduction.

Recent pharmacokinetic studies of paclitaxel demonstrated that drug clearance decreases with increasing doses (from 135 mg/m² to 300 mg/m²).32,33 This suggests a saturable elimination process, which implies that paclitaxel's pharmacokinetics are nonlinear. If this is the case, a substantial amount of variation in drug exposure (i.e., area under the curve) might exist for different doses or infusion schedules.

Single-agent Phase II trials of paclitaxel in breast cancer patients (Table 3)41,17-25 have indicated substantial activity in patients with breast cancer. Generally, the response rates are lower in those who have received extensive prior treatment. These responses have been reasonably durable, even though few have been complete. Most importantly, patients clearly have responded to paclitaxel after progression on doxorubicin. This lack of clinical cross-resistance should be exploited in designing regimens that combine both of these active agents.

Investigators at the NCI8 found that a 60-mg/m² dose of doxorubicin and an 180-mg/m² dose of paclitaxel given concurrently over 72 hours in combination with G-CSF every 3 weeks resulted in unusual toxicity. Grade 3-4 diarrhea and abdominal pain, thought to be secondary to typhlitis (thickening of the cecum on computed tomography), occurred much below the single-agent maximum tolerable dose for paclitaxel in this trial. The overall response rate was 72% (28 of 39 patients) in these doxorubicin-naïve patients, but only 10% (4 of 39 patients) had complete responses. Pharmacokinetic studies indicated that altered drug clearance does not explain the enhanced toxicity observed with this combination.

Investigators at the University of Texas M. D. Anderson Cancer Center27 encountered severe stomatitis when the administration of paclitaxel (125 mg/m² over 24 hours of continuous infusion on day 1) preceded that of doxorubicin (48 mg/m² over 48 hours of continuous infusion on days 2-5) with G-CSF (5 mg/kg/day on days 5-19). The opposite sequence currently is being studied and has allowed the escalation of the doxorubicin dose (60 mg/m²) as well as the paclitaxel dose (180 mg/m²).

A Phase III test of this combination recently has been started by the Eastern Cooperative Oncology Group. This study, in women with untreated metastatic breast cancer, is comparing single-agent doxorubicin therapy (60 mg/m² every 3 weeks for 8 cycles) versus treatment with paclitaxel (175 mg/m² every 3 weeks for

### Table 2. Suggested Single-Agent Taxol Dose (mg/m²)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose and schedule</th>
<th>No. of evaluable patients</th>
<th>Duration of response (range) (mo)</th>
<th>No. of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI</td>
<td>140 mg/m², over 96 hr infusion</td>
<td>22</td>
<td>24</td>
<td>3 (12)</td>
</tr>
<tr>
<td>BMS</td>
<td>136 mg/m², over 3 hr vs. 175 mg/m², over 3 hr</td>
<td>111</td>
<td>0-38</td>
<td>1 (5)</td>
</tr>
<tr>
<td>MDA</td>
<td>150 mg/m², over 3 hr</td>
<td>12</td>
<td>0-41</td>
<td>2 (1)</td>
</tr>
<tr>
<td>MSKCC</td>
<td>250 mg/m² + G-CSF</td>
<td>25</td>
<td>0-41</td>
<td>3 (12)</td>
</tr>
<tr>
<td>NCI</td>
<td>140 mg/m², over 96 hr infusion</td>
<td>22</td>
<td>24</td>
<td>1 (5)</td>
</tr>
<tr>
<td>BMS</td>
<td>136 mg/m², over 3 hr vs. 175 mg/m², over 3 hr</td>
<td>111</td>
<td>0-38</td>
<td>4 (1-8)</td>
</tr>
<tr>
<td>MDA</td>
<td>150 mg/m², over 3 hr</td>
<td>12</td>
<td>0-41</td>
<td>3 (12)</td>
</tr>
<tr>
<td>MSKCC</td>
<td>250 mg/m² + G-CSF</td>
<td>25</td>
<td>0-41</td>
<td>3 (12)</td>
</tr>
</tbody>
</table>

G-CSF: granulocyte-colony stimulating factor; CR: complete response; PR: partial response; NA: not available; Adj: adjacent; M: metastatic; MDA: M. D. Anderson; MSKCC: Memorial Sloan-Kettering Cancer Center; NCI: National Cancer Institute; BMS: Bristol-Myers Squibb.

* Represents overall response rate on both arms.
New Chemotherapeutic Agents for Breast Cancer/Abrams et al.

8 cycles) versus the combination of doxorubicin (50 mg/m² intravenous [IV] bolus) followed by paclitaxel (150 mg/m² over 24 hours) plus G-CSF for 8 weeks. Patients in the single-agent arms can be crossed-over to the alternate arm at progression of their disease.

Docetaxel

The effectiveness of paclitaxel has stimulated the search for other taxanes that might be easier to produce, less toxic, or more effective. Docetaxel (Taxotere, Rhône-Poulenc Rorer Pharmaceuticals, Inc., Collegeville, PA) is the first such congener to be tested in clinical trials. It is a semisynthetic molecule made in part from the needlesh of the European yew tree and then modified chemically with a synthetic side chain. Docetaxel promotes microtubule assembly in a fashion similar to that of paclitaxel, but in contrast to paclitaxel, it is more potent, resulting in lower maximum tolerated doses in Phase I trials. Pharmacologically, docetaxel is cleared from the body primarily via the biliary route, mirroring the clearance of paclitaxel. It is more water soluble than paclitaxel, however, permitting its administration in polysorbate-80 rather than cremophor. Nonetheless, HSRS have been noted. Although these are usually mild, some reactions seen with paclitaxel, premedication is recommended.

Docetaxel's toxicity profile is similar to that of paclitaxel, yet there appear to be some important differences. Neutropenia is once again the dose-limiting toxicity. Peripheral neuropathy, mucositis, alopecia, and mild nausea and vomiting likewise have been noted. Skin toxicity, however, characterized by localized or diffuse erythema with skin thickening and occasionally severe desquamation, has been found to be more common and more severe with docetaxel than with paclitaxel. Skin biopsies have revealed a toxic dermatitis. Another troubling toxicity that has occurred in multiple trials is the development of a syndrome of fluid retention, which appears to be related to cumulative dose and often requires cessation of treatment after five to six cycles. Peripheral edema, pleural effusions, and/or weight gain occur in 80% of patients. It seems that capillary leakage is responsible for this syndrome, because cardiac and renal function remain undisturbed.

Future studies will try to overcome this side effect by premedicating with corticosteroids or other membrane stabilizers.

Preliminary results from five Phase II trials in breast cancer are shown in Table 4. Although fewer patients have been treated in these studies, an overall response rate of 54–76% has been noted. Particularly significant is the high response rate seen in metastatic visceral sites, especially the liver. Thus, docetaxel possesses significant activity in breast cancer, and Phase II trials of docetaxel in combination with other active agents are being developed.

Vinorelbine

Two other microtubule interactive agents, vincristine and vinblastine, have been used in breast cancer regimens for many years. In contrast to the taxanes, these agents inhibit microtubule formation. Vinorelbine (Navelbine, Burroughs-Wellcome Co., Research Triangle Park, NC) is a new semisynthetic vinca alkaloid under development. It is the only vinca modified in the catharanthine ring rather than the vindoline ring of the molecule. In addition to inducing cellular blockade at metaphase, vinorelbine is unique among the vincas in its ability to induce an earlier blockade at prophase. Vinorelbine had demonstrated substantial activity in both in vivo and in vitro NCI screens. It has proven to be active against all tumor models previously known to be sensitive to other vincas; however, these models demonstrated cross-resistance between vinorelbine and the other vincas.

Similar to other vincas, vinorelbine is cleared predominantly through the liver. It also is taken up actively by most tissues, resulting in a long half-life (mean of 40 hours). Phase I trials have shown that the drug can be administered safely at an IV dose of 30 mg/m² weekly, with the predominant toxicities limited to leukopenia, phlebitis, alopecia, constipation, and rare peripheral neuropathy.

The response rate of patients with advanced breast cancer to IV vinorelbine administered weekly is shown in Table 5. Response rates range from 40% to 50%, with an overall 5% complete response rate. Patients with less prior treatment fare better than those who are more heavily pretreated, and complete responses do occur.

Vinorelbine also has been combined with other active agents against breast cancer, such as epirubicin, mitoxantrone, 5-fluorouracil, and doxorubicin. Meaningful complete responses (range, 20–33%) and overall response rates (66%) have been observed. Nonetheless, these trials are small, and confidence intervals for response certainly overlap those for many other active single agents. For navelbine to replace the existing vincas for breast cancer treatment, it will be necessary to prove its superiority in Phase III trials of sufficient size to detect differences at the 5–10% level.

Anthrapyrazoles

Anthrapyrazole compounds have played a prominent role in the cytotoxic treatment of breast cancer. These cell cycle-specific agents have several postulated mechanisms of action, including DNA intercalation with sub-
sequent topoisomerase (topo)-II inhibition, and free radical generation, leading to oxidation of intracellular macromolecules. The latter effect has been implicated as a cause of the delayed, cumulative cardiotoxicity observed with these compounds. In some cases, this can result in cardiomyopathy, irreversible congestive heart failure, and death. In addition, other commonly used drugs with similar mechanisms, doxorubicin and mitoxantrone, are ineffective in cells possessing the MDR phenotype. This phenomena is associated with an overexpression of P-glycoprotein, which actively expels xenobiotics (including anthracyclines) from the intracellular milieu. Therefore, recent efforts have centered on the development of analogs that have less cardiac toxicity. Loxosantrone and piroxantrone are two anthrapyrazoles that have been studied clinically. Both drugs have demonstrated activity against the MDA-1 mammary carcinoma xenograft model employed in the NCI’s drug screen as well as some degree of non-cross resistance when tested against a doxorubicin resistant 16C mammary carcinoma cell line. Phase II trials of these compounds have been conducted in women with breast cancer.

Piroxantrone has been tested, but with disappointing results. Thirty women who previously had received 1 prior chemotherapy regimen were treated with 160 mg/m^2 every 3 weeks, with 6 patients achieving a response (20%) with 1 having a complete response. Conversely, losoxantrone (50 mg/m^2 every 21 days) showed promising activity when administered to 30 evaluable women with advanced breast cancer. Equal response rates were observed in women previously treated with cytotoxics (10 of 16 patients [63%]) and cytotoxic-naïve patients (9 of 14 patients [64%]), although the only two complete responses were observed

Table 4. Phase II Trials of Docetaxel in Advanced Breast Cancer

<table>
<thead>
<tr>
<th>Institution (reference No.)</th>
<th>Dose and schedule*</th>
<th>No. of evaluable patients</th>
<th>Prior treatment</th>
<th>No. of responses</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EORTC^6</td>
<td>100 mg/m^2</td>
<td>24</td>
<td>Met-24</td>
<td>2 (8)</td>
<td>11 (46)</td>
<td>6/24 with late skin reactions</td>
<td></td>
</tr>
<tr>
<td>NCI^7</td>
<td>100 mg/m^2</td>
<td>34</td>
<td>Adj-7</td>
<td>3 (9)</td>
<td>19 (55)</td>
<td>53% nonprogressed patients had HSRs, 25% had febrile neutropenia requiring dose reductions</td>
<td></td>
</tr>
<tr>
<td>EORTC^8</td>
<td>100 mg/m^2</td>
<td>23</td>
<td>Adj-12</td>
<td>6 (18)</td>
<td>18 (55)</td>
<td>74% removed from study due to edema and/or confusion</td>
<td></td>
</tr>
<tr>
<td>MDA^9</td>
<td>100 mg/m^2</td>
<td>6</td>
<td>Adj-1</td>
<td>—</td>
<td>4 (66)</td>
<td>6/6 Grade 2 skin reactions</td>
<td></td>
</tr>
<tr>
<td>MSKCC^10</td>
<td>100 mg/m^2</td>
<td>29</td>
<td>Met-4</td>
<td>—</td>
<td>2 (7)</td>
<td>24/29 developed edema, effusions or weight gain; 28% developed HSRs (non-premedicated)</td>
<td></td>
</tr>
</tbody>
</table>

Mets: metastatic; Adj: adjacent; NA: not available; HSRs: hypersensitivity reactions; CR: complete response; PR: partial response.

* All patients received the docetaxel dose over 1 hour and it was repeated every 3 weeks.

† 4 of the 11 had a PR not yet lasting 6 weeks at time of this report.

Table 5. Phase II Trials of Weekly Nab-paclitaxel (30 mg/m^2) in Breast Cancer

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of evaluable patients</th>
<th>Prior chemotherapy for metastases (no.)</th>
<th>Response</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>Median duration (wks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canobbio^11</td>
<td>24</td>
<td>5</td>
<td>—</td>
<td>5 (21)</td>
<td>6 (25)</td>
<td>22 (9–41)</td>
</tr>
<tr>
<td>Matt^12</td>
<td>25</td>
<td>25</td>
<td>—</td>
<td>1 (4)</td>
<td>8 (32)</td>
<td>NA</td>
</tr>
<tr>
<td>Delost^13</td>
<td>141</td>
<td>0</td>
<td>—</td>
<td>18 (13)</td>
<td>69 (49)</td>
<td>42</td>
</tr>
<tr>
<td>Brun^14</td>
<td>44</td>
<td>44</td>
<td>—</td>
<td>3 (7)</td>
<td>20 (45)</td>
<td>N/A</td>
</tr>
<tr>
<td>Weber^15</td>
<td>106</td>
<td>41</td>
<td>—</td>
<td>11 (59)</td>
<td>22 (21)</td>
<td>38 (11–47)†</td>
</tr>
<tr>
<td>Razinov^16</td>
<td>20</td>
<td>0</td>
<td>—</td>
<td>1 (5)</td>
<td>9 (45)</td>
<td>28</td>
</tr>
<tr>
<td>Luck^17</td>
<td>47</td>
<td>0</td>
<td>—</td>
<td>(61)*</td>
<td>—</td>
<td>NA</td>
</tr>
</tbody>
</table>

CR: complete response; PR: partial response; NA: not available.

* Results reported only as overall response.

† Response duration reported only for patients in CR.

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in the latter cohort. Response duration was 37 weeks. Leukopenia was the dose-limiting toxicity, with no patient developing clinical evidence of cardiac toxicity. Other toxicities, such as nausea and vomiting, alopecia, and mucositis, were observed less frequently in these patients than in those treated with anthracyclines in earlier trials. Thus, with the data currently available, it appears that losoxantrone is the anthrapyrazole with the greatest clinical potential for treating breast cancer.

**Pyrazoloacridine**

Pyrazoloacridine is another DNA intercalator under development with several unique features. Similar to doxorubicin, it induces DNA strand breaks, possibly through a topo-II interaction, and inhibits RNA and DNA synthesis. It is being developed, in part, because of its in vitro and in vivo activity against solid tumor cell lines, including mammary carcinoma cells possessing the multi-drug resistance phenotype. In addition, it has a favorable activity profile against noncytosing cells and hypoxic cells. Phase II testing of this promising agent will commence in 1994.

**Edatrexate**

Edatrexate is a new dihydrofolate reductase inhibitor that has theoretical antitumor advantages compared to methotrexate. Interestingly, it is active in the MX-1 (breast carcinoma) human tumor xenograft, whereas methotrexate is ineffective. Its enhanced efficacy may be related to the increase in its polyglutamate moiety observed in tumor cells, as opposed to normal tissue, when compared to methotrexate. This apparent selective transport of edatrexate may confer an advantage to this newer antifol.

In both animals and humans, hepatic and renal clearance account for most of the excreted drug or its metabolites. Human pharmacokinetic studies indicate that edatrexate is excreted largely unchanged, with 30-37% of the drug excreted within 48-72 hours. Animal studies failed to demonstrate any schedule dependency, and Phase I trials in humans used predominantly weekly schedules. The recommended Phase II dose is 80 mg/m² weekly. As with methotrexate, stomatitis is the dose-limiting toxicity. Other side effects include nausea/vomiting, fatigue, transaminase elevation, diarrhea, and myelosuppression. A macular rash, usually appearing first on the extremities and occasionally on the trunk, occurs in about 20% of patients. Pulmonary fibrosis and pleurisy, rare side effects of methotrexate, also have been noted with edatrexate.

Two Phase II trials in breast cancer have been reported. A Dutch group studied 38 previously untreated Stage IV patients with weekly edatrexate (80 mg/m²). Three patients had a complete response, and eight had a partial response, for an overall response rate of 29%. The median duration of response was 30 weeks (range, 12-66 weeks). Treatment delays and dosage reductions occurred in more than half of the patients because of stomatitis (60% of patients with Grade 2 or 3 tumors) and myelosuppression (28% of patients with Grade 3 or 4 tumors).

Similarly, a Canadian study of 32 untreated patients with metastatic breast cancer noted 2 complete responses and 11 partial responses (overall response rate, 41%; 95% confidence interval, 24-59%). Once again, mucositis was dose limiting. Only 20% of the patients were able to receive 90% or greater of the planned dose intensity, and the median dose intensity that actually was received was only 56.7 mg/m²/week. In addition, 2 patients developed pneumonitis, and 18 patients developed a skin rash that was responsive to drug discontinuation or glucocorticoid treatment.

**ICI D1694**

A new, promising antimetabolite is ICI D1694. This compound represents a novel class of thymidylate synthetase (TS) inhibitors, which act through a folate-based mechanism. The fluoropyrimidines have enjoyed a prominent role in the cytotoxic approach to breast cancer, with single-agent response rates of 27% cited for 5-fluorouracil. The inhibition of TS is a major pathway of action for 5-fluorouracil via the ability of its metabolite (fluorodeoxyuridine monophosphate) to form a ternary complex with TS and 5,10-methylene-tetrahydrofolate. The more potent TS inhibitor D1694 targets TS through a folate route, however, potentially circumventing mechanisms of resistance, such as deoxy­uridine monophosphate accumulation. Phase I studies are nearing completion, with broad based Phase II evaluations soon to follow.

**Retinoids**

Retinoids are critical regulators of many normal human biologic functions. Relevant to oncology, they influence cell growth and differentiation and immunologic function. In vivo evidence suggests that they prevent or diminish the incidence of carcinogen-induced mammary tumors. Recently, clinical efficacy has been demonstrated for all-trans retinoic acid (TRA) in acute promyelocytic leukemia and cis-retinoic acid, in combination with alpha-interferon, in selected squamous cell malignancies. Characterization of specific retinoid receptors in the nuclei of human cells has led to the realization that different retinoids may exert their antitumor effects via mechanisms that may be organ specific. Therefore, efforts have been initiated to define
the role of retinoids in the treatment of other malignancies, including breast cancer.

**All-Trans Retinoic Acid (tRA)**

Although tRA is the natural metabolite of vitamin A (retinol), early experience in the 1970s showed that its photosensitizing activity was inconvenient. cis-Retinoic acid, had a better toxicity profile because of the lower incidence of such central nervous system symptoms as pseudotumor cerebri, headache, and nyctalopia (night blindness) associated with it. For this reason, initial studies concentrated on cis-retinoic acid. The impressive results in acute promyelocytic leukemia obtained with tRA, however, have occasioned a revival of interest.

In vitro evidence indicates that tRA is capable of inhibiting the growth of multiple human breast cancer cell lines. Fontana and Butler have studied the antitumor mechanisms of tRA and other retinoids in these cells extensively. His results indicate that retinoids inhibit tumor growth most effectively in estrogen-dependent cell lines and are capable of up-regulating the level of estrogen receptors present in these lines. It is hypothesized that, because the receptors for estrogen and retinoids belong to the same steroid "superfamily" of receptors, their ultimate cellular target indeed may be the same gene(s).

In adults with solid tumors, it is important to indicate that the oral single-agent dose will be in the 150-200 mg/m² per day range. As with other retinoids, the administration of tRA concurrent with meals increases its bioavailability. Mucocutaneous side effects, such as dry skin, cheilitis, and paronychia, have been dose limiting. Other serious but less frequently noted side effects have included headaches, nyctalopia, and liver function abnormalities.

Unlike cis-retinoic acid, RA is eliminated rapidly, and its pharmacokinetics do not remain constant with chronic dosing. After the administration of an oral dose of 45 mg/m², RA is removed from the plasma with a half-life of less than 1 hour. With chronic administration, the metabolism of tRA may be accelerated, possibly because of the induction of cytochrome P-450-mediated catabolism, which can lead to a reduction in systemic exposure. This pharmacodynamic property may be responsible, in part, for the development of resistance to tRA.

**N-(4-hydroxyphenyl)retinamide**

N-(4-hydroxyphenyl)retinamide (4HPR), or fenretinide, is a synthetic retinoid that first captured clinical interest when Moon et al. described its ability to prevent carcinogen-induced mammary carcinoma. Subsequently, others have shown that it also can induce remission in established rat mammary carcinomas. Despite a Phase II trial in patients with advanced breast cancer that showed no activity for this compound, Veronesi and colleagues have undertaken extensive studies of its potential as a chemopreventive agent. It has a superior toxicity profile compared to other retinoids, and it appears to concentrate preferentially in the adipose tissue rather than the liver, as do most retinoids.

It does not alter circulating levels of female sex hormones and has shown synergism with tamoxifen in preventing mammary carcinogenesis in murine models.

These favorable characteristics provided the impetus for an Italian pilot trial in 1986 that tested the chronic administration of 200 mg per day of 4HPR in 53 patients over 6 months. To prevent the occurrence of side effects due to the suppressive effects of 4HPR on plasma retinoid levels, patients on this trial were given a 3-day "drug holiday" each month to permit retinoid levels to recover.

Mucocutaneous side effects, liver function test abnormalities, and hyperlipidemia occurred infrequently and were quite mild. Seven patients reported some degree of night blindness, and this was confirmed in three by electrophotogram. These latter three cases were associated with low plasma retinol levels.

Based on these results, an adjuvant study in women with lymph node–negative breast cancer was initiated in Italy in 1987. This trial compares a regimen of 200 mg of 4HPR given orally daily (with 3-day drug holidays) to tamoxifen administration in women who have early-stage breast cancer. A total accrual of 3500 patients is anticipated. Clinical end points are tumor recurrence and the development of contralateral breast cancer.

Other investigators have explored the combination of 4HPR and tamoxifen based on preclinical data demonstrating a synergy between these two agents. Cobleigh et al. performed a Phase I trial in women with receptor-positive, metastatic breast cancer in which successive cohorts of patients were given progressively escalating doses to reach a total dose of 400 mg of 4HPR per day in combination with 20 mg of tamoxifen per day. Tumor responses occurred in 6 of 13 assessable patients. Nyctalopia did not occur, mucocutaneous side effects were mild, and cholesterol levels actually decreased. This toxicity profile contrasts with that observed by other investigators, who used 300 mg per day of 4-HPR alone. Patients in the combination study did not have a drug holiday, which may explain some of the differences. Alternatively, the timing of meals and 4HPR administration may be responsible for changes in drug absorption, so Cobleigh et al. are continuing their dose escalation of 4HPR until a maximum tolerated dose is attained for the combination.

A current NCI Phase II trial (J. O'Shaughnessy,
M.D., personal communication, November 1993) is evaluating potential mechanisms of action of 4HPR and tamoxifen. Similar to tamoxifen, 4HPR inhibits insulin growth factor-I and -II-mediated tumor growth and reduces the production of transforming growth factor. To determine if this mechanism occurs in breast cancer, the NCI investigators are treating women with receptor-positive and -negative advanced breast cancer with 20 mg of tamoxifen per day and 400 mg of 4HPR per day for 25 days, with a 3-day drug holiday every 4 weeks. Serum and tissue levels of insulin growth factor and transforming growth factor, are being measured in this trial.

**Topoisomerase-I Interactive Agents**

Compounds that interact with the nuclear enzyme topo-I have shown promise as antineoplastic agents. Topo-I relieves torsional strain (negative and positive supercoiling) in DNA by forming a single-strand nick, permitting the proper functioning of DNA and RNA polymerases. Topo-I differs from topo-II, which forms a transient double-stranded complex with DNA, in its lack of energy requirements and its consistent expression throughout the cell cycle. All of the agents that interact with topo-I compounds that are in clinical development are structural analogs of camptothecin, an alkaloid derived from the Chinese tree *Camptotheca acuminata*. The sodium salt of camptothecin, which consists of an open lactone ring, was evaluated in the 1960s. Its development was halted because it caused erratic and unpredictable toxicities, such as hemorrhagic cystitis and diarrhea. However, a resurgence of interest in this class of compounds has evolved with the understanding of the importance of maintaining a closed lactone ring to sustain biologic activity.

Three compounds in this class currently are being developed. Irinotecan has been the most widely studied internationally. This prodrug undergoes a deesterification reaction, presumably caused by a carboxylesterase, to yield SN-38, a metabolite with a 1000-fold greater potency in vitro compared with the parent compound. Diarrhea and myelosuppression have been the most commonly observed toxicities. Preliminary Phase II results in breast cancer patients (1 complete response in 12 evaluable patients) indicate the potential for some activity of irinotecan, although further, more mature, data from this and other trials is necessary before any firm conclusions can be drawn.

Irinotecan currently is undergoing Phase II testing, with no efficacy data yet available for breast cancer. Finally, Phase I evaluation of a very promising camptothecin analog, 9-amino camptothecin recently began. This compound is noteworthy because of its unique ability to induce disease free remissions in human cancer xenograft studies, unlike other compounds such as 5-fluorouracil, which only cause delayed growth. It is relatively insoluble in most conventional vehicles; however, if this problem can be circumvented at clinically relevant concentrations, this compound may have great promise in the treatment of many malignancies.

**Fumagillin Analog—TNP-470**

The angiogenesis inhibitor TNP-470, a synthetic analog of the antibiotic fumagillin, has the potential to be an active compound in breast cancer. Laboratory observations indicate that for any tumor's growth to exceed 10⁶ cells, the formation of new capillary blood vessels is required. This process is mediated by both the tumor and infiltrating macrophages.

TNP-470 acts by inhibiting endothelial cell proliferation, a necessary step in angiogenesis. Recently, using breast cancer tumor specimens graded according to the number of microvessels present as an indication of its angiogenic activity, it has been found that microves- sel counts and density correlate with both the presence of local (i.e., lymph node) and distant metastatic disease, and also are predictors of relapse free survival. Considering this unique mechanism of action, a compelling rationale can be constructed for studying this compound once the preliminary Phase I trials are completed later in 1994.

**Temozolomide**

Temozolomide, a cytotoxic alkylating agent in the imidazoletriazine class is being entered in clinical testing. Similar to dacarbazine, temozolomide is believed to be a prodrug of the cytotoxic triazine 3-methyl(triazen-1-y1)imidazole-4-carboxamide. Unlike dacarbazine, however, temozolomide is not dependent on metabolic N-demethylation to generate the reactive 3-methyl(triazen-1-y1)imidazole-4-carboxamide species. Rather, with temozolomide, this conversion occurs spontaneously at physiologic pH levels. It has demonstrated potent activity against a variety of solid tumors as well as L1210 leukemia. Although the initial clinical emphasis will be in central nervous system malignancies based on initial European results, studies in breast cancer would appear to be warranted.

**Penclomidine**

Penclomidine, a synthetic alpha-picoline derivative, was entered in clinical testing in 1993. It was selected for development on the basis of its in vivo activity against two breast cancer models. It demonstrated greater than 100% growth inhibition of advanced stage
CDSFI murine mammary adenocarcinomas and 90% or greater inhibition of early stage human mammary MX-1 carcinoma xenografts. In addition, it showed activity against intracerebrally implanted MX-1 xenografts. Its mechanism of action has not been defined precisely; it was ineffective in tumors resistant to alkylating agents, while tumors that express the MDR phenotype remained sensitive. Its activity is independent of whether it is given IV or orally, although initial clinical trials will use the intravenous route.

Discussion

A broad spectrum of new agents is being evaluated clinically. This list soon will be supplemented by numerous other compounds that are passing through the Developmental Therapeutics Program's revised drug screen at the NCI. The Developmental Therapeutics Program now tests promising agents with an in vivo preliminary screen composed of 60 human tumor cell lines (derived from 6 human solid tumors and 1 leukemia) followed by an in vivo screening using nude mice containing human tumor xenografts.

Although some of the current regimens, notably cyclophosphamide--doxorubicin--5-fluorouracil and cyclophosphamide--methotrexate--5-fluorouracil, offer substantial palliation to patients with metastatic breast cancer, front-line chemotherapy with these regimens offers limited possibilities for new drug development in the Stage IV setting. It may be appropriate to test new agents before administering standard regimens to avoid the development of acquired drug resistance and thus potentially miss active agents. The Cancer and Leukemia Group B has taken the lead with this approach in breast cancer patients. They randomize patients either to receive three cycles of a new agent before being switched to conventional cyclophosphamide--doxorubicin--5-fluorouracil therapy or to receive cyclophosphamide--doxorubicin--5-fluorouracil from the outset. An alternative approach, suggested by the European Organization for the Treatment of Cancer, is to screen new agents after anthracycline failure, reasoning that only those agents that demonstrate some degree of non-cross-resistance with anthracyclines or anthracyclines will have a significant impact on breast cancer treatment.

Although many methodologic issues remain to be resolved, specific clinical situations demand immediate attention. The establishment of paclitaxel's role in breast cancer treatment is quite illustrative of this need. The question of the optimal dose and schedule for this impressive new agent remains unanswered. Shorter infusion schedules are certainly more convenient and cost-effective, but longer infusion may provide greater tumor exposure and optimal cell kill. Dose-response relationships for this agent also are unclear. Two NCI-sponsored trials, one by the Cancer and Leukemia Group B and one by the National Surgical Adjuvant Breast and Bowel Project, will study specifically the questions of dose and schedule in patients with metastatic breast cancer. The implications of this question are significant in that maximal paclitaxel doses require G-CSF support, produce greater toxicity, and have a significant impact on cost-effectiveness and quality of life.

The optimal integration of paclitaxel into standard adjuvant regimens is a critical challenge if the curative potential of this agent is to be realized. Adjuvant studies initially will explore paclitaxel's benefit as a single agent when added sequentially to standard regimens; if proof of the superiority of paclitaxel combinations is demonstrated in metastatic disease, then such combinations will require Phase III testing. Furthermore, attempts to overcome taxane resistance hold substantial promise. If ongoing Phase II trials with agents that reverse multidrug resistance demonstrate activity in refractory disease states, then Phase III trials will be essential to establish the role of such agents in de novo treatment or, perhaps, as part of a "delayed" reinduction approach. Additionally, it has been shown in vitro that tumor cells are resistant to vinca alkaloids because of their enhanced ability to form microtubules, show increased sensitivity to paclitaxel. Thus, tubulin assembly-disassembly would appear to represent a dynamic equilibration that can be shifted to favor vinca alkaloid--induced disruption or paclitaxel-enhanced polymerization. Exploitation of such collateral sensitivity is being tested in clinical trials.

Future studies of the second generation taxane, docetaxel, will concentrate on overcoming the side effect of fluid retention that has limited repetitive cycles of this agent. However, differences in toxicity suggest that the two taxanes might have somewhat different mechanisms of action. Indeed, incomplete cross-resistance has been noted between the two drugs in laboratory models. Because one mechanism of taxane resistance is thought to involve altered tubulin structures, it is plausible that tumors will exhibit differential sensitivity to the varied taxanes.
Vinorelbine may be used most effectively as an oral agent. Vincas are vesicants, and an oral preparation would eliminate the hazard of extravasation. In addition, advocates of the oral route suggest that continuous exposure to low concentrations could have a positive effect on its activity. An initial phase II German study\(^9\) of 17 patients with breast cancer did not show much activity; however, additional trials in the United States have been performed with the oral formulation, and publication of these results is awaited.

Investigative efforts on edatrexate have centered on methods to overcome its dose-limiting stomatitis. Animal experiments appear to indicate that higher doses of edatrexate can be delivered safely with "leucovorin rescue" and that antitumor effect is enhanced to a much greater degree than is seen with methotrexate.\(^13\) In a Dutch study, edatrexate was given orally 24 hours after the IV administration of edatrexate to two patients who had suffered mucositis on prior cycles of the latter agent. The leucovorin was successful in abolishing the mucositis and permitted 100% of the drug dosage to be administered. A Phase I study\(^12\) recently demonstrated that intravenous doses of edatrexate can be escalated to as high as 1200 mg/m\(^2\) every 2 weeks, with only mild mucositis, when leucovorin rescue was given at doses of 20 mg orally every 6 hours for 8 doses beginning 24 hours after edatrexate administration.

The development of differentiating agents like the retinoids presents new challenges. It does not appear that these agents will be effective alone against advanced breast cancer. Similar to what has been demonstrated in patients with squamous cell malignancies, however, in vitro models suggest a synergy between certain retinoids and interferon-gamma and, to a lesser extent, interferon-alpha in breast tumor cell lines.\(^10\) Likewise, the proven ability of these agents to normalize dysplastic squamous epithelium may be translatable to hyperplastic mammary tissue, making trials with these agents in early breast disease very appealing.

Finally, a number of agents that possess varied mechanisms of action are in the formative stages of clinical development (Table 6) at the NCI. Properly conducted single-agent and combination trials in minimally pretreated patients will be essential to develop these compounds in an expeditious manner. Certainly, other important treatment approaches, including growth factors, monoclonal antibodies, and gene therapies, are also on the horizon and will make unique additions to the therapeutic armamentarium. It is unlikely, however, that these approaches will supplant chemotherapy totally. Rather, it is more likely that progress will be made best by a continued emphasis on a multimodal approach, in which chemotherapy plays a central role.

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