New Drugs in Cancer Therapy, National Tumor Institute, Naples, 17–18 June 2004

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An international meeting on 'New Drugs in Cancer Therapy' was held at the National Tumor Institute of Naples, on 17–18 June 2004. The first session of the meeting focused on analogs of conventional anti-cancer drugs, such as taxanes, platinum compounds, anthracyclines and topoisomerase I inhibitors. The data of a phase II trial of BMS-247550, an epothilone B analog, in patients with renal cell carcinoma were reported. Data were also presented on BBR-3464, a trinucleate platinum analog which was developed on the grounds of greater potency, a more rapid rate of DNA binding and the ability to induce apoptosis regardless of the p53 status of the cell. Pegylated-coated liposomal formulation doxorubicin (Caelyx) has shown efficacy in metastatic breast cancer and in advanced ovarian cancer; sabarubicin is a third-generation anthracycline with equal or superior potency to doxorubicin or idarubicin in a variety of human tumor cell lines of different histotypes. The main mechanisms of resistance to topoisomerase I inhibitors were discussed; data on diflomotecan were reported, showing a narrow therapeutic index of the drug. The second session of the meeting focused on the ErbB family as a target for anti-cancer therapy. Recent evidence of a correlation between epidermal growth factor receptor (EGFR) mutations at exons 18–21 and clinical response of advanced non-small cell lung cancer to gefitinib therapy was commented on. The issue of the association between ErbB2 expression and gefitinib activity was addressed, while clinical data of a phase II study of gefitinib in advanced breast cancer were presented. Monoclonal antibodies targeting EGFR represent another worthwhile way to interfere with EGFR-driven signal transduction. Cetuximab is reaching market registration in advanced colorectal cancer; in particular, due to the results of the BOND study. The recently presented results of the Bonner study strongly support the activity of this drug in head and neck cancer. A step forward in the research on anti-EGFR monoclonal antibodies may be represented by humanized monoclonal antibodies, such as EMD 72000 and ABX-EGF. Imatinib mesylate is probably the most outstanding example of an effective targeted therapy—its activity in gastrointestinal stromal tumors was so exciting that the drug reached the market without undergoing phase III evaluation. The third session of the meeting was on angiogenesis inhibitors. Drugs may interfere with the angiogenic process via different mechanisms and there is a sound rationale for combining anti-angiogenic agents with chemotherapy or multiple anti-angiogenic strategies. Clinical results obtained with direct anti-angiogenic agents have been negative up to now, but some exciting results have been seen with bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor (VEGF). A few VEGF-tyrosine kinase inhibiting small molecules, such as ZD6474, AZD2171 and PTK/ZK, are undergoing clinical trials. The fourth session of the meeting was on interference with intracellular signal transduction. Farnesyl transferase inhibitors exert their action by interfering with either pro-Ras or RhoB farnesylation. Several clinical studies of different phases with compounds belonging to this class have been carried out, either alone or in combination with chemotherapy; unfortunately, all of them have turned out to be negative. Cell cycle inhibitors, such as CYC-202 and BMS-387032, represent a class of interesting compounds which are in the early phase of development and whose clinical results are eagerly awaited. Another strategy to achieve cell cycle inhibition is to target heat shock protein 90, a molecular chaperone required for protein folding. Clinical data on depsipeptide, a histone deacetylase (HDAC) inhibitor with activity in T cell lymphoma, were presented. Suberoylanilide hydroxamic acid is another small molecular weight inhibitor of HDAC activity. Phase I/II clinical trials have shown low toxicity and evidence of anti-tumor activity; on the other hand, this compound has potential for synergism with radiotherapy, chemotherapy and biologicals. *Anti-Cancer Drugs* 16:211–221 © 2005 Lippincott Williams & Wilkins.

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Novel cytotoxic compounds in anti-cancer therapy

The first lecture was given by Professor Tito Fojo (National Cancer Institute, Bethesda, MD) and focused on novel taxanes. Professor Fojo mainly presented the data of his phase II trial of BMS-247550, an epothilone B analog, administered i.v. to patients with renal cell carcinoma [1]. Fifty-four patients (most with a clear cell

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histology) were enrolled in the study. The main toxicity in this study was hematologic, since grade 3–4 neutropenia occurred in 23 cycles; neurotoxicity, which was a feared side-effect, reached grade 3 in only two patients. Other grade 3 toxicities in this study were diarrhea, dysphagia, dyspnea, fatigue, mucositis, hypotension and infection. In one patient, a grade 4 pericardial effusion was observed. Nine patients achieved a partial response to treatment. Both α- and β-tubulin post-translational modifications were looked at in this study. Acetylation correlated with stable microtubules and was a reflection of the relative length of time the individual subunits are presented as a substrate for tubulin acetyltransferase. Detyrosination/tyrosination appears to reflect the length of time the individual α-tubulin substrate molecule has spent in a microtubule. Samples from patients with metastatic renal cancer showed increased levels of acetylated and Glu-terminated α-tubulin after treatment with BMS-247550. Measurement of Glu-terminated α-tubulin is a good surrogate marker to indicate that BMS-247550 has engaged its intracellular target in samples from patients with metastatic renal cancer. Patients showing tubulin alterations, but who did not respond to treatment, may have resistance mechanisms distal to the microtubule target that impact cytotoxicity. Several questions are left open. Why is BMS-247550 effective in some patients, but not in others? Does this evidence indicate that BMS-247550 has reached the tumor cells and engaged its target? If yes, then what are the mechanisms of resistance we should be investigating? Is there any feature of the tumor that can predict sensitivity to BMS-247550? Professor Fojo went over responses obtained in other trials with this molecule; in particular, highlighting a partial response obtained in a patient with cervical cancer who had already undergone three prior regimens and who had been on paclitaxel 17 months prior to enrolment on study. He concluded that the list of proteins interacting with microtubules is increasing; anti-microtubule drugs are likely to interfere with functions other than mitosis and this may allow these agents to kill cells regardless of mitotic arrest, rendering this class of drugs exciting.

Professor Graziella Pratesi (National Tumor Institute, Milan, Italy) gave a presentation on new platinum analogs. In particular, she dealt with third-generation platinum analogs that were designed to overcome cisplatin resistance, which may be due to reduced drug uptake, increased detoxification, increased DNA repair and tolerance to platin–DNA adducts [2]. JM216 (satraplatin) is a platin analog, which is active by the oral route. Satraplatin has already undergone an extensive clinical evaluation. Recently, the preliminary results of a phase III study, in which satraplatin plus prednisone were compared to prednisone alone in hormone-refractory prostate cancer (HRPC), have been presented [3]. The results are promising, although only 50 patients have been accrued. ZD 0473 is a cisplatin analog that is able to overcome glutathione-mediated inactivation, due to a reduced affinity for thiol-containing molecules compared with cisplatin [2]. This compound has already undergone phase I studies, in which myelotoxicity has qualified as dose-limiting toxicity (DLT). Combination studies with a number of other cytotoxic compounds have been undertaken. Phase II studies have been carried out in ovarian, lung, breast and prostate cancer, and in mesothelioma. A phase III study in ovarian cancer is ongoing. BBR-3464 is a trinucleate analog of cisplatin; it was developed on the grounds of a greater potency, a more rapid rate of DNA binding, the ability to form long-range DNA cross-links and the ability to induce apoptosis regardless of the p53 status of the cell [2]. Diarrhea and myelosuppression have qualified as DLT in phase I studies with this compound, whose clinical development was disappointing, probably due to the binding and degradation by human plasma proteins. New formulations of this compound, along with new analogs, are under study. SPI-77 (stealth liposomal cisplatin) is a liposomal-encapsulated cisplatin, which was developed with the aim of selectively delivering the drug into tumor tissues without toxic effects [2]. Unfortunately, low activity has been observed in clinical studies.

Professor Pierre Fumoleau (Centre G.-F. Leclerc, Dijon, France) gave a talk on novel anthracyclines in clinical development. The research on anthracycline analogs, aimed at improving the therapeutic index, has resulted up to now in about 2000 new drugs, only a few of which have reached the market. The development of new anthracyclines has involved tumor-targeted formulations, which has been carried out mainly with two strategies, i.e. development of carriers that assist preferentially distribution within tumors while not exposing healthy tissues to potentially toxic levels of the drug (liposomes), and conjugation to a carrier that specifically recognizes tumor cells (polymer-bound doxorubicin). Liposomal formulation is characterized by a reduced volume of distribution, reduced clearance, prolonged half-life, preferential accumulation in tumors characterized by leaky microvasculature and limited accumulation in healthy tissue with a normal endothelial barrier, such as the heart. The development of liposomal formulations of anthracyclines has resulted in three main drugs which have been assessed in experimental models and clinical settings, such as pegylated-coated liposomal formulation doxorubicin (Caelyx), uncoated liposomal formulation doxorubicin (Myocet) and liposomal formulation daunorubicin (DaunoXome). The peculiar characteristic of Caelyx is pegylation, which protects the liposome from detection by the mononuclear phagocyte system, thus increasing intratumor delivery and inducing pharmacokinetic peculiarities, such as reduced clearance and increased half-life [4]. Caelyx has shown efficacy in metastatic breast

cancer. In particular, it has been shown equivalent to conventional doxorubicin with respect to progression-free survival (PFS) and overall survival (OS) in first-line therapy [5]. Caelyx has a significantly reduced risk of cardiotoxicity, nausea, vomiting and alopecia compared with conventional doxorubicin, and it has a lower incidence of myelosuppression compared with commonly used salvage regimens; however, Caelyx is associated with stomatitis and palmar–plantar erythrodysesthesia [4]. Caelyx is ideally suited for specific patient populations, such as elderly patients, patients who present with hypertension, prior mediastinal irradiation or a history of heart disease, patients for whom the risk of specific toxicities are of significant concern (alopecia, myelosuppression, nausea and vomiting) and patients treated in the adjuvant setting when to be retreated for metastatic disease. In addition to being an important option in the treatment of patients with metastatic breast cancer, Caelyx is indicated in relapsed/refractory ovarian cancer, being superior to topotecan in both PFS and OS [6]. Professor Fumoleau went over a novel anthracycline (MEN 10755, sabarubicin) which was developed in phase I by the EORTC New Drug Development Group. Studies in human tumor xenografts have revealed that sabarubicin is more effective than doxorubicin in poisoning topoisomerase II and stimulating DNA fragmentation. *In vitro*, sabarubicin shows equal or superior potency to doxorubicin or idarubicin in a variety of human tumor cell lines of different histotypes including ovarian, lung, breast, cervical and colon cancer. At an optimal dose and schedule, sabarubicin is more effective that doxorubicin in human tumor xenografts in nude mice and shows activity in the doxorubicin-resistant mammary tumor MX-I, in two doxorubicin-resistant small cell lung cancer and non-small cell lung cancer (NSCLC) xenografts [7]. Finally, sabarubicin has shown reduced cardiotoxicity in rats. Phase I evaluation of sabarubicin has been carried out with two different schedules, i.e. once every 3 weeks and weekly $\times$ 3 every 4 weeks. With the first schedule, neutropenia was DLT, while cardiac toxicity was low. A dose level of 80 mg/m^2 was selected for phase II; no objective responses were observed [8]. The second study obtained results similar to the others (DLT hematological, no major cardiac side-effects, no objective responses) [9]. Phase II studies with sabarubicin are ongoing in a number of diseases (ovarian cancer, prostate cancer, lung cancer).

Dr Eric Raymond (Saint-Louis Hospital, Paris, France) gave a presentation on novel topoisomerase I inhibitors. He went over the main mechanisms of resistance to topoisomerase I inhibitors, such as point mutations, deletions and rearrangements of the TOP-1 gene; down-regulation of the TOP-1 gene, mRNA and protein expression; altered structure and function of TOP-1; and induction of multi-ubiquitin TOP-1 conjugates. The search for new camptothecin analogs with a broader anti-tumor activity, possibly extended to irinotecan- and topotecan-resistant tumors, a better toxicity profile, better pharmacokinetic parameters, and more predictable metabolic pathways represents an important need in medical oncology. Dr Raymond presented preliminary data on phase I studies on diflomotecan (BN80915), which was administered in the first trial i.v. twice 3 weeks apart to patients with advanced tumors. Taken together, these studies show that i.v. diflomotecan has a narrow therapeutic index, hematologic toxicities and fatigue being DLTs; weekly and every-other-week schedules do not improve the toxicity profile and do not allow an increase of the dose intensity of i.v. diflomotecan. Diflomotecan is also in early clinical trials with an oral formulation (once daily $\times$ 5 every 3 weeks); the finding of new orally bioavailable effective topoisomerase I inhibitors represents an additional important challenge.

ErbB family as a target for anti-cancer therapy

Dr Maurizio D'Incalci (Mario Negri Institute, Milan, Italy) gave an overview of the ErbB family, which includes four receptors, i. e. ErbB1 [epidermal growth factor receptor (EGFR)], ErbB2, ErbB3 and ErbB4. Each of these receptors, with the exception of ErbB2, has a number of ligands. EGFR is the best studied among this family of receptors and it is often dysregulated in several human cancers, due to its overexpression, or mutation, or due to increased production of one or more of its ligands, especially transforming growth factor (TGF)-α. EGFR overexpression is frequently observed in a number of solid tumors and it has prognostic significance in many of them, particularly head and neck cancer [10]. Dr D'Incalci emphasized the possibility to interfere with the EGFR-driven growth pathway at different downstream levels. Very recently, two papers have appeared in the literature in which the detection of an EGFR mutation at exons 18–21 [tyrosine kinase (TK) domain] has been associated with clinical response of advanced NSCLC to gefitinib therapy [11,12]. In both studies, the association of EGFR mutation and response was statistically significant; importantly, responding patients had very similar characteristics, such as being female, never or past smokers and having either adenocarcinoma or bronchoalveolar cancer. Several issues can be raised from this observation; in particular, what is the reason for this selective activity in patients with these anatomic and epidemiologic characteristics? Is this association with tumor response likely to be observed also with erlotinib and monoclonal antibodies? Are similar mutations present in other tumors and do they have the same significance? All these points are to be addressed while dealing with this very exciting field.

Dr Nicola Normanno (National Tumor Institute, Naples, Italy) dealt with small molecules with TK inhibitory activity. Gefitinib and erlotinib are two molecules in

advanced stage of development; they are both specific and reversible TK inhibitors; other compounds in the earlier stage of development are GW 572016 (reversible pan-HER inhibitor), EKB-569 (irreversible TK inhibitor) and CI 1033 (irreversible ErbB receptor inhibitor) [10]. Dr Normanno's presentation mainly focused on gefitinib, whose clinical activity has been mainly observed in NSCLC and in head and neck cancer. Dr Normanno discussed a number of important issues. First of all, he pointed out that no clear association exists between EGFR overexpression and sensitivity to gefitinib. More debatable is the association between ErbB2 expression and gefitinib activity [13]. The link of ligands to EGFR induces the formation of omodymers and heterodymers, each combination resulting in different downstream effects. Dr Normanno presented the preliminary data of a phase II study of gefitinib at the dose of 500 mg/day in pretreated patients with advanced breast cancer [14]. Median OS was 144 days in this study, while median PFS was 57 days. One patient had a partial response, while eight patients had a stable disease; interestingly, five of 12 patients had a marked relief of bone pain. Pretreatment with trastuzumab had been previously given in 23 patients and it had been interrupted due to further occurrence of resistance, which may be mediated by either insulin-like growth factor-I receptor or by a TGF-α/EGFR autocrine loop. Another issue was whether previous chemotherapy affected the response to gefitinib. Phase II studies in NSCLC [15,16] (IDEAL *2* in particular) did not show a correlation between the burden of previous treatment and anti-tumor activity. Dr Normanno discussed the meaning of the already mentioned EGFR mutations in NSCLC. These mutations allow us to split patients with mutated EGFR, whose tumor growth is likely to be EGFR dependent and in whom treatment with gefitinib is clearly indicated, from patients with wild-type EGFR, which are EGFR independent and in which gefitinib should not be used. The final issue was how to improve the efficacy of anti-EGFR drugs; since sensitivity/resistance to EGFR-TK inhibitors is also regulated by the occurrence of other molecular alterations which accumulate in cancer cells during tumor progression, the combined treatment with other molecular targeted approaches may result in a possible improvement of treatment results.

Dr Francesco Caponigro (National Tumor Institute, Naples, Italy), replacing Dr A. Awada, reviewed monoclonal antibodies targeting EGFR. Cetuximab is a chimerized monoclonal antibody in an advanced stage of development. The first market indication for cetuximab will be EGFR-positive, irinotecan-refractory, metastatic colorectal cancer. The European BOND study results supported this registration [17]. In this study, 329 patients with irinotecan-refractory metastatic colorectal cancer were randomized to receive either irinotecan + cetuximab or cetuximab alone. The difference in the response rate between the two arms was of borderline statistical significance (23 versus 11%), while the difference in disease control rate was clearly statistically significant; time to progression was also significantly higher in the combination arm, while there were no significant differences in survival. The toxicity matched the expectations; in particular, cetuximab did not increase the typical side-effects of chemotherapy. Possible future indications for cetuximab were discussed. In particular, ongoing studies are exploring the role of cetuximab in combination with well-established first-line regimens in front-line treatment of metastatic colorectal cancer. Cetuximab has been widely studied in head and neck cancer, mainly in combination with chemo or radiotherapy. Two phase III studies of cetuximab in head and neck cancer have been completed. In particular, a study compared cisplatin + cetuximab versus cisplatin + placebo in recurrent chemotherapy-untreated head and neck cancer, showing no significant survival advantage for the cetuximab arm [18]. In a recently presented study, cetuximab plus radiotherapy was compared to radiotherapy alone in patients with locally advanced inoperable head and neck cancer. The two main endpoints of the study were locoregional control and OS, and they both showed a significant difference in favor of the cetuximab arm, with no additional cetuximab-induced toxicities [19]. The investigation of cetuximab in NSCLC is at an earlier stage. Cetuximab has been tested in two phase II studies in association with established first-line regimens in untreated patients with advanced disease [20,21]. The Lucas study is a randomized phase II study of cisplatin and vinorelbine with or without cetuximab, whose preliminary data show an advantage for the cetuximab-including arm, at the expense of no additional toxicities [22]. A step forward in the research on anti-EGFR monoclonal antibodies may be represented by humanized monoclonal antibodies, such as EMD 72000 and ABX-EGF. EMD 72000, which has the advantage of a longer half-life and suitability for more convenient schedules, has already been tested in at least a couple of phase I trials, in which it has shown encouraging clinical activity in a number of tumors, with a toxicity profile which includes some toxicities, such as headache and fever, which are quite uncommon with cetuximab [23,24]. ABX-EGF has completed phase II evaluation in renal cancer and in colorectal cancer, with clear hints of clinical activity in both diseases [25,26].

Dr Giuseppe Frasci (National Tumor Institute, Naples, Italy) gave a lecture on trastuzumab, a monoclonal antibody targeting ErbB2. Past studies have stressed the value of trastuzumab when administered as single agent and in combination with chemotherapy, respectively [27,28]. The activity data reported in the above trials prompted evaluation of single-agent trastuzumab as first-line therapeutic approach in metastatic breast cancer with ErbB2 overexpression. This study showed a

differential clinical activity of trastuzumab according to the degree of ErbB2 expression (Dako 3 + versus 2 +). In fact, an objective response rate of 35% was observed in Dako 3 + patients, while no responses were observed in Dako 2 + patients [29]. Trastuzumab has been used in first-line treatment in combination with a number of other active drugs, with a response rate ranging between 54 and 79% [30]. Dr Frasci presented the phase II trial of cisplatin, epirubicin and paclitaxel (PET) plus trastuzumab, which he is currently carrying out. The PET regimen is given on a weekly basis with granulocyte colony stimulating factor (G-CSF) support; it has already yielded considerable activity in operable, locally advanced and metastatic breast cancer at the expense of negligible grade 4 hematologic toxicities and of 10% incidence of grade 3 anemia; cardiac toxicity is anecdotal with this regimen [31]. The way to move trastuzumab forward includes trials in the adjuvant setting, among which the Hera trial was mentioned. This latter study randomizes patients with ErbB2-positive primary breast cancer (N + and N–) to receive adjuvant treatment with trastuzumab administered every 3 weeks for 1 or 2 years. The neoadjuvant setting is not devoid of appeal for trastuzumab development as well; clinical trials with docetaxel + trastuzumab and paclitaxel + trastuzumab are ongoing. Possible further developments for trastuzumab include more convenient schedules, the possibility of administering the drug along with hormonal therapy, and, mainly, the role of trastuzumab in regimens including combinations of targeted drugs.

Main Lecture—Imatinib mesylate: a clinically effective targeted drug

Dr Paolo Casali (National Tumor Institute, Milan, Italy) gave a lecture on imatinib mesylate, which is probably the most outstanding example of an effective targeted therapy. Dr Casali mainly focused on the activity of imatinib in gastrointestinal stromal tumors (GIST), which show striking morphological and immunophenotypic similarities with interstitial cells of Cajal. The drug is electively active in c-*kit* mutated human GIST and in *bcr–abl*-positive chronic myeloid leukemia. Phase I evaluation in GIST has been carried out in Europe; 36 patients were treated at doses ranging between 400 and 1000 mg, and a partial response was observed in 53% of patients, with a 89% disease control rate [32]. A further phase II study, carried out in the USA in 147 patients, has seen a 54% partial response rate and an 86% disease control rate [33]. These exciting results have caused the drug to progress to the market without phase III evaluation. An ongoing phase III study is randomizing patients to receive imatinib at the dose of either 400 or 800 mg daily. Dr Casali emphasized that multi-modality approaches might be as important also in the molecular-targeted therapy era; so there may be a role for surgery of residual progressive disease and for surgery of responding disease. Dr Casali also reviewed the data available on SU 11248, a multi-targeted TK inhibitor, which has shown clinical activity in patients with GIST who were refractory to imatinib. A few clinical studies are ongoing with molecular targeted agents supposed to be active against imatinib-refractory GIST, given either alone (SU 11248, PTK 787 and PKC 412) or in combination with imatinib itself (PKC 412 and RAD 001). A further application of imatinib could be its use in the adjuvant setting and a trial is ongoing which compares imatinib for 2 years versus observation in patients with minimal residual disease. A Scandinavian trial in very high-risk patients (metastases removed) is comparing adjuvant imatinib for 12 versus 36 months.

Angiogenesis inhibitors as anti-cancer agents

Dr Raffaella Giavazzi (Mario Negri Institute, Bergamo, Italy) gave a general overview on angiogenesis inhibitors. She mentioned the potential clinical applications of vascular endothelial growth factor (VEGF) blocking agents, matrix metalloproteinase inhibitors and vascular targeting agents. Compounds with anti-angiogenic properties of different origin and with different mechanism of action have been identified and validated in preclinical models. There is a rationale both for combination of multiple anti-angiogenic strategies and for combination with conventional therapies. Dr Giavazzi reported preclinical data on the survival advantage of SU 6668 given in combination with paclitaxel over each of the two drugs given as a single agent [34]. The additive or synergistic effects of chemotherapeutics + anti-angiogenics may be due to increasing access/activity of chemotherapeutics as the result of changes in vascular functionality/morphology induced by the anti-angiogenic compound. The two classes of compounds may affect the tumoral and endothelial compartments through different pathways (two-compartment theory) or they may cause pro-apoptotic effects on endothelial cells by the combination of the two treatments (one-compartment theory). Clinical studies have shown that many angiogenesis inhibitors can be given safely, but lack of clinical efficacy has often been seen. Target inhibition, dormancy of endothelial cells or vascular shutdown are potential surrogate markers for anti-tumor activity. Non-invasive techniques, such as magnetic resonance imaging and positron emission tomography, for evaluating biological activity will contribute to a better understanding of anti-vascular therapy. Anti-angiogenic monotherapy might be efficacious for minimal residual disease; combination therapies including two or more anti-angiogenic drugs and conventional anti-tumor treatments might improve the treatment of progressive disease.

Dr Giampietro Gasparini (S. Filippo Neri Hospital, Rome, Italy) gave a talk on direct angiogenesis inhibitors. He went over the favorable characteristics of endothelial cells

as a target for anti-cancer therapy and the therapeutic advantages which derive. In particular, he emphasized the importance of the specificity of the target, the lack of difficulties related to the amount of drug that can reach the cellular target and the possibility to re-induce response after periods of interruption of treatment due to the non-occurrence of drug resistance. Neutralization of angiogenic peptides and therapy with endogenous anti-angiogenic peptides represent notable ways to carry out an anti-angiogenic therapy. There are important differences between anti-angiogenic therapeutic strategies; in particular, anti-angiogenic agents inhibit endothelial cell growth or production of angiogenic factors, are mainly cytostatic in nature, induce tumor dormancy or slow tumor regression. On the other hand, vascular targeting agents induce rapid destruction of existing blood vessels in tumors containing activated endothelial cells. Direct angiogenesis inhibitors (endostatin, angiostatin and TNP-470) do inhibit endothelial cell proliferation and migration in response to angiogenic peptides. Endostatin is a 20-kDa C-terminal fragment of collagen XVIII isolated from culture of mouse hemangioendothelioma; it specifically inhibits endothelial proliferation, angiogenesis and tumor growth *in vivo*. Possible mechanisms of action of endostatin include interference with the VEGF pathway, induction of endothelial cell apoptosis and inhibition of matrix metalloproteinases. Dr Gasparini gave an overview of the main phase I studies of recombinant endostatin in patients with advanced tumors. In the Herbst study [35], 25 patients received endostatin given as an i.v. bolus over 20 min once daily; the treatment was safe and well tolerated, showing linear pharmacokinetics; DLT was not found and evidence of minor anti-tumor activity was observed. In a similar phase I study [36], evidence of clinical benefit was observed in three patients. In a more recent study [37], endostatin was administered as a continuous i.v. infusion, followed by s.c. infusion. Dr Gasparini reviewed two phase I pharmacokinetic and pharmacodynamic studies of recombinant human angiostatin. The drug was well tolerated and long-term stable disease (more than 5 months) was observed in 11 of 24 patients. A recently presented phase II study has tested the activity of recombinant angiostatin in combination with paclitaxel and carboplatin in patients with NSCLC. The partial response rate was 39% and stable disease was observed in 39% as best response. The combination seems active and well tolerated [38]. TNP-470 is a fumagillin analog with a potent inhibitory action on endothelial cell proliferation. In an old study, TNP 470 was given on a weekly basis in 4-h i.v. infusions to patients with advanced solid tumors [39]. DLT in this study was neurotoxicity (dizziness, vertigo, ataxia, confusion), which appeared to be dose related and was completely reversible. A phase I dose-escalation study was carried out in patients with metastatic and androgen-independent prostate cancer [40]. DLT was a characteristic neuropsychiatric syndrome (anesthesia, gait disturbance, agitation) reversible upon cessation of therapy. No major anti-tumor activity was observed in this study. TNP-470 was also given to patients with pretreated metastatic renal cell carcinoma in a phase II study [41]. The main toxicities were also neurological in this study; only one patient achieved a partial response. There is a rationale for combining direct and indirect angiogenesis inhibitors, which mainly lies in the multi-faceted nature of the interaction between SU 5416 (a selective VEGFR-2 inhibitor) and low-dose endostatin in a number of xenografts models; future clinical trials are needed to evaluate similar combinations. Dr Gasparini then reviewed the possible reasons for negative results of early clinical trials with anti-angiogenic compounds, i.e. appropriate dose from phase I studies (optimal biologically effective dose versus maximum tolerated dose), appropriate schedule of drugs, appropriate clinical setting, optimal combination with conventional anti-cancer therapy or other biological therapies. Negative findings which have come up from previous trials include unexpectedly high rates of serious adverse events by combining angiogenesis inhibitors and chemotherapy [42], and lack of identification of eligible patients by appropriate surrogate markers. On the other hand, positive findings include the clinical synergistic interaction between bevacizumab and chemotherapy in advanced colorectal cancer, the feasibility of chronic administration with certain compounds and the promising activity of combinations of anti-angiogenic factors with metronomic chemotherapy.

Professor Giampaolo Tortora (University of Naples, Italy) gave a lecture on VEGF-targeting anti-angiogenic drugs. The VEGFs are critical tumor-secreted signaling molecules that stimulate angiogenesis and lymphangiogenesis. Five VEGF family members are known, which bind to surface receptors on endothelial cells; different VEGF family members bind and activate different VEGF receptors. Strategies to inhibit VEGF activity include toxin conjugates to VEGF, soluble VEGF receptor, peptides that interfere with VEGF binding, anti-VEGF monoclonal antibody (bevacizumab) and small TK inhibitors that block intracellular VEGF receptor signaling. The last two strategies are the best pursued. Bevacizumab is a recombinant humanized monoclonal antibody which has been shown safe when administered in combination with chemotherapy; its main toxicities are hypertension, proteinuria, hemorrhage, thrombosis and gastrointestinal perforation. Bevacizumab has been tested as a single agent in metastatic renal cell carcinoma at two different doses and a significant difference in terms of time to progression versus placebo has been observed [43]. A phase III trial comparing capecitabine plus bevacizumab versus capecitabine alone has been performed in breast cancer patients pretreated with anthracyclines and paclitaxel. Although more objective responses were observed in the combination arm, no

statistically significant differences in terms of response duration and PFS were observed [44]. The most significant trial, which has addressed the importance of bevacizumab, is a phase III study in metastatic colorectal cancer, in which significant advantages in terms of median survival, PFS, response rate and response duration were observed [45]. Further trials are planned in the advanced setting, mainly first-line phase III studies in combination with oxaliplatin-based therapy, and in the adjuvant setting. ZD 6474 is an oral small molecular weight compound, which inhibits VEGF receptor and EGFR-TK. Preclinical studies have shown that the drug has a wide spectrum of anti-tumor activity and a cooperative effect with paclitaxel on GEO xenografts [46]. ZD 6474 has shown hints of clinical activity in NSCLC. A phase I–II study of ZD6474 and docetaxel in platinum-pretreated patients with NSCLC has been recently carried out; half of patients obtained stable disease and the regimen proved feasible [47]. A double-blind randomized study is ongoing. AZD2171 is an oral therapy with potential application in multiple tumor types; AZD2171 has activity against VEGF receptors 1, 2 and 3, without activity on EGFR. Phase I clinical studies in refractory solid tumors are underway and have shown manageable toxicity. PTK/ZK is a multi-VEGF receptor TK inhibitor. PTK evaluation has been up to now carried out mainly in colorectal cancer; a phase I/II study in combination with either FOLFOX 4 or FOLFIRI has recently been presented, and it has shown low toxicity, a more than 50% objective response rate and a median survival of 16.6 months in combination with FOLFOX 4. In the FOLFIRI arm, more preliminary data are available, but clinical activity has been seen as well [48]. PTK has been evaluated as a single agent in metastatic renal cancer; in this study, median time to progression was 5.3 months, median survival was 21.5 months, correlation between reduction of blood flow and clinical outcome was observed [49]. Two large randomized trials, named CONFIRM studies, are ongoing in first- and second-line metastatic colorectal cancer, respectively. In these placebo-controlled studies, patients are randomized to receive FOLFOX 4 with or without PTK. SU 11248 is an oral, multi-targeted, RTK inhibitor with selective activity against platelet-derived growth factor receptor, VEGF receptor, KIT and FLT3. SU 11248 has been shown clinically active against leukemia, sarcomas and GIST (including those resistant to imatinib). A phase II trial in pretreated patients with metastatic renal cancer has shown a 33% response rate and a 37% stable disease, with a very acceptable toxicity profile.

Interference with intracellular signal transduction: downstream effectors

Dr Francesco Caponigro (National Tumor Institute, Naples, Italy) gave a talk on farnesyl transferase (FT) inhibitors. The best-acknowledged mechanism of action of this class of compounds is their interference with the first and most important among post-translational modifications of pro-Ras (farnesylation). Alternative mechanisms of action for this class of compounds have come up over recent years and mainly include interference with RhoB farnesylation. Lonafarnib is probably the first FT inhibitor which has entered clinical trials; it is rapidly absorbed when given by the oral route and displays linear pharmacokinetics. Four single-agent phase I studies have been carried out with this compound, with four different schedules. The dose/schedule of 200 mg b.i.d. has been chosen to be taken forward for phase II studies [50]. A phase IB study of lonafarnib as primary treatment in head and neck cancer has been recently carried out, and it has shown a partial response in three of 17 patients; in parallel, DNA-J analysis in surgical samples revealed an increase in unfarnesylated protein in patients treated with lonafarnib. Phase II evaluation of lonafarnib has been carried out in pancreatic cancer (randomized phase II versus gemcitabine). This study did not show any difference between the two treatment arms in terms of response rate, PFS and OS [50]. Preclinical studies have shown that lonafarnib has an additive or a synergistic interaction with a number of cytotoxic compounds, such as paclitaxel, gemcitabine and docetaxel; lonafarnib-induced P-glycoprotein (P-gp) inhibition is a possible mechanism underlining the synergistic interaction between lonafarnib and drugs which are P-gp substrates. The combination of lonafarnib and gemcitabine has been evaluated in a phase I study, which has clarified the toxicity pattern of the combination (nausea, vomiting, diarrhea and myelosuppression) and has shown anti-tumor activity mainly in pancreatic cancer. A phase II study of the combination has been run in advanced bladder carcinoma (second line). In this study, an interesting anti-tumor activity has been seen, with a 32% response rate and an acceptable safety profile [51]. A phase I/II study of lonafarnib and paclitaxel in taxane-resistant patients with NSCLC has shown a definite anti-tumor activity (15% partial response) [50]. The lonafarnib clinical program includes a randomized phase III trial in first-line NSCLC in combination with carboplatin/taxol and a phase I/II trial in recurrent advanced leukemia. Tipifarnib is another orally active FT inhibitor, whose DLTs are myelosuppression and neurotoxicity. Tipifarnib has single-agent activity against advanced breast cancer and acute myeloid leukemia, while its activity in glioma is hampered by concomitant use of enzyme-inducing anti-epileptic drugs. A randomized double-blind placebo-controlled trial of tipifarnib in advanced heavily pretreated colorectal cancer has shown no survival advantage for tipifarnib [52]. Tipifarnib phase I combination trials are ongoing with a number of cytotoxics, while a placebo-controlled phase III trial of tipifarnib in combination with gemcitabine versus gemcitabine alone has not shown a survival advantage for the combination arm [53]. BMS-214662 is cytotoxic at

2–10 μM concentrations, has potent apoptotic activity and has potent *in vivo* anti-tumor activity in human tumor xenografts; the major drawbacks of this drug consist in its severe gastrointestinal and liver toxicities, which prevent the achievement of adequate systemic exposures with the oral route; furthermore, an inconvenient 24-h infusion is required to enhance the duration of maximal FT inhibition [50]. BAY 43-9006 is a Raf kinase inhibitor that has been evaluated in three phase I trials [50]. Diarrhea and skin toxicity represented the main toxic effects observed with the drug, while anti-tumor activity was observed in hepatocarcinoma and in renal tumors. BAY 43-9006 has been subsequently evaluated in a phase I study in combination with gemcitabine; anti-tumor activity in a patient with ovarian cancer has been seen thus far. CI-1040 is a MEK inhibitor, whose main side-effects in phase I study have been diarrhea, skin toxicity and fatigue [50]; the drug is currently being tested in combination studies.

Dr Johann De Bono (Royal Marsden Hospital, London, UK) gave a talk on cell cycle inhibitors. They include non-specific protein kinase inhibitors, such as staurosporin and UCN-01, selective, but 'pan', cyclin-dependent kinase (CDK) antagonists, such as flavopiridol, semi-selective second-generation antagonists, which target either CDK1, 2 or 5, or CDK4. CYC-202 and BMS-387032 are CDK inhibitors in the clinic. A phase I trial of CYC-202 was performed at the Royal Marsden Hospital with CYC-202 administered orally to fasting patients twice daily for 7 days every 21 days [54]. Main toxicities in the study were nausea, vomiting, fatigue, anorexia, reversible renal and liver dysfunction. No evidence of anti-tumor activity was observed. A second phase I study was carried out in France, with CYC-202 being given orally b.i.d. for 5 days every 21 days [55]. Similar toxicity was observed in this study, i.e. DLT liver dysfunction, renal dysfunction, DLT skin rash and hypokalemia. MAG3 renal perfusion scans suggested that transient effects on creatinine are due to a reversible fall in renal blood flow, due to an uncertain mechanism. BMS-387032 has been studied in three phase I trials (1-h infusion every 21 days, 24-h infusion every 21 days and 1-h infusion weekly) [56–58]. Main toxic effects were transient early neutropenia, mild nausea/vomiting, mild constipation/diarrhea and liver enzyme derangement. Anti-tumor activity was observed in a patient with leiomyosarcoma of the bladder. Another strategy to achieve cell-cycle inhibition is to target heat shock protein (Hsp) 90, a molecular chaperone required for protein folding. Hsp90 inhibition induces decreased CDK4 levels in the clinic, and this can be pursued with 17-allylamino-17-demethoxygeldanamycin (17-AAG). DNA methyltransferase inhibitors (antisense to DNA methyltransferase, decitabine) and histone deacetylase (HDAC) inhibitors act through reversal of transcriptional repression of CDKi and increase of endogenous CDKi expression. Targeting mitotic kinases is another strategy to achieve cell cycle modulation. The kinesin superfamily includes ATPases that produce directed mechanical force along the microtubule polymer. SB-715992 is a potent highly specific small molecule inhibitor of KSP; it induces apoptosis and it has no detectable effect on microtubules *in vitro*. Phase I studies of SB-715992 have been carried out with two schedules. DLT was neutropenia, prostate-specific antigen response was observed in HRPC; the every-3-week schedule was chosen for further studies.

Dr Susan Bates (National Cancer Institute, Bethesda, MD) gave a talk on histone deacetylase inhibitors, mainly focusing on depsipeptide, which has activity in T cell lymphoma and as a modulator of molecular targets. DNA in chromatin is packaged into orderly repeating protein–DNA complexes known as nucleosomes, which consist of a core of histone proteins around which DNA wraps. Histone acetylation is a well-understood post-translational modification; the extent of acetylation depends on two enzyme families, i.e. histone acetyltransferase and histone deacetylases. Histone deacetylases inhibitors in the laboratory and in the clinic include short-chain fatty acids (butyric acid, sodium butyrate and valproic acid), bicyclic depsipeptide (depsipeptide), organic hyroxamic acids (trichostatin A, SAHA and oxamflatin), cyclic tetrapeptide (trapoxin A) and benzamides (CI-994 and MS-27-275). The mechanism of growth inhibition mediated by histone hyperacetylation include transcriptional activation of a pre-programmed set of genes, such as p21, p27, cyclin E, induction of markers of differentiation, cell cycle arrest and growth inhibition. Non-histone targets of histone deacetylase inhibitors include cellular proteins which are acetylated in addition to histone, such as Hsp90, β-catenin and p53. Depsipeptide is a histone deacetylase inhibitor, which has been tested in a phase I study with a day 1 and 5 every 21 days schedule [59]. Main toxicities in this study were nausea, vomiting, fatigue, neutropenia, thrombocytopenia, hypocalcemia, ECG changes and atrial fibrillation. Responses were observed in cutaneous T cell lymphoma (CTCL) and peripheral T cell lymphoma (PTCL). A phase II study was then run at the NCI using depsipeptide in patients with CTCL or PTCL [60]. Responses were observed in CTCL patients (11.5% complete remission plus 31% partial remission) and also in PTCL patients (26% partial remission). Dr Bates summarized the potential roles in anti-cancer therapy for histone deacetylase inhibitors, i.e. they consist of a role in cancers with known alteration of histone acetylation or deacetylation, a role in combination with conventional chemotherapy, in combination with demethylating agents and in combination with targeted agents (acting as inducers/inhibitors of molecular targets).

A second presentation on histone deacetylase inhibitors was given by Dr Alfredo Budillon (National Tumor Institute, Naples, Italy). He focused on SAHA, which is

a small-molecular-weight inhibitor of HDAC activity. Phase I/II clinical trials have shown low toxicity and evidence of anti-tumor activity; on the other hand, this compound has potential for synergism with radiotherapy, chemotherapy and biologicals. Mechanisms of SAHA anti-tumor effects include VEGF downregulation and interference with angiogenesis, differentiation effects, G_1/S arrest due to p21 upregulation and CDK inhibition, and induction of apoptosis. SAHA has been evaluated in preclinical studies in colorectal cancer. SAHA induced growth inhibition in a time- and dose-dependent manner in colorectal cancer cells at micromolar concentrations and independently of p53 status or MDR-1 expression. In these cells, SAHA induced a time-dependent cell cycle perturbation which is associated with upregulation of p27 and p21, CDK inhibitor expression, and time- and dose-dependent apoptotic cell death [61]. SAHA treatment led to down modulation of TS expression, a critical target for chemotherapeutic agents active in colorectal cancer, such as 5-fluorouracil (5-FU) and raltitrexed. Combination treatment with SAHA and either 5-FU/FA or raltitrexed produced synergistic and additive anti-proliferative effects in colorectal cancer cells. The effect of SAHA on TS and p53 expression as well as combined perturbation on cell cycle kinetic could at least in part explain the schedule-dependent interaction with 5-FU and raltitrexed on tumor cell proliferation. Overall, these results demonstrated that SAHA has anti-tumor activity in colorectal cancer cells and can be combined with cytotoxic drugs currently used for colorectal cancer treatment, indicating that it should be further investigated for therapeutic use in patients with this malignancy. Preclinical data have shown a SAHA anti-proliferative effect in a number of different squamous head and neck cancer cell lines. SAHA has been shown to have synergistic anti-proliferative and apoptotic effects with gefitinib in head and neck cancer cell lines; interestingly, this effect is evident also in cells partially resistant to gefitinib. The mechanism of SAHA and gefitinib interaction could be explained by the SAHA-induced downregulation of EGFR and ErbB2 expression and signaling. EGFR is regulated by SAHA mainly at the transcriptional level, while ErbB2 is probably regulated post-translationally. Overall these results show that SAHA, when combined with gefitinib, has a potential anti-tumor activity in head and neck cancer and should be further clinically evaluated in this disease.

Final Lecture—Academic drug development in Europe

Professor Chris Twelves (University of Leeds, Leeds, UK) gave a final lecture on academic drug development in Europe, that aims to develop new cancer treatments not driven or controlled by the pharmaceutical industry, working through individual institutions, regional or national research groups and international collaborations. Preclinical collaboration is paramount; an example is the EORTC/Cancer Research UK/NCI screening programme, with the Brussels screening laboratory established in 1970s and a programme to evaluate NCI compounds in Europe established in 1993, to compare potency, novelty of structure or mechanism and to establish 'drugability'. The above collaboration has involved approximately 1100 compounds reviewed by the EORTC/Cancer Research UK panel; up to now, 50 compounds have been selected for further evaluation, of which 26 were dropped for lack of 'drugability', two for lack of activity *in vivo* and four for other reasons. Cancer Research UK is the largest volunteer-supported cancer organization in the world; it was founded in February 2002 following a merger of the Imperial Cancer Research Fund and Cancer Research Campaign. Objectives are '…to protect and promote the health of the public by research into the nature, causes, prevention, treatment and cure of all forms of cancer…'; it is funded from voluntary donations, and it funds scientists and clinicians in universities, hospitals and institutes. The cancer research drug development process has an approximate duration of 18 months for the preclinical part and 30 months for the clinical. Cancer Research UK early clinical trials in 1980–2002 have involved 89 agents, 72 of which were in phase I trials, 13 in phase I and II trials, and four in phase II trials. During these years, Cancer Research UK licensed compounds were: temozolomide, etoposide phosphate, biantrozole and lentaron. Cancer services have been rearranged over recent years in the UK mainly because of the concern about the higher cancer mortality in the UK with respect to other industrial countries. In particular, cancer centers and units have been set up, additional funding has been planned, and coordination has been improved. The National Cancer Research Network (NCRN) was established with the desire to improve treatment for current and future patients by increasing the number of patients entering trials. The recruitment has increased over recent years and now there is a focus on quality. The National Translational Cancer Research Network (NTRAC) comprises 14 centers distributed across the UK, selected by peer review for their excellence in translational cancer research. This network has been established only recently and is still developing its identity; each center uses funding in different ways. New directions and challenges for academic drug development in Europe involve the EU directive, which will increase trial costs ($\times 6$), increase complexity of study activation ($\times 2$), delay study activation (4–11 months) and complicate the conduct of the study (adverse event reporting). On the other hand, the EU directive will not increase patient safety nor will it harmonize procedures. How to identify a 'niche' for academic drug development? Early drug development is definitely suitable; further development may include rare tumors; special populations, such as the elderly, children and people with organ dysfunction; identification of patients not worth treating; integration of new agents

into current regimens; and use of at least two unlicensed agents together in combination.

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