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Meeting Report XIII International Charles Heidelberger Symposium and 50 Years of Fluoropyrimidines in Cancer Therapy Held on September 6 to 8, 2007 at New York University Cancer Institute, Smilow Conference Center

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Abstract

This conference opened with Franco Muggia, host and principal organizer, thanking Joseph Landolph, co-Chair of the International Scientific Organizing Committee and its members (Franco Muggia, co-Chair, Max Costa, Steven Burakoff, Howard Hochster, Eliezer Huberman, John Bertram, Peter Danenberg, and Richard Moran); the members of the Local Organizing Committee (Drs. Costa, Guttenplan, Geacintov, and Hochster); and the Charles and Patricia Heidelberger Foundation for Cancer Research for developing the scientific program and for working to help him create this special symposium honoring the late Charles Heidelberger, former president of the American Association for Cancer Research, member of the National Academy of Sciences, and extraordinary scientist in the fields of carcinogenesis and cancer chemotherapy. It was most appropriate to commemorate the 50th anniversary of the patent obtained by him for 5-fluorouracil (5FU), a drug that came to symbolize the promise chemotherapy of nonhematologic malignancies. After this compound was shown to be helpful in the treatment of colorectal and breast cancers, Dr. Heidelberger proceeded to develop other fluoropyrimidines and to inspire Ph.D. students and postdoctoral fellows to investigate their mechanisms of action and to develop assays applicable to clinical specimens (what we now refer to as translational science). Steven Burakoff, director of the NYU Cancer Institute (2000 to 2008), followed with welcoming remarks. Dr. Burakoff pointed to his personal fortuitous connection to the Symposium: The famous immunologist, Michael Heidelberger, Charles' father, who was known as the Father of Immunochemistry, trained Elvin Kabat while at Columbia, who trained Baruch Benacerraf, who moved from NYU to Harvard and subsequently became Burakoff's mentor. The renowned NYU Division of Immunology carries the name Michael Heidelberger because he spent more than 30 years in the Department of Pathology at the NYU School of Medicine after retiring from Columbia University.

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5FU as a Prototype for the Design of Targeted Therapy

Targeted therapy has become the ideal of therapy in general and of cancer therapy in particular. From this perspective, 5FU and methotrexate (MTX) can be considered as the first rationally designed targeted anticancer drugs. Furthermore, the development of 5FU emerges as a prototype of translational research, not only in the last two decades when such research has been emphasized but, thanks to Heidelberger, even in the first few years after its discovery and introduction into the clinic. 5FU was developed on the basis of findings in the 1950s that cancer cells incorporated a larger amount of the uracil base into the DNA than normal cells (1, 2). From the halogen-substituted uracils, 5FU appeared to be the most active and promising drug (3), and clinical studies were immediately initiated (4), in which not only plasma concentrations were measured but also tissues' disposition using radioactive 5FU (5). It was rapidly recognized that 5FU was metabolized by enzymes from normal pyrimidine metabolism (6) and that it was incorporated both into RNA and DNA (5), although it has long been debated what accounted for its antitumor effects. Also degradation pathways of 5FU were similar to the natural bases (7), demonstrating an important role for dihydropyrimidine dehydrogenase (DPD). 5FU can be activated by the subsequent action of uridine phosphorylase and uridine kinase (8), while Heidelberger already postulated that a activation via the thymidine phosphorylase and thymidine kinase pathway is unlikely due to lack of the co-substrate deoxyribose-1-phosphate. Later some significant differences were found: in contrast to uracil, 5FU is an excellent substrate for mammalian orotate phosphoribosyltransferase (OPRT) (ref. 9), for which an association with 5FU sensitivity was observed both in experimental models and in clinical samples. It was also recognized by Heidelberger's group that the 5FU metabolite 5-fluoro-2′-deoxyuridine monophosphate (fdUMP) is a very potent suicide inhibitor of the pyrimidine *de novo* enzyme thymidylate synthase (TS) (refs. 6, 10, 11) and resistance to 5FU was already associated with disturbances in TS (12). In the early 1980s a breakthrough in this research was achieved by the Heidelberger laboratory in applying sensitive biochemical assays to determine the role of TS activity and the extent of TS inhibition in relation to the response to 5FU in animal models (13), followed by similar studies in patients (14). This led to more extensive studies that clearly demonstrated that response to 5FU was related to TS activity (15, 16). Two novel technical developments enabled the laboratory to perform retrospective studies on the role of TS in large patient populations: the development of specific antibodies against TS (17) and sensitive reverse transcriptase (RT)-PCR assays (18). In a retrospective study, Peter Danenberg's group demonstrated that low tumor TS and DPD levels were associated with a very high response rate and survival compared to the high TS and/or DPD group (19). Application of this knowledge in a prospective study by measurement of pretreatment TS and DPD levels demonstrated that it is possible to double the response and survival to single agent 5FU (with leucovorin) by selecting patients with both a low TS and DPD level (20). Recent developments in therapy of colorectal cancer have demonstrated the additional benefit of concomitant chemotherapy with e.g., oxaliplatin (21) and irinotecan (22), as well as the benefit of anti-angiogenic treatment with the vascular endothelial growth factor (VEGF) antagonist Bevacizumab (23). Therefore, predicting response to 5FU combination treatment on the basis of TS and DPD analysis in selecting patients may need to be revisited.

What can we learn from these studies for current targeted drug development? Sledge (24) defined targeted therapy in its simplest form as "drug plus a molecular target"; hence targeted therapy implies therapy toward a molecularly defined target. A targeted therapy should attack a biologically important process (usually but not necessarily a single molecule) preferably one central to a hallmark of cancer. The target should be validated in the laboratory but subsequently needs validation in the clinic as well; the target should be measured reliably in order to prove its validity. When 5FU was developed a substantial amount of information on its potential metabolism was known compared to other drugs

developed in that period. Its target(s) were rapidly characterized. However, available techniques were insufficient to properly validate the target in patients, although some preliminary measurements were performed (5). The latter, target validation in the tumor and normal tissues/body fluids, can be considered as essential for developing novel targeted drugs. For this purpose we can now use gene expression, gene polymorphisms, and protein expression [activity, immunohistochemistry, enzyme-linked immunosorbent assays (ELISAs)]. In several studies 5FU's anticancer activity has been shown to be related to TS expression (25). However, current research has clearly shown that this relation is not straightforward. In untreated patients high TS is a poor prognostic factor, but in adjuvant therapy this may be favorable because TS is also linked to proliferation, which makes a tumor sensitive to antiproliferative drugs. However, in advanced disease a low TS is favorable because it is easier to inhibit a low TS activity completely compared to a high TS. This inhibition has been shown in various clinical studies (26), and similar data were also shown in this symposium. Comparison of studies performed in the same patients with different methods (activity, protein expression, gene expression, gene polymorphisms) has also shown that one should be careful in extrapolating data from laboratory models to a patient. For example, in cell-free models a clear linear correlation may exist between activity, gene expression, and immunohistochemistry as was shown for the triple repeat of the TS enhancing region (TSER) in the promoter and gene expression (27). However, regulation of TS in a tumor is far more complicated, since TS translation is regulated by TS itself (28), while the transcription may also be regulated by p53 (29), which is obviously deregulated in tumors with a mutant p53. Hence, TS polymorphisms in lymphocytes are unlikely to predict the response of a tumor (30). They may, however, predict toxicity. Similarly, a low systemic DPD activity led to severe, sometimes life-threatening 5FU toxicity (31). Although several polymorphisms were linked with this low activity, the relation was not sufficiently strong to use sequence analysis for screening of patients, leaving enzyme activity analysis as the most reliable biomarker for DPD (32, 33). Although single nucleotide polymorphism (SNP) analysis is becoming a major tool in defining differences between individuals, one should be careful in extrapolating data from normal cells to the tumors. Nevertheless, quick and accurate genotypic and phenotypic assays should be made available to allow clinicians to select patients for adverse events. Research with 5FU has clearly shown that analysis of both the tumor and normal tissues is essential to select patients for optimal personalized treatment.

One important lesson from the past 50 years is that the target and its regulation should be clearly defined at all levels, such as gene copy number, gene expression, epigenetic regulation, posttranslational and posttranscriptional regulation, differential expression in tumor/normal tissue, and for enzymes kinetic parameters and intracellular regulation by signal transduction, normal metabolites, and other drugs. Next to that, essential pharmacokinetic parameters, such as bioavailability for oral drugs, metabolism, and tissue penetration and retention should be evaluated as well. Since most drugs are given in combinations, interactions with other drugs should be characterized, both at the tumoral level and in normal tissues (e.g., with endothelial cells for anti-angiogenic compounds), in addition to potential chemical interaction. Obviously, defining a novel target is an important first step, leading to successful treatment when carefully developed.

Session I: Environmental Carcinogenesis, Cancer Epidemiology, and Cancer Detection (Curtis Harris, Chair)

Curtis Harris discussed selected mechanisms that account for certain cancers arising from presumed environmental or endogenous carcinogens. Moon-sho Tang's work has focused on p53 mutations found in lung and liver cancers (34). Mutation "hot spots" in TP53 occur as a result of mutations in codon 249 in liver cancer, and in codons 157/ 158 in lung cancer

arising in smokers. His prior work had demonstrated that methylation of CpG sites by polyaromatic hydrocarbons in cigarette smoke explained the “hot spots” associated with lung cancer, whereas the affinity of aflatoxin B1 (AFB1) epoxide from aflatoxin for 249 AAG explained the “hot spot” in liver cancer. Exploring for other common carcinogens in smoke that have the potential to cause mutations by binding to methylated sites led to postulating a possible role for acrolein. In fact, this reactive molecule is commonly produced in the type of cooking by Taiwanese women, and is capable of binding to codon 249 and other methylated sites. Moreover, its abundance leads one to predict that it extensively forms adducts with thiols, depleting reduced glutathione and, in turn, inhibiting nuclear excision repair (NER) pathways. Presentations by Joseph Guttenplan and Nicholas Geacintov dealt with possible mechanisms of estrogen-induced carcinogenesis (35, 36). While cellular proliferation stimulated by estrogens in estrogen-responsive tissues such as the breast may lead to increased likelihood of DNA damage and cancer susceptibility, the Guttenplan laboratory has examined consequences of peroxidation of estradiol to reactive quinones, which have been shown to lead to rapid generation of apurinic sites, in mammary tissue and demonstrated metabolites that are mutagenic. Prevention of mutagenesis with free radical scavengers is currently being pursued. In the Geacintov laboratory, the chemistry of premarin (consisting mostly of equine estradiol differing in one double bond) and interactions with DNA are being pursued at a structural level. Studies are ongoing on how adduct conformation influences the efficiency of NER. The p53-inducible gene *wip1* as a marker of carcinogenesis is being studied by Al Fornace (37). Interest in this phosphatase-encoding gene by his laboratory ensued after its up-regulation following radiation stress signaling. *Wip1* is often up-regulated in colon and breast cancers that are mostly p53 wild type; on the other hand, its depletion leads to senescence but not cancer, presumably by activation of p38 (that is normally inhibited by *wip1*). How this gene relates to ataxia telangiectasia mutated (ATM) phosphatase is a major future question.

Emerging carcinogenic mechanisms in human cancer were addressed by the next three talks. Curtis Harris began by emphasizing the evidence for the importance of proinflammatory cytokines not only in cancer causation but also cancer progression, thus altering prognosis (38). For the latter, any study of prognostic factors in human cancers needs to first develop a training set, and once established, to validate its significance in test cohorts. As an example, he cited exploring the prognostic value of 10 cytokine levels (reduced from 15 cytokines in the training set) in patients who had undergone resection for stage II lung adenocarcinomas. More recently, he has focused on micro RNAs (miRNAs) and identified five, among them miR21, that may be associated with poor survival (hazard ratios of 4.7 for high levels relative to low levels), and whether chemotherapy may alter its prognostic significance. Bill Rom described his biomarker program for the early detection of lung cancer relying on serum determinations, analysis of hydrocarbons in breath, and examination of bronchial specimens including cytology, RT-PCR, and DNA microarrays. An 80-gene assay with a better prediction of the presence of a cancer than routine bronchoscopy has been developed (39). Fredicka Robertson used a number of new molecular tools to define key steps in skin carcinogenesis that follow the classical pathway of initiation (e.g., by ultraviolet light), promotion (e.g., by phorbol esters), and progression to altered terminal differentiation resulting in squamous cell carcinomas. While studying angiogenesis in the benign papillomas resulting from promotion, the laboratory identified a new splice variant of VEGF (VEGF 205*), and went on to study its function in human umbilical vascular endothelial cell (HUVEC) cells, where it is not mitogenic but leads to tubule formation through actions on an integrin-linked kinase. This isoform’s effect on survival of endothelial cells relates to VEGFR2 activation of PI3Kinase, and is blocked by rapamycin [inhibiting the downstream activation of mammalian target of rapamycin (mTOR)] (ref. 40).

Session II: Cancer Biology (Eli Huberman, Chair)

From epidemiology of hormone-dependent cancers in women, Malcolm Pike derived some conclusions in relation to the factors implicated in enhancing or decreasing cancer susceptibility. Specifically, he has related the risk of breast cancer to the number of cells present; these proliferate during the luteal phase of the menstrual cycle when both estrogen and progesterone are present. Mammographic density relates to risk, because it bears a relationship to epithelial cell number (although the density may be in large part derived from “active stroma”). Body mass index (BMI) is a risk factor after menopause, and presumably BMIs over 30 slow down the normal involution of the breast with age. With menopause, thin women have a lower risk but their risk may be altered with estrogen replacement. The role of progesterone may be more complex: replacement with both estrogen and progesterone after menopause continues to have the effect on cell proliferation that it had during the luteal phase prior to menopause. However, high doses may promote differentiation in the presence of prolactin. This might explain why newer oral contraceptives with higher progestin seem to be associated with lower breast cancer risks. Epidemiologic data indicate that parity, number of pregnancies, and oral contraceptives diminish ovarian cancer risk, but as presented by Louis Dubeau, the likely interaction between hormones and heredity remain obscure. In particular, the ethnic variations, the degree of protection of pregnancy vis-a`-vis oral contraceptives, the rise in incidence after menopause, and the lasting protective effect of a late pregnancy are unexplained. To explain the role of BRCA1 in ovarian carcinogenesis, Dubeau has postulated that loss of BRCA1 function leads to dysregulation in granulose cell function and in turn the altered hormonal function is ultimately involved in the ovarian cancer predisposition. He has obtained evidence in conditional BRCA1 knockouts in granulosa cells (and also in 10% of pituitary cells) of dysregulated menses in mice that eventually develop adenomas in mullerian epithelium (41).

Shifting to the subject of stem cells, Eli Huberman described the pluropotential capacity of blood mononuclear cells, and how under certain stimuli, they take up a wide variety of differentiation pathways: lipopolysaccharides induce macrophage differentiation, interleukin 2 (IL-2) leads to lymphocytic differentiation, epidermal growth factor initiates epithelial lineages, VEGF leads to endothelium, hepatocyte growth factor gives rise to hepatic cells, insulin growth factor to islet cells, and nerve growth factor to neuronal differentiation. Phenotypic changes are associated with markers of differentiation, but biological differences may exist depending on the cell of origin that was exposed to the differentiating agent (42). The polo-like kinases involved in centrosome regulation were the subject of Wei Dai’s presentation, as he investigated their regulation of HIF-1α. Plk3 functions as a tumor suppressor gene partly by repressing HIF-1α, an action that is antagonized by plk1 (43). John Bertram updated concepts on the action of retinoids in inhibiting carcinogenesis. Such inhibitory actions were first demonstrated in the early 1970s by Cathy Reznikoff in 10T1/2 cells from the Heidelberger laboratory; the clinical utility of all-trans retinoic acid (ATRA) in acute promyelocytic leukemia and the reversion of premalignant oral leukoplakia changes highlighted the potential of retinoid action in reversing malignant or premalignant changes. Synthetic ligands to retinoid X receptor (RXR) nuclear receptors offer the promise of overcoming some of the troublesome side effects of retinoids when given in clinically therapeutic doses. Bertram’s effort have focused on identifying their positive effects by induction of connexin 43, as well as expression of collagen, sebum, and other markers of differentiation in skin. Slowing down of HeLa cell growth has been shown by inducing expression of connexin 43 (44). Robert Schneider described locally advanced breast cancer as an example of how tumor growth and progression involves the uncoupling of translation and the usual inhibitory signals that regulate normal cell growth and survival. In collaborative studies with Silvia Formenti, he has shown that expression of eIF4G and

4EBP1 are increased in these large tumors with considerable consequences on the regulation of protein synthesis by mTOR. This kinase phosphorylates 4EBP1 and when inactivated, leads to cap-dependent mRNA translation. When there is an excess of unphosphorylated 4EBP1, then through an alternate ribosomal splicing mechanism (IRES) only some RNAs go on to be translated through the actions of eIF4G (45).

Epigenomics was the subject of Ari Melnick's presentation. He has set up extensive collaborations to begin identifying the relevant epigenetic changes that play a major role in acute leukemias. Such studies include determination of the status of DNA methylation through the *Hpa*II-tiny fragment enrichment by ligation-mediated PCR (HELP) assay, the status of histones in the cellular genome by chromatin immunoprecipitation (CHIP)-on-chip techniques, and characterization of the genomic by comparative genomic hybridization (CGH). Such information can be predicted to greatly aid efforts in epigenetic therapy that have just begun.

Session III: Models of Cancer Causation and Treatment Interventions (Joseph Landolph and Max Costa, Chairs)

Joseph Landolph showed that insoluble nickel compounds (nickel subsulfide, NiS, green/black NiO) are phagocytosed into C3H/10T1/2 cells and induce a combination of genotoxic events-chromosome breakage, micronucleus formation, and gene amplification-and epigenetic events (silencing of gene expression). Nickel compounds cause mutations/amplifications/rearrangements of four proto-oncogenes to activate them to oncogenes, and inactivation of four tumor suppressor genes by mutation, methylation of gene promoters, and chromosomal breakage and loss, leading to global disruption of control of gene expression. This results in differential expression of 130 genes in nickel-transformed 10T1/2 cells, including over-expression of calnexin, Wdr, and ect-2 genes and silencing of expression of the DRIP/TRAP80 and β-2 centaurin genes. This global disruption of gene expression then results in induction and maintenance of morphological and neoplastic cell transformation (46). Max Costa introduced the subject of nickel's effects on biological systems. This metal has no role in the animal kingdom and doesn't bind to metallothionein or ferritin; its ions have profound acute effects on Iκ B kinase (IκK)/nuclear factor κB (NFκB) and on hypoxia-inducible factor (HIF); these ions usually arise through phagocytosis of nickel present in particulate air pollution. Of greatest concern is the ability of Ni to cause changes in DNA methylation, changes that are related to dimethyl- and monomethylation of histone3 lysine position 9, presumably by its inhibition of enzymes that demethylate lysine. These demethylases are iron dependent, but nickel and cobalt bind better than iron, leading to global epigenetic effects. These are compounded by hypoxia and effects on decreasing dihydrofolate reductase (DHFR) and MLH1 through demethylation. The potential of nickel to disrupt epigenetic homeostasis and global DNA methylation highlights the need to control air pollutants containing this metal (47).

Shirley Taylor described actions of DNA methyl transferases (DNMTs) and their role in maintaining gene expression. DNMT1 has several p53 binding sites, and its expression increases with loss of p53. This relationship explains in part the global hypermethylation that occurs in culture during the process of immortalization, accounting for losses of various alleles such as p16 that are frequently reported in cell lines (48). Of course, these epigenetic changes may take place *in vivo* during cancer pathogenesis. The development of kinase inhibitors for the treatment of cancer was introduced by Robert Kramer, who traced the recent remarkable therapeutic accomplishments since the introduction of trastuzumab and imatinib in the past decade, and now joined by cetuximab, erlotinib, sorafenib, sunitinib, dasatinib, and lapatinib, with more to come. One may consider broad spectrum kinome inhibition versus single target, small molecules versus antibodies, and intra- versus extra-

cellular-receptor targets as potential approaches. Targets under active development include AKT, aurora kinase, IKK, p38, PI3K, and met. Their development is challenging researchers to come up with more information on tumor biology, the pathways that predominate, and the ligands within the tumor environment. The path to clinical use is long, however: from identification of a potential target to a “druggable” compound is often 10 years.

Daniela Dinulescu discussed treatment interventions both empirically derived and related to the ovarian cancer model in genetically engineered mice (49). In these orthotopic models, PTEN deleted and Kras or PI3K–mutated cells are injected into the infundubular bursa of mice, and endometrioid cancers arise in a background of endometriosis. The tumors are highly sensitive to cisplatin, but recurrences take place and are increasingly less sensitive. She went on to describe the isolation of slowly cycling (stem) cells that are more common in ascites, are c-myc amplified, and overexpress BCRP1 (50).

Session IV: Therapeutic Targets and Novel Strategies in Gastrointestinal Cancer (Richard Moran and Joseph Bertino, Chairs)

These last two sessions related to the chemotherapy legacy of Charles Heidelberger. Giuseppe Pizzorno discussed uridine phosphorylase as a negative prognostic factor, but perhaps also as a reason for selectivity of 5FU prodrugs such as capecitabine, although studies have focused more on thymidine phosphorylase. Joseph Bertino emphasized the dual mechanisms of 5FU action: RNA damage being favored after bolus administration, and DNA damage after continuous exposure. His laboratory developed a cell line that was resistant to short (bolus) exposures and this led to decreased incorporation into RNA, decreased uridine monophosphate kinase, and decreased folate polyglutamylation. He went on to explore the decrease in kinase activity and found that there was promoter methylation, and that its expression could be increased by 5-azacytidine.

Yves Pommier and Leroy Liu, central participants to prior topoisomerase conferences held at NYU, returned to provide an update on topoisomerase I as a validated antitumor target. Pommier described new topoisomerase I poisons that may have an advantage over camptothecins. The binding of these drugs to large molecular complexes between DNA and the enzyme is being extensively studied: the cleavage patterns of the indolocarbazoles, as an example, are remarkably different than the camptothecins; they are also not substrates of ABCG2. Liu studied a ubiquitin-like protein ISG15 that appears to be closely related to camptothecin sensitivity. Ubiquitin-like proteins are often induced by viral and bacterial infections, in the endometrium during first pregnancy, and in tumors such as endometrial and colon cancers. ISG15 expression is inversely correlated with topoisomerase I down-regulation during exposure to camptothecins; in addition, cell lines selected for camptothecin resistance have low ISG15 (51).

Clinical use of FUDR, the second fluoropyrimidine introduced by Heidelberger, was described by Nancy Kemeny and Franco Muggia. Kemeny provided evidence through phase II and phase III studies on the impact of intra-arterial FUDR in the survival of patients with liver metastases. Even in the presence of more effective systemic therapy its use in second line therapy of metastases impacted on 2-year survival rates; in addition intra-arterial use after resection of the metastases shows a diminution in recurrences. The pharmacologic properties of FUDR versus 5FU also render it suitable for intraperitoneal administration (IP). Muggia is pursuing postoperative IP FUDR as part of combined modality therapy in gastric cancer. Surgical oncologists from Germany, Karl-Heinrich Link, and from the United States, Elliott Newman, elaborated on the current role of surgery in the advances of the treatment of colorectal and gastric cancers, respectively. Link, a Heidelberger trainee, has also devoted part of his career to identifying predictors of fluoropyrimidine sensitivity. The

session ended with presentations on the emerging molecular classification of colon cancer by Stan Hamilton, historical overviews to the present on the treatments of colorectal cancer by Eddie Chu, and of gastric cancer by Howard Hochster. Hamilton emphasized the role of molecular markers to improve staging, identify micrometastatic disease, and as potential surrogate markers of outcome; also the hope is to be able to delineate a metastatic phenotype. Chu described the long hiatus between 1957 and 1996 [from first use of 5FU to when irinotecan gained Food and Drug Administration (FDA) approval] as being the era of 5FU alone or with "modulators" in treating colorectal cancer-an unprecedented long run for any treatment-and went on to describe the strides made in overall survival by the recent addition of oxaliplatin and the new "biologic" anti-angiogenic and anti-EGFR therapies. Although the improving outcome of gastric cancer in relation to new systemic therapies beyond 5FU has been less well delineated, Hochster pointed to interest in the oral 5FU prodrug S1 in leading the way to new adjuvant strategies. In addition to IP FUDR described earlier, the platinum drugs cisplatin or oxaliplatin in combination with 5FU or capecitabine, anthracyclines, taxanes, and the topoisomerase I-targeted drug irinotecan, are all part of clinical trials in locally advanced and disseminated gastric cancers alone or in combination with radiation.

Session V: Therapeutic Targets and Novel Strategies in Gastrointestinal and Breast Cancers (Masatoshi Watanabe and Franco Muggia, Chairs)

Watanabe described his laboratory's attempt to study prostate cancer *in vitro* and replicate the tissue environment in an effort to have more reliable surrogates of sensitivity to therapeutic interventions. In spheroids he has shown that cells make VEGF more than in two-dimensional cultures; also oxaliplatin is more effective in spheroids, although efficacy varies with zone: central, which is not viable; the intermediate zone, which is mostly quiescent and shows increased p27 and PARP; and the proliferating zone; the intermediate zone is under hypoxic stress and shows many methylated genes and increased resistance. In an effort to replicate the *in vivo* environment, he is attempting to establish spheroid cultures with cocultures of stroma, extracellular matrix, and adipocytes.

Peter Houghton focused on insulin-like growth factor-1 (IGFR1) receptor as a therapeutic target in childhood sarcoma. Interest in this area has derived from the known t(2;13) translocation that turns on IGF2. Other sarcomas also may be influenced by this pathway: IGF1 is a mitogen for osteosarcoma and IGF-BP3 is increased in Ewing's where T(11;22) is present. Accordingly, Houghton has been interested in IGF1R inhibitors that are being considered for clinical trials, and in their possible synergy with mTOR inhibitors: IGFs appear to protect against rapamycin-induced apoptosis; therefore, a dual blockade may enhance the efficacy of rapamycin (52).

Robert Ladner shifted the discussion to one gene: dUTPase. This is an S-phase dependent gene that exerts a marked influence on dUTP pools, that are usually tightly controlled. When dUTPase is overexpressed, small interfering RNA (siRNA) leads to up to 60 × sensitivity to FUDR in MCF7 and SW620 cells. Oxaliplatin also reduces dUTPase expression, possibly an explanation to the known oxaliplatin/5FU synergy. Other inhibitors of dUTPase are being sought (53).

TS polymorphisms have been among several determinants of drug action studied by Heinz-Joseph Lenz. High TS activity is associated with shorter overall survival in various colorectal cancer trials. Its down-regulation by a drug such as vorinostat is being tested clinically in refractory disease. The significance of germ-line polymorphisms in the promoter is also being investigated. Focusing on identifying factors leading to decreased

response to fluoropyrimidines may lead one to treat with other agents, in fact, in colon cancer, high TS expression seems to be associated with better response to irinotecan.

Three talks focused on folates. David Goldman described the unique features of pemetrexed, and perhaps most relevant that it is a 300 × better substrate of folyl polyglutamyl synthetase (FPGS) than methotrexate. An additional feature of pemetrexed may be that it is a substrate of a newly described protein-coupled folate transporter (PCFT) that is commonly expressed in spleen, liver, and the upper gastrointestinal tract, and functions best at an acidic pH. This channel for pemetrexed was discovered by comparing its activity in wild-type or reduced folate carrier (RFC)-null cells: collateral sensitivity to pemetrexed was found in the latter (54). Since PCFT is widely expressed in tumors, the selectivity of pemetrexed over other antifolates may be enhanced in these tumors; moreover, its efficacy may be affected by the level of folate. Ann Jackman provided an overview of the potential for TS folate inhibitors tracing its historical origins on CB3717, raltitrexed, and pemetrexed, and then focusing on two novel agents. In addition to their activity against the enzyme and other enzymes where folate are cofactors or being regenerated to reduced folates by DHFR, knowledge of transport and their affinity for FPGS are key features in predicting their spectrum of activity. An antifolate being developed is BGC 9331, which is transported via the RFC, but in contrast with the other TS inhibitors is not a substrate for FPGS. Phase I and II clinical studies are complete with activity observed in several tumor types, e.g., gastric cancer. Another compound, BGC 945, with high affinity to the α folate receptor and very low affinity to the RFC is being developed for treatment of tumors expressing high folate receptor levels; very little systemic toxicity is expected (55). Clinical studies are planned and a range of biomarkers are being developed (e.g., folate receptor levels). Furthermore, TS inhibition may be studied by using 18Fluorothymidine as a reagent for positron emission tomography (PET) imaging. Richard Moran studied the epigenetic control of tissue-specific expression for FPGS. He examined the contrast between the promoters in liver and kidney, and the promoters in proliferating tissues and tumor that utilized IRES and are unmethylated (56).

Gemcitabine was the subject of Bill Plunkett's and Fritz Peters' talks. This fluoropyrimidine drug was selected for development at Lilly by the late Gerald Grindey and was the first drug to show consistent clinical benefit in clinical trials of patients with pancreatic cancer. Plunkett provided the rationale for the development of fixed-dose rate schedules that have been tested with varying results in the clinic. He also pointed out that gemcitabine diphosphates inhibit ribonucleotide reductase, although the major antitumor effects probably derive from activation to triphosphates and DNA incorporation. Peters and his group have done comprehensive studies on gemcitabine nucleotides and their perturbation in various clinically relevant combinations (cisplatin, taxanes, radiation, and more recently bortezomib), although mostly confined to determination in peripheral blood mononuclear cells. He is also exploring ribonucleotide inhibitors to potentiate the effects of gemcitabine, and the role of cytidine deaminase in predicting the likelihood of response; lower levels signify a better response, but it also is a marker of toxicity, and patients with overexpression may be undertreated.

Kathy Danenberg's presentation focused on how to better predict response of various tumors to both chemotherapy and "targeted" therapies. Predicting response from determinations on formalin-embedded tissues has been central to the effort of Response Genetics, a company that uses DNA and RNA arrays to determine emerging predictive markers. For example ERCC1 has recently emerged as a marker of platinum sensitivity in lung cancer. Investigators are utilizing determination of this protein's expression by RT-PCR to guide clinical trials.

Finally, gene expression profiles to direct therapeutic decisions was also the topic of Joffre Baker. Oncotype Dx has been widely incorporated into breast therapeutics and in clinical trials, in the subset of patients that are estrogen receptor positive and lymph node negative. The future for validated gene expression profiles appears bright in the formulation of new treatment strategies.

This symposium was representative of the legacy of Charles Heidelberger ranging from cancer causation to mechanisms of drug action and to laboratory-guided clinical applications. This legacy derived not only from his accomplishments as a scientist, but also from making his laboratory a fertile ground for training. A forthcoming issue of *Molecular Cancer Therapeutics* will expand on items covered in this symposium proving the continued viability of the Heidelberger view of anticancer drug development: recruiting students and collaborators to pursue biological findings in the laboratory and in the clinic.

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