Nineteenth Annual Pezcoller Symposium: Hypothesis-Driven Clinical Investigation in Cancer

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Abstract

This symposium was held in Trento, Italy, from June 14 to 16, 2007, and was cochaired by William G. Kaelin, Enrico Mihich, and Charles L. Sawyers. A session was devoted to the proof of concept derived from successes in target-oriented therapeutics. Molecular targets must be identified in each patient because they are equally present in all patients with the same tumor type. A session was devoted to the identification of markers of drug effectiveness. Two sessions were focused on opportunities for developing new molecular target-oriented therapies. [Cancer Res 2007;67(23):11102–5]

Introduction

Progress in target cancer chemotherapy unveiled the advantages and limitations of the approach. Ways to optimize this chemotherapy, the identification of new sites of intervention, the development of markers of drug effectiveness, and the rational design of combination treatments are all discussed herein.

Report

Charles L. Sawyers stated that 85% of patients with relapsed chronic myelogenous leukemia (CML) have mutations in the ABL kinase domain altering imatinib sensitivity; the majority of them occur at residues altering the conformational flexibility of ABL such that it cannot achieve the closed conformation required for optimal imatinib binding. Dasatinib binds ABL in either conformation and is effective in imatinib-resistant patients. Resistance to dasatinib occurs almost exclusively through mutations at drug contact residues; some confer resistance to dasatinib but not to imatinib. The data favors combinations with these two compounds for CML and for other cancers. Preliminary data suggests that resistance to both of these inhibitors could be overcome by VX680. In general, the magnitude of kinase inhibition and size of the inhibitor’s area under the curve correspond to clinical responses. The responses could also be affected by mutations in “modifier” genes reducing kinase dependence. Thus, patients with glioblastoma fail to respond to epidermal growth factor receptor (EGFR) inhibitors if the tumor also contains a Pten mutation. Negative feedback loop blockade could lead to hyperactivated signaling pathways: mammalian target of rapamycin (mTOR) inhibitors could cause increased activity of Akt in some tumors, and this may counteract therapeutic effects. These difficulties may be solved through appropriate combination therapies. Obviously, patient-tailored kinase inhibitor therapy requires the evaluation of diverse molecular mechanisms for optimal response.

George D. Demetri emphasized that imatinib provided remarkable progress in the treatment of gastrointestinal stromal tumors (GIST). Mutations in the PDGFRα proto-oncogene of a KIT-negative GIST subset justified the development of new agents targeting related signaling molecules. Imatinib inhibits most mutated variants of KIT and a small subset of mutated PDGFRα kinases and induces a 3-fold increase in patient survival. Resistance follows secondary mutations in the tumor genome. The multi-targeted kinase inhibitor, Sunitinib, improved responses in patients with imatinib-resistant GIST. Other multikinase inhibitors and combinations of agents are being tested to inhibit other pathways, including phosphatidylinositol-3-kinase (PI3K) and mTOR.

Imaging techniques and response biomarkers are being developed. Inhibition of heat shock protein-90 (Hsp90) selectively destroys numerous structural mutants of KIT or PDGFRα. Early clinical data of the Hsp90 inhibitor IPI-504 suggests that destruction of KIT is possible by this mechanism. Based on the GIST example, translational research could be implemented to control other common cancers.

William Pao stated that gefitinib and erlotinib induce major responses in certain patients with non–small cell lung cancers (NSCLC). EGFR tyrosine kinase somatic mutations are more commonly associated with sensitivity to gefitinib and erlotinib in patients who never smoked cigarettes, whereas mutations in KRAS associated with primary resistance were more frequent in patients with significant tobacco exposure. Second site EGFR mutations occur in about half of the patients with acquired resistance to these drugs. Substitution of methionine for threonine at position 790 (T790M) is linked to genetic susceptibility to lung cancer. Transgenic mice with EGFRT790M alone or with drug-sensitive L858R mutation were developed and both developed lung adenocarcinomas requiring mutant EGFR for tumor maintenance and were resistant to an EGFR kinase inhibitor. The models should be useful for developing improved therapies for NSCLC harboring the EGFRT790M mutation.

William Kaelin indicated that clear cell renal carcinoma is usually linked to loss of the VHL tumor suppressor gene and that restoration of VHL function suppresses tumor formation. pVHL regulates hypoxia-inducible factor (HIF) polyubiquitination. When pVHL is defective, or oxygen levels are low, HIF accumulates and activates 100 to 200 genes involved in adaptation to hypoxia, such as vascular endothelial growth factor (VEGF). HIF down-regulation is necessary and sufficient for pVHL to suppress VHL−/− renal carcinoma in vivo. Expression of HIFα and inactivation of VHL phenocopy similar pathologic changes. The risk of renal carcinoma linked to VHL alleles correlates with induced HIF deregulation. Thus, HIF and HIF-responsive gene products are validated therapeutic targets in kidney cancer. Drugs inhibiting VEGF, or KDR, have activity against renal carcinoma and several
adoptive immunotherapy are being studied with PET imaging. Such as tyrosine kinase inhibitors, tumoricidal antibodies, and receptor. The pharmacology and pharmacodynamics of agents such as [18F] 2-fluoro-2-deoxy-D-glucose, which measures glycolysis biomarkers. Positron emission tomography (PET) is well-suited for such as mTOR, integrin-linked kinase, phosphoinositide-dependent 'oncogene addiction' rendering them sensitive to PI3K inhibition. The challenge is identifying PI3K inhibitors with a usable therapeutic platelet-derived growth factor receptor, and Bcr-abl. The main Drugs developed for other purposes target PI3K, such as rapamycin or the downstream regulator Akt have thus far been approved. Despite the validation of the PI3K pathway, no drugs targeting PI3K or the downstream regulator Akt have achieved substantial benefit from targeted therapy. Some patients without EGFR mutations achieve significant benefit. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was used to study pretreatment unfractionated sera and identify NSCLC patients with improved survival following gefitinib and erlotinib treatment. Independently acquired mass spectra gave concordant results, and an algorithm predictive of time to progression and survival was generated and might be useful in selecting therapies for patients with advanced NSCLC. Assessment of eicosanoid expression in individual lung cancer patients seemed to identify those who would benefit from cyclooxygenase inhibition. However, this pathway is complex in lung cancer as some eicosanoids have antitumor activities. Gordon Mills discussed the possibility that 'oncogenic addiction' may provide targets for specific tumor growth inhibition. Despite the validation of the PI3K pathway, no drugs targeting PI3K or the downstream regulator Akt have thus far been approved. Drugs developed for other purposes target PI3K, such as rapamycin analogues, ether lipids, and inhibitors of EGFR, HER2, c-kit, platelet-derived growth factor receptor, and Bcr-abl. The main challenge is identifying PI3K inhibitors with a usable therapeutic index. Tumors with PI3K pathway aberrations may undergo "oncogene addiction" rendering them sensitive to PI3K inhibition. Intervention sites in the PI3K pathway include PI3K itself, the downstream regulator Akt, and other downstream components such as mTOR, integrin-linked kinase, phosphoinositide-dependent kinase-1, p70S6 kinase, and Forkhead/FOXO1. Steven Larson stressed that better-targeted therapies are likely to be guided by molecular imaging through the use of radiolabeled biomarkers. Positron emission tomography (PET) is well-suited for this purpose. Tumor responses have been studied with biomarkers such as [18F] 2-fluoro-2-deoxy-D-glucose, which measures glycolysis and [18F]fluorothymidine (FLT), which measures proliferation. Selective anticancer drug effects on key molecules may also be imaged with [18F]fluorodihydrotestosterone, an androgen receptor-binding agent and [186G]gallium-Fab2 herceptin, binding to HER2 receptor. The pharmacology and pharmacodynamics of agents such as tyrosine kinase inhibitors, tumoricidal antibodies, and adoptive immunotherapy are being studied with PET imaging. Todd Golub discussed the development of target-specific therapies based on the molecular characterization of a tumor at the level of DNA, RNA, protein, or other biomolecules. Gene expression profiling could be used to classify acute leukemias, childhood brain tumors, prostate cancer, lymphoma, and lung cancer. Gene expression profiling indicates which components of the signature might be worthy of conventional biochemically based high-throughput screens. In some cases, no single gene/protein, but a group of signature genes are required to explain the phenotype under study. In other cases, the target is found to be "undruggable." A small library could be screened to identify compounds modulating a defined gene expression signature of biological interest. This gene expression–based high-throughput screening was applied to discover compounds inducing the differentiation of acute myeloid leukemia (AML) cells and compounds inhibiting the activity of the Ewing sarcoma oncogene (EWS/FLI), or abrogating androgen receptor signaling in prostate cancer. The feasibility of using gene expression profile databases to systematically connect signatures of diseases to the signatures of gene product function or of drug action was established. Relevant connections were discovered in dexamethasone-resistant childhood leukemia, androgen response in prostate, and HDAC inhibition in various cell types. Neal Rosen outlined the rational design of combination therapies based on biomarkers indicating their combined molecular action and emphasized that the efficient clinical development of these therapies requires a knowledge of the kinetics of target inhibition by the agents employed. Immunohistochemical assays are not quantitative and it is impossible to obtain multiple biopsies over short time periods to ascertain the kinetics of inhibition. Target inhibition is determined in surrogate normal tissue, and only occasionally in tumor biopsies obtained before and after drug administration. These problems could be overcome with functional imaging. Hsp90 inhibitors cause the degradation of client proteins, HER2 being among the most sensitive. 17-AAG has significant anti-tumor activity in patients with advanced breast cancers over-expressing HER2. Herceptin Fab fragment with a 2 h half-life was chelated to positron emitting isotopes, including 68Ga. The reagent imaged the 17-AAG effects on surface HER2 expression in tumor xenografts as a function of dose and time after drug administration and is now being used in phase 2 clinical trials of 17-AAG and Herceptin. Tumors with mutant BRAF were quite sensitive to MEK inhibitors. Treatment of melanoma with MEK inhibitor PD0325901 resulted in objective responses and disease stabilization. In BRAF tumors, MEK inhibition was associated with D-cyclin loss, p27 induction, RB-phosphorylation loss and concomitant G1 arrest. As 18-FIT transport into tumor cells is phase-dependent, 18-FIT PET could constitute a marker of early response to MEK inhibitors. The FLT PET image declined after MEK inhibitor dosing and after drug discontinuation, the image stayed low until the tumor began to increase in size. The PET method is useful for assessing agents that cause stable disease and the heterogeneous responses of tumor metastases. Kevin Shannon discussed the use of genetically engineered mice to investigate treatment responses and provided evidence that hyperactive Ras is a promising therapeutic target. However, the critical target(s) of hyperactive Ras are largely unknown. Mice carrying conditional mutant alleles of the Nf1 tumor suppressor gene encoding neurofibromin (Nf1) or oncogenic Kras help in understanding how cells remodel signaling in response to hyperactive Ras. The Msi1-Cre transgene ablating Nf1 or activating KrasG12D expression in hematopoietic cells results in a fatal
myeloproliferative disorder (MPD) reminiscent of juvenile myelomonocytic leukemia and CML. Retroviral insertional mutagenesis identifies mutations cooperating with hyperactive Ras to induce progression from MPD to AML. CI-1040, a potent MEK inhibitor, had no effects in Nfl mice with MPD. However, MEK inhibition induced the regression of Nfl-deficient AMLs which uniformly developed resistance to CI-1040 in vivo, despite equivalent target inhibition in sensitive and resistant clones. Retroviral insertions in resistant AMLs were consistent with the outgrowth of preexisting clones during CI-1040 administration.

Barbara Weber emphasized that oncology drug development should be increasingly focused on molecularly targeted small molecules and should involve testing only in patients mostly likely to respond. Response prediction biomarkers are identified based on the hypothesis that all cancer abnormalities are fixed in the genome by genetic or epigenetic alterations, which are the targets and response determinants of targeted cancer therapeutics. At her institution, the IC50 for every compound in development was determined in 300 human cancer cell lines representing the spectrum of adult cancer patients. All cell lines were also profiled for DNA copy number, RNA transcript profiles and targeted genomic sequencing. The incidence and prevalence of cell line sensitivity predictors in primary human tumors were assessed. The overall data determined the clinical studies design. As targeted agents may also be more effective in combination, short hairpin RNA technology was used to identify lethal phenotypes that predict both effective targeted combinations and new targets. Combinations of targeted agents with standard cytotoxicities were also evaluated.

David Livingston indicated that short hairpin RNA library screening was initiated for proteins that, when depleted from human ovarian cancer cell lines, lead to inhibition of cell proliferation and may serve as ovarian cancer drug targets. With a hairpin library encompassing all 92 human tyrosine kinases in three different ovarian cancer cell lines, erbB3 was detected as a protein that, when depleted, was associated with a proliferative defect. The erbB3 protein was activated through phosphorylation and coimmunoprecipitated with P13K. Analogous results were obtained with fresh tumor cells from patients with late stage ovarian cancer. ErbB3 engaged P13K pathways, leading to AKT and mTOR modulations.

David Tuveson outlined a model of pancreatic cancer and its potential use in experimental therapeutics. Mutant mice were generated that developed preinvasive intraepithelial neoplasia (PanIN). Mice with pancreatic ductal carcinoma (PDA) slowly progressing to invasive and metastatic tumors had 16 months median survival. PanIN and PDA closely resembled their human counterparts, with cachexia and analogous metastatic pattern. Activation/expression of the Notch, Cox-2, erbB1/B2, and hedgehog pathways were seen and represent potential therapeutic targets. By incorporating additional conditional mutations in tumor suppressor genes including p53 and Ink4a/Arf, advanced and metastatic PDA models were generated. The pancreatic-specific concomitant expression of endogenous KrasG12D and Trp53R172H allele resulted in metastatic PDA, and tumor cells showed widespread numerical and structural chromosomal instability. Mice with PDA were refractory to gemcitabine, whereas mice harboring transplanted human or murine PDA were sensitive to it, consistent with the striking difference in gemcitabine triphosphate levels seen within the tumors. The exploration of additional therapeutics in this new model is warranted.

Arul Chinnaiyan discovered oncogenic chromosomal aberrations based on outlier genes ERG and ETV1 which were overexpressed in the majority of prostate cancers, being mutually exclusive across several gene expression data sets. By RNA ligase-mediated rapid amplification of cDNA ends, a recurring fusion of prostate-specific, androgen-regulated genes TMPRSS2 to ERG or ETV1 occurred in cases which overexpressed the respective ETS family member. Using fluorescence in situ hybridization, 23 of 29 prostate cancer samples were found to harbor rearrangements in ERG or ETV1. Androgen-responsive promoter elements of TMPRSS2 identified three prostate cancer subtypes, the androgen-regulated TMPRSS2:ERG, TMPRSS2:ETV1, and TMPRSS2:ETV4. Dysregulation of ETS family member expression through fusions with TMPRSS2 may be a generalized mechanism for prostate cancer progression. Novel 5′ fusion partners with outlier expression of ETS family members were identified. The role of ETS gene rearrangements in prostate cancer progression was confirmed. The identification of androgen-insensitive 5′ fusion partners has implications for the antiandrogen treatment of advanced prostate cancer.

Anna Bagnato outlined the endothelin axis role in cancer and the possibility of affecting it by target chemotherapy. In ovarian cancer, ligand-mediated ETAR engagement activated different tumor-promoting pathways including protein kinase C, P13K, and mitogen-activated protein kinase and transactivates EGFR. ETAR was identified as a metastasis-associated gene correlating with resistance to chemotherapy, and as a relevant therapeutic target. The lack of clinical responses to EGFR inhibition by ZD4054 suggests that through signaling via alternative receptors, such as ETAR, the tumor can override the EGFR inhibition. The cross-talk between the EGFR and ETAR pathways, and the improved therapeutic efficacy of targeting ETAR by ZD4054, plus EGFR by gefitinib in preclinical models, encourages clinical evaluations of this combination. ET-1 and ET-3 trigger pathways affecting normal host-tumor interactions and tumor progression.

Nathaneal Gray emphasized that most kinase inhibitors developed to date target the ATP binding site in its "active" conformation, in which the activation loop is phosphorylated (type I). The crystal structures of inhibitors such as imatinib, BIRB796, and sorafenib revealed new binding modes at additional sites immediately adjacent to the ATP region (type II). New cellular Bcr-abl inhibitors like GNF-2 target the myristate-binding site of abl, and allosterically inhibit kinase activity. GNF-2 exhibits antiproliferative activity in Bcr-abl-transformed cells and acted synergistically with ATP-competitive inhibitors in cell culture and in a murine CML model. Combinations with Gleevec reduced the development of resistance.

Massimo Santoro indicated that the RET receptor is rearranged in ~30% of papillary thyroid carcinomas (PTC). RET germ line point mutations cause multiple endocrine neoplasia type 2 ( MEN 2 ) syndromes and RET somatic mutations were found in sporadic medullary thyroid carcinoma (MTC). RET/PTC and RET/MEN 2 are constitutively active tyrosine kinases inducing anchorage-independent growth and tumorigenicity in nude mice when introduced in NIH 3T3 cells, and carcinogenesis when introduced in transgenic mice. In vitro, some pyrazolo-pyrimidines inhibited RET/PTC and RET/MEN 2 autophosphorylation in a dose-dependent manner. Zactima and Sorafenib also inhibited RET. Zactima binds RET kinase, had cytostatic effects in RET-positive human thyroid carcinoma cells, and had significant activity against xenografted RET mutation–positive MTC TT. Both Zactima and Sorafenib are being tested in patients with thyroid cancer. Clinical evaluation coupled with measurements of RET phosphorylation levels or RET pathway activity will be crucial.
to assess target inhibition and clinical value of RET inhibition in MTC and PTC.

Prospects

As discussed at this symposium, the success achieved in targeted chemotherapy provides ample proof of principle and firmly establishes this approach in cancer chemotherapy. Difficulties in optimizing treatments may be overcome through pharmacokinetic and pharmacodynamic studies rendered possible by the increasing availability of imaging technologies. Tumor molecular characterization in individual patients is necessary to obviate heterogeneity and to proceed further towards individualized therapies. Future emphasis will no doubt be given to the identification of novel sites of intervention and related markers and to target determined combination treatments.

Acknowledgments

Received 8/22/2007; accepted 10/10/2007.

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doi:10.1158/0008-5472.CAN-07-3230

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