Twenty-fourth Annual Pezcoller Symposium: Molecular Basis for Resistance to Targeted Agents

Richard Marais¹, William Sellers², David Livingston³ and Enrico Mihich³

Authors’ Affiliations: ¹The Paterson Institute for Cancer Research, Manchester, England; ²Novartis Institute for Biomedical Research, Cambridge, Massachusetts; ³Dana Farber Cancer Institute, Boston, Massachusetts

Note: The findings of this article were presented in the 24th annual Pezcoller Symposium titled, “Molecular Basis for Resistance to Targeted Agents” which was held in Trento, Italy on June 14-16, 2012.

Corresponding Author: Enrico Mihich, Dana Farber Cancer Institute, 450 Brookline Ave, Boston, MA 02115. Phone: 716-316-7782; Fax: 617-582-8550; Email: enrico_mihich@dfci.harvard.edu
Abstract

The mechanisms of genetically determined mechanisms of resistance to several target drugs were discussed in breast cancer, melanoma, colorectal and prostate cancers, CML, SCLC and medulloblastoma. In each case, heterogeneity of mechanisms was emphasized. In melanoma therapeutic interference with the effects of BRAF mutations was repeatedly discussed. It was also reported that anti CTLA4 antibodies provided the first treatment improving survival of patients with stage IV melanoma. Epithelial Mesenchimal Transition (EMT) was introduced as a mechanism of resistance particularly in lung and pancreatic cancer, where the role of microenvironment factors was also indicated. In colorectal and prostate cancers the use of liquid biopsies, namely, measurements of tumor nucleic acid in blood was indicated as a way to obtain whole tumor assessment instead of the partial assessment obtainable with traditional biopsies. Knowledge of the mechanisms of drug action and resistance was stressed to be essential for the design of new agents and combination of agents aimed at increasing antitumor effectiveness and overcoming resistance.

Findings Presented

Jeffrey Engelman (Massachusetts General Hospital, Boston, MA) discussed natural resistance to kinase inhibitors, the mechanisms of resistance and strategies to overcome it. He focused on resistance to RAF inhibitors in BRAF mutant colorectal tumors. Gene alteration and bypass mechanisms of resistance were discussed with examples of ALK and EFGR genes. Heterogenicity of mechanisms of resistance were emphasized as challenges to overcoming resistance in the clinic. EMT yielding a new type of cells after inhibition of target in the original cell was also indicated as a mechanism of resistance.

Resistance in Breast Cancer

Jos Jonkers (Netherlands Cancer Institute, Amsterdam, The Netherlands) studied genetically engineered mouse models (GEMMs) and patient-derived tumor graft models for BRCA-deficient breast cancer. These mice develop mammary tumors with genomic instability and hypersensitivity to DNA-damaging agents, including platinum drugs and PARP inhibitors. Response and resistance to platinum drugs and the clinical PARP inhibitor olaparib were found to be affected by drug efflux transporter activity, type of BRCA1 founder mutation and 53BP1 status. BRCA1 re-expression contributes to therapy of resistance in patient-derived tumor graft models of BRCA1-deficient triple-negative breast cancer.

Elaine Mardis (The Genome Institute at Washington University, St. Louis MO) studied breast cancer samples from a clinical trial of aromatase inhibitors (AI). She used whole genome sequencing of paired tumor and normal DNA to correlate genomic alterations...
with clinical response. Sequencing of known variant regions identified impacts of heterogeneity of tumor cells on -response and -resistance. The remaining tumor cells often display known driver mutations not detected in the pre-treatment cell populations.

Jose Baselga (Massachusetts General Hospital, Boston, MA) discussed the reversal of endocrine resistance in breast cancer by targeting the mTOR pathway. A mechanism of endocrine resistance in hormone receptor positive (HR+) breast cancer is aberrant signaling via the phosphatidylinositol 3-kinase (PI3K/Akt/mammalian target of rapamycin (mTOR) pathway. A phase III study was concluded comparing the mTOR inhibitor everolimus and exemestane to exemestane and placebo in 724 patients with HR+ breast cancer refractory to nonsteroidal aromatase inhibitors. Everolimus plus exemestane resulted in synergistic improvement in progression-free survival. Going forward new studies are being designed with PI3K inhibitors in combination with ER degraders that in preclinical models have shown synergism.

**Resistance in BRAF Mutant Melanoma**

Levi Garraway (Dana Farber Cancer Institute, Boston, MA) discussed the development of selective RAF and MEK inhibitors which has resulted in prolonged survival of melanoma patients whose tumors harbor BRAF(V600E/K) mutations. Resistance to kinase inhibitors may be grouped into three categories: 1, “target-oriented” resistance mechanisms (e.g., secondary mutation, amplification, or dysregulation of the target oncoprotein); 2, “bypass” mechanisms (engagement of a signaling module that circumvents the target oncoprotein); or 3, alterations in downstream effectors (e.g., key signaling proteins that are activated by the target oncoprotein). Mechanisms of resistance in BRAF-mutant melanoma are pertinent to each of these categories. Examples include discovery of several mechanisms that re-establish RAF signaling, bypass mechanisms such as the COT kinase or various receptor tyrosine kinases, and activating mutations of MEK1, which signals downstream of mutated B-RAF. In most cases, the resistance mechanisms result in sustained ERK signaling—hence restoring activation of the oncogenic MAP kinase pathway that is dysregulated by mutant BRAF in melanoma. Multiple mechanisms of MAP kinase activation, dysregulation of the oncogenic MAP kinase transcriptional output, and possible ERK-independent mechanisms of resistance were identified by functional screens; although 150-200 individual resistance effectors have been nominated by these screens, many of these seem to converge upon a narrower set of key cellular effectors. Thus, parsimonious therapeutic combinations (e.g., 3-4 drugs) could be developed that impede many individual “upstream” resistance mechanisms. This knowledge and a detailed understanding of “steady state” tumor dependencies and clinical genomic characterization of relapsing tumors, may lead to novel therapeutic combinations worthy of clinical evaluation.
Richard Marais (The Peterson Institute for Cancer Research, Manchester, UK) indicated that BRAF regulates cell growth and survival through the MEK/ERK signaling pathway. BRAF is mutated in about half of melanoma cases. Drugs targeting BRAF achieve responses in about 60% of melanoma patients, validating this approach to treatment of these patients. However, these drugs induce non-melanoma skin lesions (keratoacanthomas and cutaneous squamous cell carcinomas) in about 30% of patients because they drive paradoxical activation of the MAP kinase pathways in cells with RAS mutations, the upstream activator of BRAF. This activation is driven by BRAF dimerization with a related protein called CRAF. These side-effects can be blocked by MEK inhibitors. Other drugs can drive this paradox. Nilotinib induced RAF dimerization and pathway activation in drug-resistant CML cells, but the combination of nilotinib with a MEK inhibitor induces the death of these cells.

Jeffrey Sosman (Vanderbilt University, Nashville, TN) outlined BRAF inhibitor (BRAFi) effects on resistance in melanoma. Most melanoma patients with $\text{BRAF}^{V600}$ mutations treated with BRAFi, including vemurafenib or dabrafenib have rapid clinical responses with median progression-free survival of 6.8 months and survival improved to 13-16 months. MEK-dependent mechanisms include activating mutations in upstream NRAS or downstream MEK1; overexpression of COT, which activates MEK, and either alternate splicing, or amplification of the mutant $\text{BRAF}^{V600}$ gene. MEK-independent resistance includes activation of RTKs (PDGFR, IGF1R) or their ligands (HGF for met). Many of these cases may also require re-activation of the MAP kinase pathway. Once resistance occurs MEKi alone are ineffective while the addition of a MEKi to BRAFi appears to re-induce responses in about 20% of patients. Other approaches include the combination of a MEKi with AKTi; BRAFi with PI3K inhibitor, BRAFi+HGF/met inhibitor, and an ERK inhibitor. A different approach is the combination of an immune activating agent such as anti-CTLA4, anti-PD1, or Interleukin-2 with a BRAFi.

Charlotte Ariyan (Memorial Sloan Kettering Cancer Center, New York, NY) discussed the potential value of immune checkpoint blockade. Anti-CTLA-4 antibody blockade was the first treatment shown to improve survival in a randomized control trial in patients with Stage IV melanoma. While the response rates were low, the durability of response was years. To improve the effects of immunotherapy combinations with other drugs are being studied. It was found that BRAF inhibitors are not immunosuppressive, and may have stimulatory effects on vaccine responses in vivo. Characterization of targeted therapies will be needed to optimize combination treatments.

**The Use of Liquid Biopsies in Colorectal and Prostate Cancer**

Alberto Bardelli (Institute for Cancer Research and Treatment, Candiolo, Torino, Italy) outlined the mechanisms of resistance to EGFR targeted agents in colorectal cancer. To monitor resistance measurements of tumor DNA in blood, “liquid biopsies”, are used,
thus capturing a whole tumor marker. Clinical findings are correlated with results obtained in mouse colon carcinoma xenografts: K-ras mutations correspond to anti-EGFR resistance. Metastatic colorectal cancers respond differently to EGFR-targeted agents and monotherapy with cetuximab and panitumumab is effective only in 10-20% of cases. Oncogenic activation of EGFR downstream effectors such as KRAS, BRAF, PIK3CA and PTEN has a role on response to therapy. Predictive biomarkers are translated to couple EGFR-targeted antibodies to patients.

Johann de Bono (Institute of Cancer Research and Royal Marsden Hospital, London, UK) discussed prostate cancer therapy. New agents have improved overall survival, including two novel endocrine agents with different mechanisms of action (abiraterone and enzalutamide), a cytotoxic (cabazitaxel) and a systemic alpha-particle emitting radionuclide (Radium 223). Studies with the CYP17 inhibitor abiraterone and the novel AR antagonist enzalutamide have confirmed that advanced prostate cancer is not hormone refractory; in castrate cancer patients the prostate contains high hormone concentrations. The personalized prostate cancer treatment is likely complicated by the intra-patient and inter-patient heterogeneity of this disease, with more than 20 clonal subtypes of prostate cancer being identified. Circulating tumor cells and plasma nucleic acids are being evaluated as multi-purpose biomarkers.

BCR-ABL Inhibitors in CML

Giulio Superti-Furga (CeMM Research Center for Molecular Medicine, Vienna, Austria) discussed the complexity of factors conditioning drug-target interactions and the activity of a given drug. Occurrence of the BCR-ABL T315I gatekeeper mutation is one challenge in CML therapy. BCR-ABL inhibitors have multiple targets and pleiotropic effects with synergistic potential. A strong synergy between danusertib and bosutinib exclusively affected CML cells harboring BCR-ABL T315I. Both compounds targeted MAPK pathways downstream of BCR-ABL resulting in impaired activity of c-Myc and downregulation of c-Myc target genes. The contribution of danusertib and bosutinib could be mimicked individually by specific MAPK inhibitors and collectively by downregulation of c-Myc through Brd4 inhibition.

William Sellers (Novartis Institute for Biomedical Research, Cambridge, MA) discussed understanding resistance and related applications to drug discovery. Therapeutics against cancer genetic underpinnings have had clinical impact as shown by imatinib in BCR-ABL driven CML, erlotinib and gefitinib in EGFR mutated lung cancer, crizotinib in ALK translocated lung cancer and vemurafenib in BRAF-mutant melanoma. The efficacy of small molecule inhibitors of KIT, PDGFR, HER2, EGFR, BRAF and ALK in a diversity of malignances including GIST, lung cancer and melanoma indicates that a new generation of efficacious drugs is emerging. Understanding of resistance has led to second generation inhibitors, including nilotinib in imatinib-resistance CML and then in
the first-line CML therapy. Targeting BCR-ABL, the Smoothened receptor, the MET receptor and the ALK kinase with novel combinations was discussed.

Thomas O’Hare (University of Utah School of Medicine / Huntsman Cancer Institute, Salt Lake City, UT) discussed resistance to tyrosine kinase inhibitors (TKIs) in CML. Imatinib resistance due to point mutations in the BCR-ABL kinase domain is largely controlled by nilotinib and dasatinib. The most problematic point mutation is the frequent BCR-ABL T315I ‘gatekeeper’ mutation, which is insensitive to all three approved therapies. Ponatinib is a high-affinity BCR-ABL TKI with activity against all known single kinase domain mutants. However, ponatinib binding is adversely affected by ≥ two mutations in the same BCR-ABL molecule. BCR-ABL compound mutation resistance profiles were established for imatinib, nilotinib, dasatinib, bosutinib, ponatinib and DCC-2036. Evidence supporting the hypothesis that ponatinib resistance will fall into two main categories was presented: 1) BCR-ABL compound mutation-mediated resistance in which BCR-ABL remains an appropriate sole target and, 2) resistance despite inhibition of BCR-ABL, requiring concurrent inhibition of newly identified co-critical targets.

Correlations of Genomic Changes to Resistance

Roman Thomas (University Hospital Cologne, Cologne, Germany) indicated that lung cancer therapeutics targeting specific signaling pathways activated by genetic lesions has shown clinical success, as exemplified by EGFR mutations as well as fusions affecting ALK and ROS1. Genetic alterations have also been identified in squamous cell carcinomas and small cell lung cancer, which may bear therapeutic potential. Annotating lung tumor samples with cancer genomics data may enhance diagnostic resolution by establishing a more biologically oriented, cancer genomics-based, taxonomy of lung cancer.

Silvia Buonamici (Novartis, Cambridge, MA) discussed resistance to smoothened inhibitors. The 12-pass transmembrane protein Patched (Ptc) inhibits Smoothened (Smo), a G-protein coupled receptor (GPCR)-like molecule. When Ptc inhibition is attenuated, Smo signals via a cytosolic complex of proteins leading to activation of the Gli family of transcription factors. Somatic mutations in Ptc and Smo lead to constitutive pathway activation and are found in sporadic medulloblastoma (MB) and basal cell carcinoma (BCC). NVP-LDE225 inhibits Smo-dependent signaling in vitro and in vivo and shows dose-related anti-tumor activity in genetically defined MB models. Following long-term dosing of NVP-LDE225 in medulloblastoma allograft models, resistance was observed. Three different mechanisms of resistance were identified: chromosomal amplification of Gli2, mutations in smo and increased phosphatidylinositol-3-kinase (PI3K) signaling. Combination of NVP-LDE225 with PI3K/mTor inhibitors markedly delayed the development of resistance.
EMT and Microenvironment as Determinants of Resistance

Rafaella Sordella (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) discussed lung cancer resistance targeted therapy. In lung cancer cell lines resistant to erlotinib EGFR (EGFR T790M) secondary mutations and MET oncogene amplification were two principal mechanisms of acquired resistance. Cells intrinsically resistant to erlotinib displayed features suggesting an EMT. TGF-β–mediated signaling was sufficient to induce these phenotypes. Increased TGF-β–dependent IL-6 secretion unleashed previously addicted lung tumor cells from their EGFR dependency. Inflammation stimulated IL-6 secretion and decreased tumor response to erlotinib. Thus, both tumor cell-autonomous mechanisms and/or activation of tumor microenvironment contributed to erlotinib resistance; treatments based only on EGFR inhibition may not be effective in lung-cancer patients harboring mutant EGFR.

Ben Stanger (University of Pennsylvania, Philadelphia, PA) discussed the dynamics of tumor spread in pancreatic cancer realizing that metastasis formation is relatively rare. During stochastic tumor evolution, cells undergo EMT – associated with the acquisition of stem cell-like features well before frank malignancy, suggesting that EMT enables early spread of pancreatic tumor cells. Circulating pancreatic cancer cells which pass the basement membrane are E-cadherin negative and at a premalignant stage. EMT and invasive behavior are associated with areas of inflammation; an inhibitor of inflammation (dexamethasone) blocks cell entry into the bloodstream. The importance of soil for the establishment of metastasis was clearly indicated.

David Tuveson (Cambridge Research Institute, Cambridge, UK) discussed how tumor stroma influences the therapeutic response of pancreatic tumors. Pancreatic ductal adenocarcinoma (PDAC) frequently harbors somatic mutations in 4 genes (KRAS, P16, Tp53, and SMAD4). Transplanted tumor models have superior tissue perfusion and delivery of gemcitabine compared to primary murine PDAC, this inversely correlating with stromal content. Human PDAC is hypovascular and contains a compressed residual vasculature. Smoothened inhibitors such as IPI-926 depleted the PDAC stroma, and transiently increased vascular density and prolonged survival. Systemic administration of polymerized hyaluronidase (PEGPH20) caused dilatation of intratumoral vasculature and delivery of chemotherapy prolonging survival of mice with PDAC. Alternative targeting the tumor stroma may also be beneficial in PDAC, including the use of an albumin-Paclitaxel formulation which elevated the gemcitabine levels in the mouse PDAC tumors. Paclitaxel elicits the liberation of reactive oxygen species (ROS), which induces the destruction of cytidine deaminase, the major pathway of gemcitabine inactivation in cells. PDAC is resistant to VEGF targeted therapeutics. The notch pathway implicated in vessel morphogenesis, is inhibited with gamma secretase inhibitors which induced the rapid destabilization of the PDAC vasculature, promoting
the death of intratumoral endothelial cells. These effects were exacerbated by concomitant exposure to gemcitabine.

Todd Golub (Broad Institute at MIT, Cambridge, MA) discussed approaches to discovering microenvironment mediated drug resistance. A co-culture system was developed to systematically assay the ability of 23 stromal cell types to influence the innate resistance of 45 cancer cell lines to 35 anti-cancer drugs. The stroma-mediated resistance of BRAF-mutant melanoma to RAF inhibition was characterized. Stromal secretion of hepatocyte growth factor (HGF) resulted in activation of the HGF receptor MET, reactivation of the MAPK and PI3K/AKT pathways, and immediate resistance to RAF inhibition. In patients with BRAF-mutant melanoma there was a statistically significant correlation between stromal HGF expression and innate resistance to treatment. Dual inhibition of RAF and MET caused reversal of drug resistance. Thus, the systematic dissection of tumor-microenvironment interactions may reveal important mechanisms underlying drug resistance.

Best Poster Presentation

Hadas Reuveni (NovoTyr Therapeutics Ltd, Tel Hai/Jerusalem Hebrew University, Israel) gave an oral presentation of an outstanding poster on targeting insulin receptor substrates as a therapeutic target for drug resistant cancers. The phosphorylation of insulin receptor substrates IRS1/2 on serine residues leads to IRS1/2 degradation. mTOR and EGFR inhibitors inhibited the phosphorylation of IRS1. This resulted in the stimulation of the IGF1R-IRS1 to PKB/Akt pathway, enhancing cancer cell survival and developing resistance to the drugs. An inhibitor of mutated BRAF\textsuperscript{V600E} inhibited serine phosphorylation of IRS1/2 and led to an increase in IRS1/2 levels in BRAF\textsuperscript{V600E} mutated 451-LU-BR melanoma cells. Melanoma cells that became resistant to PLX4032, have significantly higher levels of IRS1 and/or IRS2 compared to PLX4032-sensitive melanoma cells. Inhibitors, developed by NovoTyr and the Hebrew University, induce IRS1/2 phosphorylation on serine residues and degradation of IRS1/2, with long lasting anti-tumor effects. The lead compound inhibited various drug-resistant cancer cell types, and possessed antitumor and anti-metastatic effects on human melanoma in nude mice.

Summary

A significant benefit derived from this meeting was the unequivocal confirmation that the use of molecular target oriented anticancer drugs and combination of drugs can lead to clinical benefits.

A common theme throughout the symposium was that resistance to target oriented agents has heterogeneous molecular mechanisms which are correlated with genetic changes and that this clearly determines important clinical challenges.
The possibility was emphasized of developing new agents and combination of agents which would overcome the resistance determining effects of primary and sequential mutations and thus restore responses. It was recognized that knowledge of the mechanisms of drug action and resistance is essential for a successful development of these treatments.

The use of tumor cells circulating in blood as markers of response was discussed and compared with the advantages of the so-called “liquid biopsies”, namely, the measurements of circulating tumor nucleic acid reflecting the status of a whole tumor instead of the partial visualization provided by traditional biopsies. Two other important emerging themes were that EMT could be at the basis of resistance and that the microenvironment of tumor cells can play an important role in the development of resistance.

The information discussed supported the possibility of ultimately developing individualized treatments of cancer based on a knowledge of determinants of drug action and resistance in individual tumors.
Twenty-fourth Annual Pezcoller Symposium: Molecular Basis for Resistance to Targeted Agents


*Published OnlineFirst on December 7, 2012.*

**Updated version**

Access the most recent version of this article at:

doi:10.1158/0008-5472.CAN-12-3236

**Author Manuscript**

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

**E-mail alerts**

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

**Reprints and Subscriptions**

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

**Permissions**

To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/early/2012/12/06/0008-5472.CAN-12-3236. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.