

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

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### Abstract

The mechanisms of genetically determined mechanisms of resistance to several target drugs were discussed in breast cancer, melanoma, colorectal and prostate cancers, chronic myelogenous leukemia, small cell lung cancer, 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-mesenchymal 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, were 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. *Cancer Res*; 73(3); 1046-9. ©2012 AACR.

### 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 *EGFR* genes. Heterogeneity of mechanisms of resistance was emphasized as challenges to overcoming resistance in the clinic. Epithelial-mesenchymal transition (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 (GEMM) 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. 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 pretreatment cell populations.

Jose Baselga (Massachusetts General Hospital) 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 phosphoinositide 3-kinase (PI3K)/Akt/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 estrogen receptor (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

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inhibitors, which has resulted in prolonged survival of patients with melanoma whose tumors harbor *BRAF(V600E/K)* mutations. Resistance to kinase inhibitors may be grouped into 3 categories: (i) "target-oriented" resistance mechanisms (e.g., secondary mutation, amplification, or dysregulation of the target oncoprotein); (ii) "bypass" mechanisms (engagement of a signaling module that circumvents the target oncoprotein); or (iii) 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 *BRAF*. In most cases, the resistance mechanisms result in sustained extracellular signal-regulated kinase (ERK) signaling—hence restoring activation of the oncogenic mitogen-activated protein kinase (MAPK) pathway that is dysregulated by mutant *BRAF* in melanoma. Multiple mechanisms of MAPK activation, dysregulation of the oncogenic MAPK transcriptional output, and possible ERK-independent mechanisms of resistance were identified by functional screens; although 150 to 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 patients with melanoma, 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 MAPK 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 chronic myelogenous leukemia (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 patients with melanoma with *BRAF*<sup>V600</sup> 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 to 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 *BRAF*<sup>V600</sup> gene. MEK-independent resis-

tance includes activation of receptor tyrosine kinases [RTK; platelet-derived growth factor receptor (PDGFR) and insulin-like growth factor-I receptor (IGF-IR)] or their ligands [hepatocyte growth factor (HGF) for met]. Many of these cases may also require re-activation of the MAPK pathway. Once resistance occurs MEKi alone are ineffective, whereas the addition of a MEKi to BRAFi appears to re-induce responses in about 20% of patients. Other approaches include the combination of an 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 (IL)-2 with a BRAFi.

Charlotte Ariyan (Memorial Sloan Kettering Cancer Center, New York, NY) discussed the potential value of immune checkpoint blockade. Anti-CTLA4 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 BRAFi 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% to 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 2 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 androgen receptor antagonist enzalutamide have confirmed that advanced prostate cancer is not hormone refractory; in patients with castrate cancer, the prostate contains high hormone concentrations. The personalized prostate cancer treatment is likely complicated by the intra- and interpatient 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 dasinertib 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 dasinertib 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 CMLs, 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 malignancies including gastrointestinal stromal tumor, 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-resistant CML and then in the first-line CML therapy. Targeting BCR-ABL, the Smoothed 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 (TKI) in CMLs. 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 3 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 2 or more 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 2 main categories was presented: (i) *BCR-ABL* compound mutation-mediated resistance in which BCR-ABL remains an appropriate sole target and (ii) 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 have shown clinical success, as exemplified by *EGFR* mutations as well 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) discussed resistance to smoothed inhibitors. The 12-pass transmembrane protein Patched (Ptch) inhibits Smoothed (Smo), a G-protein-coupled receptor (GPCR)-like molecule. When Ptch inhibition is attenuated, Smo signals via a cytosolic complex of proteins leading to activation of the Gli family of transcription factors. Somatic mutations in *Ptch* and *Smo* lead to constitutive pathway activation and are found in sporadic medulloblastoma and basal cell carcinoma (BCC). NVP-LDE225 inhibits Smo-dependent signaling *in vitro* and *in vivo* and shows dose-related antitumor activity in genetically defined medulloblastoma 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 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 2 principal mechanisms of acquired resistance. Cells intrinsically resistant to erlotinib displayed features suggesting an EMT. TGF- $\beta$ -mediated signaling was sufficient to induce these phenotypes. Increased TGF- $\beta$ -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 patients with lung cancer 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 that 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 than primary murine PDACs, 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 PDACs. Alternative targeting the tumor stroma may also be beneficial in PDACs, 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 coculture 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 anticancer drugs. The stroma-mediated resistance of *BRAF*-mutant melanoma to RAF inhibition was characterized. Stromal secretion of 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 (IRS) 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 devel-

oping resistance to the drugs. An inhibitor of mutated *BRAF*<sup>V600E</sup> inhibited serine phosphorylation of IRS1/2 and led to an increase in IRS1/2 levels in *BRAF*<sup>V600E</sup>-mutated 451-LU-BR melanoma cells. Melanoma cells that became resistant to PLX4032 have significantly higher levels of IRS1 and/or IRS2 than 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 antitumor 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 that 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 that 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 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 knowledge of determinants of drug action and resistance in individual tumors.

### Disclosure of Potential Conflicts of Interest

W. Sellers has employment (other than primary affiliation; e.g., consulting) in Novartis Institutes for BioMedical Research as VP/Global Head of Oncology; has ownership interest (including patents) in Novartis Pharmaceuticals; and is a consultant/advisory board member of MSKCC and St. Jude's Hospital. D.M. Livingston has employment (other than primary affiliation; e.g., consulting) in Novartis Institute for Biomedical Research as consultant; has a commercial research grant from Novartis Institute for Biomedical Research; and is a consultant/advisory board member for Nextech. No potential conflicts of interest were disclosed by the other authors.

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