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Co-Treatment with Panitumumab and Trastuzumab Augments Response to the MEK Inhibitor Trametinib in a Patient-Derived Xenograft Model of Pancreatic Cancer[1]

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Abstract

Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations and epidermal growth factor receptor (EGFR) family signaling are drivers of tumorigenesis in pancreatic ductal adenocarcinoma (PDAC). Previous studies have demonstrated that combinatorial treatment of PDAC xenografts with the mitogen-activated protein kinase–extracellular-signal-regulated kinase (ERK) kinase1/2 (MEK1/2) inhibitor trametinib and the dual EGFR/human epidermal growth factor receptor 2 (HER2) inhibitor lapatinib provided more effective inhibition than either treatment alone. In this study, we have used the therapeutic antibodies, panitumumab (specific for EGFR) and trastuzumab (specific for HER2), to probe the role of EGFR and HER2 signaling in the proliferation of patient-derived xenograft (PDX) tumors. We show that dual anti-EGFR and anti-HER2 therapy significantly augmented the growth inhibitory effects of the MEK1/2 inhibitor trametinib in three different PDX tumors. While significant growth inhibition was observed in both *KRAS* mutant xenograft groups receiving trametinib and dual antibody therapy (tumors 366 and 608), tumor regression was observed in the *KRAS* wild-type xenografts (tumor 738) treated in the same manner. Dual antibody therapy in conjunction with trametinib was equally or more effective at inhibiting tumor growth and with lower apparent toxicity than trametinib plus lapatinib. Together, these studies provide further support for a role for EGFR and HER2 in pancreatic cancer proliferation and underscore the importance of therapeutic intervention in both the KRAS–rapidly accelerated fibrosarcoma kinase (RAF)–MEK–ERK and EGFR-HER2 pathways to achieve maximal therapeutic efficacy in patients.

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Introduction

Pancreatic cancer is the 10th most common US cancer and the 4th leading cause of cancer death in the United States. The 5-year survival from this disease has barely improved from 2% to 6% in the last 40 years [1]. These poor outcomes coupled with a projected increase in disease incidence of 55% in the next 20 years highlight the pressing need for improved systemic therapies for this disease [2].

Central to the failure of existing treatment strategies is the marked genetic heterogeneity and resultant molecular signaling complexity observed in pancreatic cancers [3–5]. Despite this diversity, a

Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; HER2, human epidermal growth factor receptor 2; KRAS, Kirsten rat sarcoma viral oncogene homolog; MEK1/2, mitogen-activated protein kinase–extracellular-signal-regulated (ERK) kinase1/2; PDAC, pancreatic ductal adenocarcinoma; PDX, patient-derived xenograft; pEGFR, phospho-EGFR; pHER2, phospho-HER2; pJNK, phospho–c-Jun N-terminal kinase; pRTK, phospho–receptor tyrosine kinase; RAF, rapidly accelerated fibrosarcoma kinase; Tra + P + T, trametinib plus panitumumab plus trastuzumab

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conserved sequence of acquired genetic alterations is observed in a majority of pancreatic ductal epithelial cells during their malignant transformation to adenocarcinoma. Activating mutations of the *Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) have been reported in 75% to 95% of pancreatic ductal adenocarcinomas (PDAC), and the importance of this oncogene in pancreatic tumorigenesis has been demonstrated in genetically engineered mouse models [6–11]. Developing strategies to target RAS pathway signaling at the preclinical and clinical levels has been emphasized as a high priority in pancreatic cancer research [12]. Small molecule inhibitors of both rapidly accelerated fibrosarcoma kinase (RAF) and mitogen-activated protein kinase (MAPK)–extracellular-signal-regulated (ERK) kinase1/2 (MEK1/2) within the RAS-RAF-MEK-ERK signaling cascade are in clinical development with promising early results in mutant RAS/RAF-driven tumors [13,14].

The epidermal growth factor receptor (EGFR) families have also been identified as promising targets for PDAC treatment. The observation that 40% to 70% of PDAC tumors overexpress EGFR led to the testing and US Food and Drug Administration (FDA) approval of erlotinib, an EGFR-specific tyrosine kinase inhibitor, in combination with gemcitabine for the treatment of patients with advanced disease [15,16]. Additionally, human epidermal growth factor receptor 2 (HER2) has been identified as overexpressed in approximately 20% of PDAC tumors [17]. Its overexpression has been associated with worse patient outcomes, and anti-HER2 therapy has exhibited therapeutic synergism with anti-EGFR agents in pancreatic xenografts expressing moderate and even low levels of HER2 [18–20].

Despite promising early results with RAS pathway inhibitors and anti-EGFR family therapy, the genetic heterogeneity, signaling redundancy, and plasticity of pancreatic tumor cells suggest that monotherapy or single pathway treatment strategies are unlikely to result in significant and durable responses [5]. Importantly, preclinical studies evaluating combination therapy with EGFR and RAS pathway inhibitors in pancreatic cancer xenografts have shown promising results [21–23].

To develop and test rational approaches to therapy for PDAC, we have established a patient-derived xenograft (PDX) model of pancreatic cancer with orthotopic implantation of tumors into immunocompromised mice. Genetic and molecular profiling of the initial 15 PDXs in this tumor bank revealed a high frequency of tumors with *KRAS* mutations and activated EGFR and a smaller cohort with activated HER2 [24]. In this preclinical model, we evaluated combination therapy with trametinib (GSK1120212), a selective allosteric inhibitor of MEK1/2 [16,25,26], plus lapatinib, an inhibitor of both EGFR and HER2 receptor tyrosine kinase (RTK) activity [27–29]. We reported that trametinib-mediated tumor growth inhibition was significantly enhanced by concomitant lapatinib therapy in four of five patient-derived tumors assessed [23].

While this combination therapy was highly efficacious and considered for a clinical trial, there were concerns about potential patient toxicity as a recent phase I/Ib study evaluating trametinib in combination with erlotinib in patients with non–small cell lung and pancreatic cancers reported treatment-limiting gastrointestinal toxicity [30]. We therefore sought to identify alternate agents targeting EGFR and HER2 to use in combination with trametinib.

In this study, we have used two well-studied therapeutic antibodies, panitumumab (specific for EGFR) and trastuzumab (specific for HER2), to probe the role of EGFR and HER2 signaling in the proliferation of PDX tumors bearing mutant and wild-type *KRAS* alleles. We show that dual anti-EGFR and anti-HER2 therapy significantly augmented the growth inhibitory effects of the MEK1/2 inhibitor trametinib in different PDX tumors. Particularly noteworthy was the observation that two different tumors bearing wild-type *KRAS* alleles were particularly sensitive to trametinib plus dual antibody therapy exhibiting significant tumor regression. *In vitro* and *in vivo* studies confirmed that treatment with panitumumab or trastuzumab effectively inhibited the epidermal growth factor (EGF)–dependent autophosphorylation of EGFR and HER2, respectively. These studies using PDX tumors support the role for EGFR and HER2 in pancreatic cancer proliferation and underscore the importance of therapeutic intervention in both the KRAS-RAF-MEK-ERK and EGFR-HER2 pathways to achieve maximal therapeutic efficacy *in vivo*.

Materials and Methods

Orthotopic PDXs and Cell Lines

PDAC cell line and tumor samples MAD 08-608, 08-738, 09-366, and 10-215 (T608, T738, T366, and T215, respectively) were generated from fresh human tumor specimens collected with the approval of the University of Virginia's Institutional Review Board and Animal Care and Use Committee following informed consent from each patient as previously described [23,31]. Six- to 8-week-old male athymic nude mice were used for all *in vivo* experiments. For *in vitro* experiments, cells were maintained in Roswell Park Memorial Institute 1640 medium containing 10% FBS and 1% penicillin/streptomycin and cultured in a humidified (37 °C, 5% CO_2) incubator. Fresh cell aliquots were thawed, propagated, and used for experiments every 6 months. Cell lines were authenticated in 2010 by the University of Virginia Biomedical Research Facility as previously described [23].

MEK1/2 Inhibitor, EGFR/HER2 Inhibitor, and Antibodies

Trametinib (GSK1120212), a selective allosteric inhibitor of MEK1/2 [16,25,26], and lapatinib, a dual tyrosine kinase inhibitor of EGFR and HER2, were kindly provided by GlaxoSmithKline (Brentford, United Kingdom). Panitumumab (Amgen, Thousand Oaks, CA) is a fully humanized, anti-EGFR monoclonal antibody that is FDA approved for the treatment of metastatic colorectal cancer with disease progression on standard therapy [32]. Trastuzumab (Genentech, San Francisco, CA) is a humanized anti-HER2 monoclonal antibody that is FDA approved for the treatment of HER2 overexpressing breast cancer [33]. Pertuzumab (Roche, Basel, Switzerland) is a humanized anti-HER2 monoclonal antibody that inhibits HER2 dimerization and has improved survival for patients with metastatic breast cancer in combination with trastuzumab and docetaxel [34]. The University of Virginia Medical Center's Investigational Drugs Pharmacy kindly provided panitumumab, trastuzumab, and pertuzumab.

In Vitro Molecular Response Assays

To assess the molecular responses to EGF stimulation, tumor 366 cells were plated and allowed to adhere overnight in a six-well plate in regular culture conditions. Cells were then starved in serum-free media for 4 hours before the addition of drug combinations (panitumumab, 5 μg/ml; trastuzumab, 20 μg/ml; trametinib, 10 nM) or phosphate-buffered saline (PBS)/DMSO control. One cohort of cells remained in 10% FBS-containing media as a control population. After 1 hour of drug treatment, one cohort of cells was stimulated with 100 ng/ml human EGF or PBS control. Thirty minutes later, samples were lysed and Western blot performed with previously described techniques and antibodies [23].