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RESEARCH REPORT

# Testing *ERBB2* p.L755S kinase domain mutation as a druggable target in a patient with advanced colorectal cancer

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**Abstract** Recent advances in molecular profiling technologies allow genetic driver events in individual tumors to be identified. The hypothesis behind this ongoing molecular profiling effort is that improvement in patients' clinical outcomes will be achieved by inhibiting these discovered genetic driver events with matched targeted drugs. This hypothesis is currently being tested in oncology clinics with variable early results. Herein, we present our experience with a case of advanced colorectal cancer (CRC) with an *ERBB2* p.L755S kinase domain mutation, a *BRAF* p.N581S mutation, and an *APC* p.Q1429fs mutation, together with a brief review of the literature describing the biological and clinical significance of *ERRB2* kinase domain mutations in CRC. The patient was treated with trastuzumab combined with infusional 5-fluorouracil and leucovorin based on the presence of *ERBB2* p.L755S kinase mutation in the tumor and based on the available evidence at the time when standard treatment options had been exhausted. However, there was no therapeutic response illustrating the challenges we face in managing patients with potentially targetable mutations where results from functional in vitro and in vivo studies lag behind those of genomic sequencing studies. Also lagging behind are clinical utility data from oncology clinics, hampering rapid therapeutic advances. Our case also highlights the logistical barriers associated with getting the most optimal therapeutic agents to the right patient in this era of personalized therapeutics based on cancer genomics.

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**Ontology terms:** neoplasm of the gastrointestinal tract

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## INTRODUCTION

In patients with advanced colorectal cancer (CRC), median survival of ~21 mo is achieved by using 5-fluorouracil (5-Fu)-based combination chemotherapies, FOLFIRI (5-Fu/leucovorin/irinotecan), and FOLFOX (5-Fu/leucovorin/oxaliplatin) in a sequential manner (de Gramont et al. 2000; Douillard et al. 2000; Saltz et al. 2000; Tournigand et al. 2004). Addition of biological agents to chemotherapy (bevacizumab/aflibercept in nonselected populations and cetuximab/panitumumab in patients with *KRAS*/*NRAS* wild-type tumors) has also been shown to improve clinical outcomes including survival (Hurwitz et al. 2004; Van Cutsem

et al. 2009, 2012; Douillard et al. 2010, 2013). In chemotherapy refractory setting, regorafenib, an oral multikinase inhibitor, improved survival when compared with best supportive care (Grothey et al. 2013). However, despite the advances made in management of advanced CRC, beyond the selection of patients with *RAS* wild-type tumors for epidermal growth factor receptor (EGFR)-targeted therapies, personalized treatment options are still very much limited.

With recent advances in next-generation DNA sequencing (NGS) technologies, it is now feasible to perform molecular profiling of tumors within an acceptable time frame at a reasonable cost. As such, there are several ongoing molecular profiling studies using NGS across multiple tumor types to identify actionable genetic driver events in individual tumors. The hypothesis behind these studies is that improved clinical outcomes will be achieved by targeting these actionable genetic aberrations with matched targeted therapies. However, there are barriers in development of this new treatment paradigm, especially when results from functional in vitro and in vivo studies lag behind those of genomic sequencing studies and utility of genomic data in the oncology clinic is still very experimental. Herein, we present a case of metastatic rectal cancer with an *ERBB2* p.L755S kinase mutation treated with trastuzumab and 5-Fu/leucovorin illustrating the challenges we face in development of personalized medicine based on genomic profiles.

## RESULTS

### Clinical History

A 35-yr-old male, who was treated with a laparoscopic low anterior resection in June 2008 for a stage I (pT2N0M0) *KRAS* and *NRAS* wild-type, moderately differentiated, microsatellite stable rectal adenocarcinoma, developed liver metastases in July 2011 and underwent a right hepatectomy followed by 6 mo of pseudoadjuvant FOLFOX chemotherapy. In September 2012, his disease recurred with new liver and lung metastases and was treated with FOLFIRI and bevacizumab for ∼10 mo with an initial disease response. On disease progression in July 2013, he was treated with panitumumab and progressed rapidly. He declined regorafenib because of concerns around toxicities. During the course of his illness, his primary rectal tumor and liver metastases were profiled within the Princess Margaret Cancer Genomic Program (CGP) and three somatic mutations, *APC* c.4285delC (p.Gln1429fs/ p.Q1429fs), *BRAF* c.1742A>G (p.Asn581Ser/p.N581S), and *ERBB2* c.2264T>C (p.Leu755Ser /p.L755S) were detected in the tumors (detailed in Genomic Analyses and Methods sections and summarized in Table 1). As the *ERBB2* p.L755S kinase domain mutation is likely to be an activating mutation, he was subsequently treated with three weekly intravenous doses of trastuzumab (8 mg/kg loading dose at the first cycle followed by 6 mg/kg at subsequent cycles), combined with infusional 5-Fu and leucovorin in

**Table 1.** Summary of variants identified

| Gene | Chr No. | HGVS DNA ref | HGVS protein ref | dbSNP/ dbVar ID | Variant type | Genotype | Predicted effect |
|---|---|---|---|---|---|---|---|
| *APC* | 5 | c.4285delC | p.Gln1429fs | Not listed | Deletion | Heterozygous | Not known |
| *BRAF* | 7 | c.1742A>G | p.Asn581Ser | rs121913370 | Substitution | Heterozygous | Not known |
| *ERBB2* | 17 | c.2264T>C | p.Leu755Ser | rs121913470 | Substitution | Heterozygous | *ERBB2* kinase activation |

HGVS, Human Genome Variation Society; dbSNP, Database for Short Genetic Variations.

November 2013, but his liver disease progressed after two cycles. He then received ziv-aflibercept plus a selective angiopoietin-2 monoclonal antibody within a clinical trial. The treatment ceased in March 2014 because of poor tolerance. He continued with best supportive care and expired in August 2014.

## Genomic Analyses

Patient's written consent was obtained for molecular profiling. All genomic analyses detailed here were performed within the Research Ethics Board approved Princess Margaret Cancer Genomic Program's clinical research studies (REB reference numbers 11-0962 and 12-0361) and in a laboratory accredited by the College of American Pathologists and certified to meet Clinical Laboratory Improvement Amendments. Formalin-fixed, paraffin-embedded (FFPE) sections from the primary rectal tumor resected in 2008 (sample 1), 2011 liver resection (sample 2), and a liver biopsy performed in October 2013 (sample 3) were profiled. Tumor cell content of the samples 1, 2, and 3 was assessed by a qualified pathologist using a hematoxylin and eosin slide from each sample. Profiling of paired germline DNA extracted from peripheral blood was also performed to differentiate somatic and germline variants.

In June 2013, profiling of sample 2 (tumor cell content 80%) using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (detailed in the Methods section) showed a heterozygous somatic *ERBB2* p.L755S kinase domain mutation with an allele frequency of 57%. In October 2013, next-generation sequencing of Sample 3 (tumor cell content 80%) using the Illumina TruSeq Amplicon Cancer Panel (detailed in the Methods section) revealed the known *ERBB2* p.L755S mutation and two additional heterozygous somatic mutations, *APC* p.Q1429fs and *BRAF* p.N518S, that were not detectable previously by MALDI-TOF mass spectrometry because the custom panel we used did not include them. To investigate whether these mutations were present in the primary rectal tumor, sample 1 (tumor cell content 70%) was profiled using Illumina TruSeq revealing the presence of all three identical mutations in the primary tumor. Interestingly, increases in allele frequencies of all three mutations were observed over time, suggesting temporal clonal progression (Table 2). The biological effect of detected mutations was predicted using algorithms as previously described (Sukhai et al. 2016). Two detected mutations, *BRAF* p.N581S

**Table 2.** Summary of mutations identified in serial tumor samples (S1, S2, and S3) using MALDI-TOF mass spectrometry and Illumina TruSeq sequencing and their allele frequencies

| | Methods | | Allele frequency (%) | | | Target coverage at variant site | | | Mean target coverage | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mutations identified | MALDI-TOF | TruSeq | S1 | S2 | S3 | S1 | S2 | S3 | S1 | S2 | S3 |
| *APC* c.4285delC (p.Gln1429fs) | Not tested by panel | Detected | 52 | NA | 85 | 7378× | NA | 6420× | 5115× | NA | 4332× |
| *BRAF* c.1742A>G (p.Asn581Ser) | Not tested by panel | Detected | 30 | NA | 42 | 8771× | NA | 9260× | 5115× | NA | 4332× |
| *ERBB2*c.2264T>C (p.Leu755Ser) | Detected | Detected | 33 | 57 | 68 | 8171× | NA | 7056× | 5115× | NA | 4332× |

S1, S2, and S3 represent sample 1 (primary rectal tumor collected in 2008), sample 2 (tumor from 2011 liver resection), and sample 3 (tumor from 2013 liver biopsy), respectively. S1 and S3 were profiled using Illumina TruSeq sequencing and S2 was profiled using MALDI-TOF mass spectrometry.
MALDI-TOF, matrix-assisted laser desorption/ionization time of flight; NA, not applicable.

and *ERBB2* p.L755S, were verified by Sanger sequencing. As the *APC* mutation is not actionable, no attempt was made to verify it using an orthogonal technology.

## DISCUSSION

Unlike data reported in a previous study where all three patients with *ERBB2* mutant CRCs had a *KRAS* mutation (Lee et al. 2006), our case had an *ERBB2* kinase domain mutation p.L755S with a rare *BRAF* p.N581S and an *APC* p.Q1429fs mutation. *BRAF* p.N581S and *APC* p.Q1429fs mutations are found infrequently in CRCs (http://cancer.sanger.ac.uk/cosmic) and their clinical significance in this disease was not known. *ERBB2*, on the other hand, is mutated in ~3%–5% of CRC (http://cancer.sanger.ac.uk/cosmic) (Cancer Genome Atlas Network 2012). Mutations (small insertions and missense mutations) in the kinase domain of the *ERBB2* were observed across multiple solid tumors including CRCs (Lee et al. 2006), and these mutations were shown to activate *ERBB2* (Herter-Sprie et al. 2013). It was also known that L755 is one of the critical amino acid residues that determine resistance to lapatinib, a reversible blocker of EGFR and ERBB2 tyrosine kinases (Kancha et al. 2011). As lapatinib inhibits only the inactive conformation of ERBB2 kinase, p.L755 mutations stabilize the kinase in active conformation, thereby conferring resistance to lapatinib (Kancha et al. 2011). In contrast, irreversible ERBB2 and EGFR inhibitors such as neratinib, and irreversible pan-ERBB inhibitor such as afatinib, bind to active conformation of the ERBB2 kinase thus likely to be more effective for treating tumors with p.L755 mutations (Kancha et al. 2011; De Greve et al. 2012). However, it was not known whether p.L755 mutations sensitize to trastuzumab response in CRC at the time of molecular profiling.

Considering the best available evidence at the time, it would have been most logical to treat our patient with an irreversible ERBB2 inhibitor. However, when this patient progressed on standard therapies, there was no matched clinical trial with an irreversible ERBB2 inhibitor. Request for regulatory approval via a Special Access Program for neratinib was declined because it was not licensed for use outside of clinical trial setting at the time in Canada. There was also no access to off-label use of approved agents such as afatinib. He was eventually treated with trastuzumab combined with infusional 5-fluorouracil and leucovorin chemotherapy (paid for by our research fund) based on evidence that in non-small-cell lung cancer with *ERBB2* kinase domain mutations (mainly exon 20 insertions), partial responses were seen in nine of 15 patients (60%) treated with trastuzumab plus chemotherapy (Mazieres et al. 2013).

Although there was evidence showing that combination treatment of pertuzumab and lapatinib produced sustained tumor regression in quadruple-negative (negative for activating mutations in *KRAS*, *NRAS*, *BRAF*, or *PIK3CA*), *ERBB2*-amplified, cetuximab-resistant CRC patient–derived xenografts (Bertotti et al. 2011), our case was considered to have an *ERBB2*-activating kinase domain mutation rather than amplification, and as such combination treatment of pertuzumab or trastuzumab and lapatinib was not thought to be logical in our patient. Immunohistochemistry examination of the patient's resected primary cancer indeed showed that HER-2 immunohistochemical staining was zero, confirming that ERBB2 was not amplified in this case. The biological significance of the *BRAF* p.N581S mutation was also not known, and as such treatment with BRAF inhibitors or MEK inhibitors, either alone or in combination, was not considered.

The molecular profile of our patient is unique in that an *ERBB2* p.L755S mutation was identified together with a *BRAF* p.N581S mutation. The biological and clinical significance of this unique profile was not known, and it was not possible to exploit this profile fully for genotype-matched therapy for our patient. Neratinib or another irreversible ERBB2 inhibitor was not accessible either because they were not approved for use outside of the clinical trial setting or not funded for off-license indications. It was not easily feasible to set up an *N* of 1

trial specifically for this individual case. Although trastuzumab was not the optimum drug for our patient, it was chosen mainly because of logistical reasons. This case clearly highlights the barriers of getting the optimum targeted drugs to patients in this era of personalized cancer therapeutics. These barriers, however, are not unique to Canada and require a wider global solution.

Since our patient was treated in 2013, there have been recent reports on the biological significance of *ERBB2* kinase domain mutations in CRCs. These mutations, particularly p.L755S and p.V842I variants, are more frequently seen in Lynch or Lynch-like CRCs (Kloth et al. 2016) and are activating mutations in preclinical models (Kavuri et al. 2015; Kloth et al. 2016). There was no family history suggestive of Lynch syndrome in our patient and microsatellite status of our patient's tumor, tested by both fluorescent polymerase chain reaction (PCR) and immunohistochemistry, was stable. In vitro functional analyses indicate the superiority of more potent, irreversible pan-ERBB inhibitors over reversible ERBB inhibitors for inhibiting *ERBB2* mutant CRC cell growth (Kloth et al. 2016). The *ERBB2* p.L755S mutant CW2 cell line showed a strong sensitivity to irreversible pan-ERBB inhibitors but no sensitivity to lapatinib, cetuximab, or trastuzumab (Kloth et al. 2016). In CRC patient–derived xenografts, single-agent ERBB2-targeted therapy (trastuzumab, neratinib, or lapatinib) delayed the growth of *ERBB2* p.S310Y and p.L866M mutant tumors, but durable tumor regression was only seen with dual ERBB2 blockade (trastuzumab combined with neratinib or lapatinib) (Kavuri et al. 2015). Failure of our patient's response to trastuzumab combined with 5-fluoroacil and leucovorin seems to concur with this emerging preclinical data. Activation of ERBB2 via mutations including p.L755S was also reported to mediate resistance to anti-EGFR therapy in vitro (Kavuri et al. 2015). The observation that our patient had primary resistance to panitumumab seems to anecdotally support this notion. However, no current preclinical evidence indicates that the *BRAF* p.N581S mutation contributed resistance to panitumumab or trastuzumab. Considering the preclinical results seen with CRC patient–derived xenografts, dual ERBB2 inhibition such as the combination of trastuzumab with neratinib may be more effective treatment for patients with *ERBB2*-mutated CRCs, but this hypothesis requires clinical evaluation.

## METHODS

### DNA Extraction and Quantification

DNA was extracted from FFPE tumor sections using the QIAamp DNA FFPE Tissue Kit (QIAGEN) and germline DNA from peripheral blood mononuclear cells (PBMCs) using automated extraction (MagAttract DNA Mini M48 kit; QIAGEN). DNA was quantified using the Qubit dsDNA Assay kit on the Qubit 2.0 Fluorometer (ThermoFisher Scientific).

### Mutation Profiling by MALDI-TOF Mass Spectrometry

This analysis was performed using a custom panel to detect 279 mutations in 23 oncogenes as described elsewhere (Thomas et al. 2007). Briefly, DNA is amplified using a PCR primer mix, and a single base extension reaction is performed using extension primers that hybridize immediately adjacent to the mutation. From multiplexed reactions, peaks with different mass are resolved by MALDI-TOF mass spectrometry (MassARRAY, Agena Bioscience) using MassArray software as well as manual analysis. The lower limit of sensitivity for the assay is 10%–15%. Neoplastic cells must be present at a minimum of 30% for mutation detection.

### Illumina TruSeq Amplicon Cancer Panel Sequencing

This was performed using the Illumina TruSeq Amplicon Cancer Panel (TSACP, Illumina), covering 212 amplicons in 48 cancer-associated genes (http://www.illumina.com/Documents/products/datasheets/datasheet_truseq_amplicon_cancer_panel.pdf), on the Illumina MiSeq sequencing platform. The postsequencing bioinformatics analysis pipeline using NeXTGene v2.3.1 software (SoftGenetics) allows the mapping of each amplicon to the reference human genome, the determination of coverage depth, and the identification of the sequence therein. Data were also visualized using the Integrative Genomics Viewer (IGV, Broad Institute). The lower limit of sensitivity for the assay is 5%–10%.

## ADDITIONAL INFORMATION

### Data Deposition and Access

NGS data have been deposited at the European Genome-phenome Archive (EGA; https://www.ebi.ac.uk/ega/) under accession number EGAS00001001897. The reported variants have been submitted to COSMIC (Catalogue of Somatic Mutations in Cancer; http://cancer.sanger.ac.uk/cosmic). The variant ID numbers for *ERBB2* p.L755S, *BRAF* p.N581S, and *APC* p.Q1429fs are COSM14060, COSM462, and COSM41625, respectively.

### Ethics Statement

Molecular analyses were performed within the Research Ethics Board–approved Princess Margaret Cancer Genomic Program's clinical research studies (REB reference numbers 11-0962 and 12-0361) with the patient's written informed consent.

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### Author Contributions

All authors are members of the Princess Margaret Cancer Genomics Program. T.L.S. and S.K.-R. are responsible for accuracy of genomic data. K.L.A. and L.L.S. are responsible for the accuracy of clinical data. All authors contributed to writing the manuscript and approved the final manuscript.

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Competing Interest Statement

The authors have declared no competing interest.

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