Response to Trastuzumab and Lapatinib in a Metastatic Colorectal Cancer Harboring *HER2* Amplification and *HER2* S310F Mutation

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ABSTRACT

Dual *HER2*-targeted therapy has been associated with clinical responses and prolonged progression-free survival and overall survival in *RAS*-wild type *HER2*-amplified colorectal cancer (CRC). However, no clinical benefits have been reported in patients with CRC with *HER2* mutations. Activated *HER2* mutations have been largely deemed resistant to trastuzumab and to dual *HER2* targeting. This report describes a patient with metastatic CRC with concurrent *HER2* amplification and a *HER2* S310F mutation, which is an active mutation located in the extracellular dimerization domain of *HER2*. Treatment with trastuzumab + lapatinib resulted in an excellent response that lasted for 10 months. Upon disease progression, treatment with the antibody–drug conjugate trastuzumab–deruxtecan resulted in a short-lived response. This is the first case report of successful *HER2* targeting in metastatic CRC with concurrent *HER2* amplification and a *HER2* S310F mutation.

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***HER2* amplification occurs** in 2% to 3% of patients with metastatic colorectal cancer (CRC).^{1–3} Recent studies have shown that *HER2* amplification is associated with relative resistance to anti-epidermal growth factor receptor (EGFR) targeting in *RAS* wild-type (WT) CRC.^{4–6} Preclinical studies have conferred the sensitivity of *HER2*-amplified *RAS* WT colorectal patient-derived xenograft (PDX) models to dual *HER2* targeting, leading to several phase II clinical trials in patients with *RAS* WT metastatic CRC.⁷ The combinations of trastuzumab + lapatinib, trastuzumab + pertuzumab, and trastuzumab–deruxtecan have all been associated with significant activity in *HER2*-amplified *RAS* WT CRC, leading to their endorsement in the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Colon Cancer as a treatment option for this patient population.^{8–11}

HER2 mutations are equally rare in metastatic CRC, occurring in approximately 2% of patients.¹² For example, concurrent *HER2* amplification and mutation was found in 0.5% of patients with CRC.¹² Preclinical studies have shown that select activated *HER2* mutations are sensitive to the combination of the *HER2* antibody + *HER2* tyrosine kinase inhibitors (TKIs).¹³ Responses to *HER2*-targeted therapy have been reported in *HER2*-mutated breast cancer.¹⁴ However, no convincing clinical activity has been reported yet in metastatic CRC. A comprehensive investigation of *HER2* alterations in metastatic CRC showed that *HER2* mutations were clustered in the tyrosine kinase domain, the juxtamembrane domain, the transmembrane domain, and the extracellular domain (ECD).¹² Mutations in the tyrosine kinase domain, such as L755S, V842I, D769Y, and K753E, lead to increased kinase activity and have been associated with resistance to both anti-*HER2* antibodies and small-molecule *HER2* kinase inhibitors.^{15–18} Responses to *HER2* targeting have been reported in juxtamembrane domain/transmembrane domain mutations in preclinical and clinical studies.^{19,20} Mutations in the ECD, such as S310F and S310Y, lead to the increased dimerization of *HER2* and subsequent activation of the downstream signaling pathways. Prior

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studies have shown that these ECD mutations do not affect trastuzumab binding. Cells overexpressing these mutated proteins remain sensitive to both trastuzumab and HER2 TKIs, such as lapatinib, neratinib, and afatinib.^{21,22} Given the unique mechanism of activation of S310F *HER2* mutations, it is more likely that these mutations may be amenable to inhibition in metastatic CRC. This report describes the first patient with *RAS* WT metastatic CRC and a concurrent *HER2* amplification and *HER2* S310F mutation who experienced a clinical response to dual HER2 targeting.

Case Presentation

A male patient aged 59 years presented with rectal bleeding, leading to diagnosis of a clinically T3N0 poorly differentiated rectal adenocarcinoma in November 2016. He received capecitabine-based chemoradiation between November 2016 and January 2017 and experienced a complete clinical response, after which he opted for watchful waiting and no further treatment. In June 2017, he was found to have disease recurrence with distant lymph node metastases. Biopsy of a supraclavicular lymph node revealed a *RAS* and *BRAF* WT microsatellite-stable metastatic adenocarcinoma, consistent with his primary tumor. He was treated with infused 5-FU/folinic acid/oxaliplatin (FOLFOX) + bevacizumab between July 2017 and November 2017, with an excellent response, followed by further consolidation radiation therapy to the supraclavicular lymph node area. The patient experienced further disease progression in May 2018 with confirmed metastatic disease to the liver; he was started on 5-FU/folinic acid/irinotecan (FOLFIRI) + panitumumab and completed 6 cycles of treatment. Imaging studies showed response to treatment, and radiofrequency ablation of 3 hepatic lesions was performed in late August 2018. In November 2018, he was diagnosed with bowel obstruction secondary to progressive local recurrence that required a diverting ileostomy. Intraoperative findings were significant for the presence of peritoneal carcinomatosis, and this was confirmed through biopsy. The

patient was referred to our center at that point for consideration for clinical trials.

A circulating tumor DNA (ctDNA) assay was performed (Guardant360; Guardant Health, Inc.) to explore the presence of other potential driver mutations or potential mechanisms of resistance to anti-EGFR therapy. Results showed a plasma *HER2* copy number of 8.1 and a *HER2* S310F mutation (variant allele frequency [VAF], 72.3%) in addition to *TP53* S127fs and *MYC* amplification. Given the recently documented predictive value of the *HER2* ctDNA copy number and response to trastuzumab + lapatinib, and knowing the potential sensitivity of S310F to HER2 targeting, the patient was offered prompt treatment with dual HER2 targeting.²³ In December 2018, he was started on trastuzumab at an intravenous loading dose of 8 mg/kg, followed by 6 mg/kg intravenously every 3 weeks plus oral lapatinib at 1,000 mg once daily.

Within 2 months, the patient's performance status dramatically improved from ECOG 2 to 0, along with a significant decline in tumor markers (Figure 1A). Significant tumor shrinkage was documented on his imaging studies (Figure 1B, C) after 8 cycles of treatment. He continued this treatment for 13 cycles with an excellent response, but experienced disease progression in August 2019. Repeat Guardant360 ctDNA assay at the time of progression revealed an increased plasma copy number of *HER2* amplification (14.8), increased allele frequency of the *HER2* S310F mutation (VAF, 85.6%), *HER2* G1189fs mutation (variant of uncertain significance), and *FGFR2* amplification (plasma copy number 3.0), in addition to *KRAS* G12V, *PIK3CA* R88Q, *PIK3CA* E545K, and *TP53* S127fs mutations and *MYC* amplification (Figure 2A, B). A liver biopsy confirmed ongoing *HER2* amplification and the presence of the *HER2* S310F mutation, in addition to a *PIK3R1* E17fs mutation, *FANCD2* mutation, and *MYC* amplification in late September 2019. The patient was re-challenged with FOLFOX + bevacizumab (September 2019 to March 2020), but experienced disease progression in the liver, distant lymph node, and primary tumor. He then received 3 cycles of trastuzumab + pertuzumab

Figure 1. Laboratory and imaging results after treatment with trastuzumab + lapatinib. (A) Graph showing the dynamics of CA19-9 tumor marker response during treatment. (B) CT image from PET/CT scan before trastuzumab + lapatinib (red arrow: liver lesion, 2.4 × 1.4 cm). (C) MRI scan after 8 cycles of trastuzumab + lapatinib (red arrow: the same lesion, 0.7 × 0.9 cm).

Figure 2. ctDNA change after trastuzumab and lapatinib treatment. (A) Plasma copy number change of *HER2*, *MYC*, and *FGFR2*: copy numbers of *HER2*, *MYC*, and *FGFR2* at baseline were 8.1, 2.7, and 0, respectively, and at the time of disease progression were 14.8, 5.5, and 3.0, respectively. (B) VAF change in *HER2*, *TP53*, *PIK3CA*, and *KRAS*: VAFs of the *HER2* S310F, *HER2* G1189fs, *TP53* S127fs, *PIK3CA* R88Q, *PIK3CA* E545K, and *KRAS* G12V mutations at baseline were 72.3%, 0%, 16.5%, 0%, 0%, and 0%, respectively, and at the time of disease progression were 85.6%, 3.4%, 44.2%, 0.4%, 0.08%, and 0.04%, respectively. Abbreviations: ctDNA, circulating tumor DNA; VAF, variant allele frequency.

between April 2020 and June 2020, with continued disease progression. Given the reported activity with trastuzumab–deruxtecan in the DESTINY-CRC01 trial in patients with prior trastuzumab-naïve and pretreated metastatic *HER2*-amplified metastatic CRC,¹¹ the patient was treated with trastuzumab–deruxtecan from June 2020 through October 2020, and experienced a brief response in tumor markers (Figure 3A) and with tumor shrinkage in measurable lesions (Figure 3B, C), followed by disease progression.

Discussion

This case report presents the first patient with CRC with concurrent *HER2* amplification and an *HER2* S310F mutation who experienced remarkable response to trastuzumab + lapatinib. After 10 months, his disease progressed and treatment with trastuzumab–deruxtecan produced a short-lived response.

Dual *HER2* targeting with trastuzumab + *HER2*-specific TKIs (lapatinib or tucatinib) or trastuzumab + pertuzumab has achieved significant clinical activity in

multiple phase II trials in *RAS/RAF* WT, *HER2*-amplified CRC.^{8,9,24,25} Targeting *HER2* mutations in CRC is not well-defined. Prior studies evaluating *HER2* mutations in breast cancer and non-small cell lung cancer have shown that most of the activated *HER2* mutations fall into residues in the ECD and the kinase domain.^{21,22} The *HER2* S310F mutation in our patient was an activated mutation located in the extracellular dimerization domain and did not affect trastuzumab binding. It resulted in the noncovalent dimerization of *HER2* and subsequent activation of downstream signaling.²¹ Preclinical studies with CRC PDX harboring an *HER2* S310Y mutation, which functions the same way as the S310F mutation, showed that dual *HER2*-targeted therapy with trastuzumab + lapatinib leads to significant tumor regression.¹³ Responses to single *HER2* tyrosine kinase targeting have been reported in *HER2*-mutated breast cancer.¹⁴ The 10-month time to progression and objective radiographic response documented in our patient confirms the clinical relevance of targeting this mutation in the setting of *HER2* amplification with trastuzumab + lapatinib in patients with *RAS* WT metastatic CRC.

Figure 3. Laboratory and imaging results with trastuzumab–deruxtecan treatment. (A) Graph showing the dynamics of CA19-9 tumor marker response during treatment. (B) MRI scan before trastuzumab–deruxtecan therapy (red arrow: posterior hepatic dome lesion, 2.0 × 1.9 cm). (C) MRI scan after 4 cycles of trastuzumab–deruxtecan treatment (red arrow: the same lesion, 1.3 × 0.7 cm).

ctDNA *HER2* amplification is a reliable alternative to tissue-based genomic analysis, with an observed plasma copy number of >2.4 predicting response to *HER2*-targeted therapy.²³ Indeed, our patient had a *HER2* plasma copy number of 8.1 and responded to dual *HER2* targeting. The genomic analysis of the liver biopsy was concordant with ctDNA assay at the time of disease progression in detecting both *HER2* amplification and *HER2* mutation. However, ctDNA analysis detected more genomic alterations than tumor biopsy genomic analysis, showing the limitation of tumor biopsies in mapping out genomic heterogeneity. The increase in the *HER2* copy number and mutant allele frequency of the *HER2* S310F mutation at the time of disease progression probably reflects the increased tumor burden at the time of progression. A novel clone of *HER2* (G1189; VAF, 3.4%), a truncate mutation localized at the distal portion of the C-terminal tail, was noticed at the time of disease progression. We did not find any reports correlating this specific mutation to *HER2* resistance, and several studies have suggested that deletions in this region are not likely to affect *HER2* phosphorylation.^{26,27}

The emergence of *KRAS* and *PIK3CA* mutations has been associated with acquired resistance to *HER2* targeting in metastatic CRC with *HER2* amplification.²⁸ Preclinical PDX models have also linked *RAS* mutations with resistance to anti-*HER2* therapy in *HER2*-amplified CRC.²⁸ It is possible that the *KRAS* and *PIK3CA* mutations may have contributed partially to the resistance to *HER2* targeting. However, the VAFs of *KRAS* and *PIK3CA* (0.04% and 0.4%, respectively) were extremely low compared with that of *TP53* (VAF, 44.2%), indicating that these mutations may not represent the predominant mechanism of resistance to *HER2* targeting in our patient. Other studies have shown that the amplification of *FGFR* signaling represents another mechanism of resistance to *HER2* targeting in *HER2*-amplified tumors.^{29,30} Our case report suggests the emergence of the *KRAS* mutation, the activation of the *PI3K-AKT* pathway, and *FGFR2* amplification as separate and heterogeneous mechanisms of resistance to *HER2* targeting.

Trastuzumab–deruxtecan is an antibody–drug conjugate that conjugates trastuzumab through a tetrapeptide-based cleavable linker to a topoisomerase I inhibitor payload. The DESTINY-CRC01 trial¹¹ has shown a response rate to trastuzumab–deruxtecan of 45% and a median progression-free survival of 6.9 months in patients with *RAS* WT *HER2*-amplified metastatic CRC. The response rate in patients with prior *HER2*-targeted therapy was 43.8%, similar to the response rate in those who were *HER2*-targeting-naïve. The clinical benefit seen in our patient, albeit short, further supports the value of trastuzumab–deruxtecan beyond progression with dual *HER2*-targeting.

Conclusions

This is the first report of a patient with metastatic CRC with concurrent *HER2* amplification and a *HER2* S310F mutation who achieved an excellent clinical response to dual *HER2* targeting with trastuzumab + lapatinib. He received a total of 13 cycles of trastuzumab + lapatinib, with a 10-month time to progression. Our data confirm the relevance of *HER2* targeting for this specific *HER2* alteration and does not extend to other *HER2* tyrosine kinase alterations in metastatic CRC. Treatment with trastuzumab–deruxtecan resulted in additional clinical benefit, suggesting the relevance of this agent in tumors harboring concurrent *HER2* amplification and mutation. Our case report highlights the need for *HER2* assessment in all patients with CRC and provides support for further investigating *HER2* targeting in *HER2*-mutated CRC.

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