Supplementary Materials:

**Data file S1. Patient Histories**

*Patient 1 (Standard-of-Care ORR 2nd Line = 20-30%)*

A 73-year-old man with history notable for years of heartburn and nocturnal cough refractory to proton pump inhibitors who presented with abdominal fullness and early satiety and esophagoduodenoscopy revealed a partially obstructive, fungating, poorly differentiated distal esophageal adenocarcinoma with mixed diffuse and intestinal type histology. His supraclavicular lymph node biopsy confirmed metastatic disease (cT3N3M1) and he began systemic FOLFOX, which was de-escalated to 5-FU maintenance after 4 cycles due to neuropathy, but his scans at the time revealed radiographic response in his adenopathy. After 9 additional cycles of 5-FU, he progressed in his liver and primary lesion and received durvalumab (PD-L1 inhibitor) in combination with tremelimumab (CTLA-4 inhibitor) on protocol, but developed progression and new radiographic carcinomatosis after 4 cycles. At diagnosis 10/2014, his metastatic biopsy of the lymph node at original presentation did have *EGFR* amplification by NGS (CN=145). Of note, he also had *MYC* amplification (63 copies) and a *TP53* R273C mutation (**Supplemental Figure 1A**). His primary tumor at diagnosis was *EGFR* amplified by FISH and insufficient for NGS, which was confirmed to have high IHC expression. His primary tumor biopsy NGS just prior to IO therapy 7/2015 did not reveal *EGFR* amplification NGS and was insufficient for FISH or other studies. Baseline NGS on ctDNA revealed *EGFR* amplification (++, 3.53 copies), TP53 R273C mutation (1.1% allele frequency, MAF), and CDKN2A A34P (0.6% MAF). After FOLFOX/5FU his EGFR ctDNA dropped to (+, 2.21). After quick progression on IO (we do not consider this a line of therapy given that it is non-standard and he didn’t derive benefit), his ctDNA had risen to (+++, 15.19 copies) on IO therapy, and then (+++, 33.76 copies) just prior to starting FOLFIRI with cetuximab. Given the *EGFR* amplification, he began FOLFIRI with cetuximab, and he developed a classic cetuximab rash on his face and torso, and clinically, he had a radiographic complete response after 10 weeks of therapy. His subsequent ctDNA NGS demonstrated dramatic reduction in disease (*EGFR* amplification undetectable, 0.2% MAF TP53 R273C). Given the dramatic response, he received 8 cycles of maintenance 5FU-cetuximab therapy, and interval restaging CTs demonstrated stable disease. However, concomitant ctDNA sequencing after 4 and 8 maintenance cycles suggested early recurrence with a rising somatic alteration burden and actual return of EGFR amplification (+++, 16.09 copies). Repeat endoscopy for dysphagia identified a second primary lesion adjacent to his previous (what we consider a ‘skip’ lesion), which exhibited persistent *EGFR* amplficication but de novo *PTEN* deletion by NGS and IHC, which accounts for his acquired cetuximab resistance. Notably, the original region of the primary tumor more proximally was *EGFR* non-amplified at this time. Irinotecan was reinitiated along with 5-FU and cetuximab to address this tumor heterogeneity for 6 cycles in attempt to regain control which did demonstrate some benefit with decrease in *EGFR* amplified ctDNA to (+++, 6.24 copies), before he developed concurrent bilateral pulmonary emboli and upper gastrointestinal bleeding, despite arterial embolization. Progression-free and overall survival were 10 months from cetuximab initiation. This case highlighted not only intra-tumoral heterogeneity in the setting of selective pressure, but also the limitation of ctDNA in detecting acquired somatic deletions where we noted persistent *EGFR* amplification in the plasma, but these amplified clones had acquired *PTEN* deletion as determined in the tissue.

*Patient 2 (Standard-of-Care ORR 1st Line = 40%)*

A 48-year-old man who presented with pancytopenia and widely metastatic disease to his liver, lungs, and skeleton (including marrow replacement). He was initiated on FOLFOX with transfusion support (hemoglobin 7 and platelets 10 on presentation due to diffuse marrow replacement), and ABT-806, an EGFR inhibitor, was added per the PANGEA protocol once his NGS reported *EGFR* amplification (primary: CN=67, retroperitoneal: CN=111). *EGFR* amplification correlated well with protein expression by IHC and SRM. He achieved a complete response by RECIST after 4 cycles of therapy (2 with ABT806) and was no longer transfusion-dependent. Of note, he also had amplifications of *ERBB2* (CN=31), *JAK2* (CN=11), *PD-L2* (CN=16), and *PD-L1* (CN=16) in the primary lesion, but not in the retroperitoneal node or bone marrow biopses. *EGFR* and *ERBB2* co-amplification occurs in less than 1% of gastric cancer patients in TCGA (4/443) (CbioPortal). They were not amplified in the same cellular clones, but rather in two independent regions of the tumor account for ~50% each. This primary intra-tumoral heterogeneity, which was initially composed of 50% HER2+ and 50% EGFR expressing clones, after EGFR-directed therapy we observed that the HER2+ clone represented ~75% of the tumor, while the *EGFR* amplified clone was no longer present. Serial ctDNA demonstrated absence of *EGFR* amplification by 3 months of therapy with gradual increasing *KRAS* amplification burden over time. After 42 weeks of therapy, he developed progressive hydronephrosis and his peritoneal biopsy revealed *KRAS* amplification and absence of *EGFR* amplification in this tissue and by ctDNA (**Supplemental Figure 2B**). Per protocol, he was initially transitioned to FOLFIRI with continued ABT-806 until we received the biopsy results, at which point given subsequent *KRAS* amplification and absence of *EGFR* amplification, he then received FOLFIRI with ramucirumab per protocol, which he remains on to date more than 12 months later (see treatment assignment algorithm Supplemental Figure 1).

*Patient 3 (Standard-of-Care ORR 3rd Line = 10%)*

 This is a 72-year-old man with progressive dysphagia and heartburn who was found to have a *HER2* non-amplified gastroesophageal adenocarcinoma. He underwent 6 cycles of ‘neoadjuvant’ EOX, but later declined surgery and was continued on capecitabine for 7 months. Therapy was transitioned to second line paclitaxel with ramucirumab due to rising tumor markers, but paclitaxel was stopped after 4 months due to neuropathy. Osseous and solid organ metastases were seen 11 months after ramucirumab initiation, and therapy was stopped. The natural transition from clinical stage III at diagnosis to clinical stage IV clearly occurred during these first/second line therapies. NGS of his primary lesion at diagnosis and also after second line therapy demonstrated *EGFR* amplification (CN=167) as well as *TP53* R248Q and *PIK3R1* mutations. Just prior to starting therapy for 3rd line cetuximab monotherapy, his ctDNA in the blood was (+++, 20.77 copies). Of note, he had developed a progressive cough in the months leading up to initiation that was his main reported clinical cancer-associated symptom. Cycle one was complicated by acute worsening cough and dyspnea during cetuximab infusion leading to hospitaliation, which was aborted with ~75% of drug completed treated with steroids and antihistamines (in case of infusion allergic reaction). His cough completely resolved one week after initial infusion. It was our sense that he had an intense ‘therapy-related reaction’ that first cycle with exacerbation of the cough at first, then with resolution of the cough. He did not develop infusion reaction with any cycle thereafter. He developed a significant grade 3stereotypical cetuximab-induced acneiform rash. His ctDNA EGFR dropped after 4 doses (+, 2.42 copies). Upon repeat routine EGD after 6 months of therapy, his residual primary tumor no longer demonstrated *EGFR* amplification, but had acquired a new *SMAD4* L533R mutation. Of note, his ctDNA revealed residual low level *EGFR* amplification which gradually continued to increase over time every 2 months, but did not identify the *SMAD4* mutation. After 9 months of cetuximab monotherapy, CT imaging and clinical status were stable, yet serum tumor markers and ctDNA alteration burden began rising, and after 12 months he developed recurrent dyspnea on exertion and finally with objective radiographic progression in his lungs and mediastinal lymph nodes identified at 14 months from initiation. His ctDNA at the time of progression demonstrated persistent *EGFR* amplification (+++, 34.8 copies) and also *BRAF* and *MET* amplifications (**Figure 4C,** **Supplemental Figure 1C**). Given persistent circulating *EGFR* amplification, cetuximab was planned to be continued with concomitant FOLFIRI initiation, but he developed falls and found to also have new multiple brain lesions. He received palliative radiotherapy to these lesions, but declined rapidly and died.

*Patient 4 (Standard-of-Care ORR 4th Line = <5%)*

 A 67 year old man with remote history of prostate cancer resection who presented with abdominal distention, reflux, epigastric discomfort and dysphagia. An EGD demonstrated a partially obstructive circumferential mass from the distal esophageal to the gastric cardia with biopsy revealing poorly differentiated HER2 negative adenocarcinoma. His initial staging also revealed liver and osseous metastases, and he initially received mFOLFOX6 for 12 cycles with radiographic and clinical response. This was followed by capecitabine monotherapy due to neuropathy, but 5 months later, his CT demonstrated progression in his hepatic metastases and he was started on second line docetaxel and ramucirumab with mixed response after 3 cycles. He continued on paclitaxel monotherapy for 3 months, and was transitioned to third line irinotecan monotherapy due to progression before this too two months later was discontinued due to progression and worsening performance status. At that time, he was seen in consultation and had an ECOG PS of 3 and total bilirubin of 2.1. His initial diagnostic tumor NGS revealed *EGFR* amplification (54 copies), *NRAS* amplification (60 copies), *FGF3/4/19* amplification (16 copies), *CCND1* amplification (16 copies), *ERBB2* amplification (8 copies) and *TP53* R273C mutation (MAF 67%). Repeat NGS of ctDNA at this time (after three lines of therapy) confirmed persistent *EGFR* amplification (+++, 58.5 copies), along with amplifications of *CCND1* (+++), *MYC* (+++), *ERBB2* (++), *PDGFRA* (+), *CCND2* (+), *KRAS* (+), *BRAF* (+), *AR* (+) and *TP53* mutation R273C (MAF 62.9%) and *NRAS* D126N (MAF 2.9%). He was offered cetuximab monotherapy versus hospice care, and after one dose of cetuximab, he noted improvement in energy, appetite, and functional status with resolution of right upper quadrant pain (from extensive liver tumor involvement) and normalization of bilirubin. He achieved at partial RECIST response at 2 months, and ctDNA demonstrated decreased EGFR amplification (++, 3.01 copies). An on treatment biopsy at ~2 months of the liver revealed persistent EGFR amplification by NGS (18 copies). He developed rapid disease progression by 14 weeks with hyperbilirubinemia in the setting of increasing *NRAS* amplification and other genomic aberrations including CCND2 amplification, HER2 amplification and GNAS mutation by ctDNA, and EGFR amplification although persistent, remained relatively low (+++, 5.01 copies) (**Figure 4E, Supplemental Figure 1D**).

Patient 5 (*Standard-of-Care ORR 1st Line = 40%)*

 This is a 74-year old woman who presented with abdominal pain and dysphagia and her EGD with EUS identified a uT3N2 moderately differentiated, HER2 negative gastroesophageal junction adenocarcinoma. PET identified metastatic spread to the liver, which was confirmed by biopsy. Her primary tumor NGS demonstrated *EGFR* (copy number = 54), *MET* (copy number =15), *CDK6* (copy number = 6), and *BRAF* (copy number = 6) amplifications as well as mutations in *TP53* (P250L allelic frequency 7.9%, R342\* allelic frequency 41%), and *ARID1A* (Q1334\_R1335insQ allelic frequency 25.1%) and an *EGFR-SEPT14* fusion. Her baseline ctDNA was notable for *TP53* R342\* (MAF 1.4%), *KRAS* G12D (MAF 0.3%), *EGFR* L858M (MAF 0.2%), *MET* amplification (++), and *EGFR­* amplification (+, 2.41 copies). She received 1 month of FOLFOX with continued rise in her CEA and CA19-9 and ABT-806 was added per full profiling per the PANGEA trial (see supplementary Figure 1). Although serum tumor markers continued to rise during first line therapy, her dysphagia and abdominal discomfort dramatically improved. She was deemed clinically progressing with persistently and rapidly increasing tumor markers, however, after 4 months of FOLFOX-ABT806 therapy, and at which time her ctDNA no longer revealed *EGFR (*nor *MET* amplification) but increasing allele frequency of *KRAS* G12D mutation that was present at diagnosis. EGD at this time with biopsy of the primary tumor revealed no *EGFR* amplification, and repeat liver biopsy no *EGFR* amplification but *KRAS* mutation. Therefore therapy was transitioned to second line FOLFIRI with ramucirumab per PANGEA protocol. After this therapy, all ctDNA burden declined including the *KRAS* mutation, which is attributed to this adjusted therapy. This case again demonstrates intrapatient tumor heterogeneity where the primary tumor initially was EGFR amplified, with low level EGFR amplification in the plasma, and ultimately ‘loss’ or ‘eradication’ of this clone in all compartments (primary tumor, metastatic tumor, and ctDNA) analyzed post-therapy at 4 months while tumor markers and other ctDNA was rising, suggesting the need for change and ultimately limited time frame of benefit from anti-EGFR therapy.

*Patient 6 (Standard-of-Care ORR 3rd Line = 10%)*

 This is a 58-year-old woman who initially presented with cervical adenopathy and was found to have metastatic gastric adenocarcinoma. She received 6 months of first line FOLFOX followed by 3 months of 5-FU maintenance before resuming FOLFOX for another 6 months. Upon disease progression, she transitioned to second line ramucirumab and paclitaxel for 7 months before again progression in her liver and peritoneum. Somatic NGS on her metastatic lymph node at diagnosis demonstrated *EGFR, MYC and CCNE1* amplification and *PTCH 1* and *EZH2* mutations. NGS of her primary lesion after this second line therapy identified amplifications in *EGFR* (copy number = 98), *CCNE1* (copy number=14), *MYC* (copy number=40) and mutations in *PTCH1* (T1064M, allelic frequency 62%), *EZH2,* and *MAP3K1*. Baseline ctDNA revealed amplifications in *EGFR* (+++, 5.14 copies), *KRAS* (+++)*, CCNE1*(++), and *MYC* (++) as well as mutations in *GNAS* (R201C, allelic frequency 1.1%) and *TP53* (R273H, allelic frequency 0.2%, H178P; allelic frequency 0.2%). The *KRAS* amplification in her ctDNA was not identified in either her baseline somatic NGS prior to cetuximab, nor her previous NGS from initial diagnosis two years prior, which points to inter-tumoral heterogeneity. She received one dose of cetuximab monotherapy with development of an acneiform rash, but was unable to obtain further drug due to insurance disapproval. She did have a CT 6 weeks after the one dose of cetuximab therapy which was stable. She was transitioned to FOLFIRI subsequently with stable disease also. Upon peritoneal progression six months later, she demonstrated persistent *EGFR* (++, 4.60 copies) and *KRAS* amplifications by ctDNA. She did receive two doses of compassionate use cetuximab at this time without clinical benefit, prior to hospice transition.

Patient 7 (*Standard-of-Care ORR 1st Line = 40%)*

This is a 54-year-old man with past history of diabetes mellitus who presented with early satiety and vomiting in the setting of a 25 pound weight loss. His imaging revealed a 5cm distal-esophageal mass as well as extensive liver and bone involvement. The liver lesions were confirmed by biopsy that identified moderately differentiated HER2 negative adenocarcinoma in both his primary lesion and liver. His primary lesion NGS was notable for *EGFR* amplification (copy number = 77) and deletions in *CDKN2A/B* and mutation in *PIK3CB*, but NGS on his liver revealed the *CDKN2A/B* and *PIK3CB* alterations, and lacked *EGFR* amplification. IHC of his primary tumor confirmed high EGFR expression in 30 percent of the sampled primary tumor. Of note, ctDNA identified mutations in *TP53* (S241F, allelic frequency 0.3%) and *PDGFRA* (N995N, allelic frequency 0.2%), but also failed to identify *EGFR* amplification. Per PANGEA protocol, he was assigned to the ABT806 arm due to EGFR expression in his liver by mass spectrometry and lack of other targetable lesions (see supplementary figure 1). He had received 4 cycles of FOLFOX per protocol while awaiting all molecular profiling. He had persistent/refractory dysphagia during this time. CT after these 4 cycles was ‘stable’. Prior to considering stent/radiation and/or change of chemotherapy given his progressing dysphagia, and given the molecular results, ABT806 was added at C5 per protocol. He experience rapid improvement in dysphagia after the first cycle. He remained on FOLFOX and ABT-806 (though oxaliplatin discontinued after 8 cycles for neuropathy) with clinical and radiographic therapeutic response at 8 months

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Patient 8 *(Standard-of-Care ORR 2nd Line = 20-30%)*

 This is a 58-year-old man who presented with epigastric pain and was found to have gastric cancer with gastric outlet obstruction and peritoneal carcinomatosis. He received eight cycles of FOLFOX followed by a palliative gastrectomy that revealed ypT4bN2 disease with minimal residual tumor. Six months later, he was admitted for small bowel obstruction due to peritoneal disease progression and he received an additional six cycles of FOLFOX as well as biliary stenting. Upon presentation to our clinic at second line therapy evaluation, sequencing identified amplification of *MET* (CN=11), *EGFR* (CN=159), and *ERBB2* (CN=27). However, his poor performance status precluded further therapy by that time and so no targeted agents were administered.