Supplementary File 1

Clinical information

In 2014, Ospedale Niguarda collected urine from two young CRC patients (**Tables S1, S2**). One (CRC-UD21) was a 39-yo male with rectal carcinoma in situ (*KRAS* WT, *BRAF* p.V600E, *HER2* IHC score 0) experiencing a disease stabilization lasting ~ 4 months as best response with first line FOLFIRI/ Bevacizumab from February to June 2014 with FOLFIRI plus Bevacizumab as first line, with a stable disease at first CT scan (April 2014) and progression at the following evaluation (July 2014). In addition to the primary tumor, lung, liver nodes metastases and peritoneal carcinosis were present at the time of urine sample sampling collection were detected. The second patient (CRC-UD24) was a 40-yo female with a left colorectal cancer and liver metastasis resected in December 2013 (pT3N2bM1, *KRAS* p.G13D on primary tumor and liver, *BRAF*, ALK/ROS1/TRKA WT). A first line of FOLFIRI plus bevacizumab was administered from February to April 2014 with a rapid hepatic disease progression. A second standard chemotherapy regimen with FOLFOX plus bevacizumab was administered from May to September 2014, with a first liver SD at echography after 3 cycles followed by radiological hepatic PD and poor clinical conditions.

Two further colorectal cancer patients (**Table S1** and **Table S2**) were treated at Istituto Nazionale Tumori (INT), where urine samples were collected in 2016. The first patient (CRC-UD02) was a 42 years old male at the time of diagnosis, with a surgically amenable rectal adenocarcinoma. In 2014, the patient was treated according to standard therapy with neoadjuvant chemo-radiation followed by surgery and adjuvant therapy with CAPOX. Recurrence in lung and abdominal extra-regional lymph node was diagnosed by imaging on January 2016. Due to the presence of *KRAS* p.G12D mutation detected on the primary tumor, the patient received first line treatment with FOLFOX plus Bevacizumab.

Urine samples were collected at baseline prior to cycle 1 day 1. The patient CRC-UD09 showed local relapse and onset of liver metastases two years after a multimodality treatment for his *BRAF* p.V600E –mutated rectal cancer. On June 2014, first-line therapy with FOLFOXIRI was started with partial response. After 9 months of disease control, the CT scan performed on June 2015 showed liver progression and the patient underwent an off-label treatment with panitumumab and vemurafenib with initial response and then disease progression after 4 months. The patient failed to respond to subsequent line with trifluridin-tipiracil was started soon thereafter on November 2015, achieving disease progression as

best response after two months of treatment. An off-label regimen with vemurafenib plus crizotinib was then administered from January 2016 up to May 2016. Radiological assessment done in May 2016 showed liver and lymphnodes disease progression followed by a rapid worsening of performance status.