EGFR Amplification in Metastatic Colorectal Cancer

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Supplementary Table 1. List of screening sources for *EGFR*-amplified cases and controls with relative assays used for *EGFR* amplification and/or extended next-generation sequencing.

Screening Source	***EGFR* amplification assay**
EGFR-amplified cases	
TRIBE-2 trial	Caris MI TumorSeek™ [1] and dual-color silver in-situ hybridization
VALENTINO trial	Foundation One® [2] and dual-color silver in-situ hybridization
PICCOLO trial	Affymetrix OncoScan array [3]
Istituto Nazionale dei Tumori	Foundation One® [2] and dual-color silver in-situ hybridization
Memorial Sloan Kettering Cancer Center	MSK-IMPACT™ [4]* and fluorescence in situ hybridization
Vall d'Hebron Institute of Oncology	Oncomine™ Comprehensive/Focus Assay [5] and fluorescence in-situ hybridization†
Samsung Medical Center	Oncomine™ Comprehensive Assay [5]
EGFR-negative cases	
TRIBE-2 trial	Caris MI TumorSeek™
VALENTINO trial	Dual-color silver in-situ hybridization
PICCOLO trial	Affymetrix OncoScan array
Istituto Nazionale dei Tumori	Dual-color silver in-situ hybridization
Memorial Sloan Kettering Cancer Center	MSK-IMPACT™

Vall d'Hebron Institute of Oncology	Oncomine^{TM} Comprehensive/Focus Assay
Samsung Medical Center	Oncomine^{TM} Comprehensive Assay

* *EGFR* gene copy-number (GCN) was assessed by means of allele-specific copy number analysis (FACETS software) for patients from the Memorial Sloan Kettering Cancer Center screening source as previously reported [6].

† FISH results were not available in 2 out of 4 cases to due tissue block exhaustion and inconclusive results, respectively.

Supplementary References

[1]. Puccini A, Poorman K, Salem ME, et al. Comprehensive genomic profiling of gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs). *Clin Cancer Res*. 2020; 26(22):5943-5951.

[2]. Pietrantonio F, Di Nicolantonio F, Schrock AB, et al. ALK, ROS1, and NTRK Rearrangements in Metastatic Colorectal Cancer. *J Natl Cancer Inst*. 2017; 109(12).

[3]. Seligmann J, Wood H, Richman S, et al. Epidermal growth factor receptor (EGFR) copy number (CN) as a biomarker of prognosis and panitumumab (Pan) benefit in RAS-wt advanced colorectal cancer (aCRC). *Ann Oncol*. 2017;28:v185.

[4]. Mondaca S, Walch H, Nandakumar S, et al. Specific Mutations in APC, but Not Alterations in DNA Damage Response, Associate With Outcomes of Patients With Metastatic Colorectal Cancer. *Gastroenterology*. 2020;159(5):1975-1978.

[5]. Yeh Y-M, Lee C-H, Chen S-H, et al. Comprehensive assessment of HER2 alteration in a colorectal cancer cohort: from next-generation sequencing to clinical significance. *Cancer Management Res*. 2019;11:7867-7875.

[6]. Shen R, Seshan VE. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res*. 2016;44(16):e131.

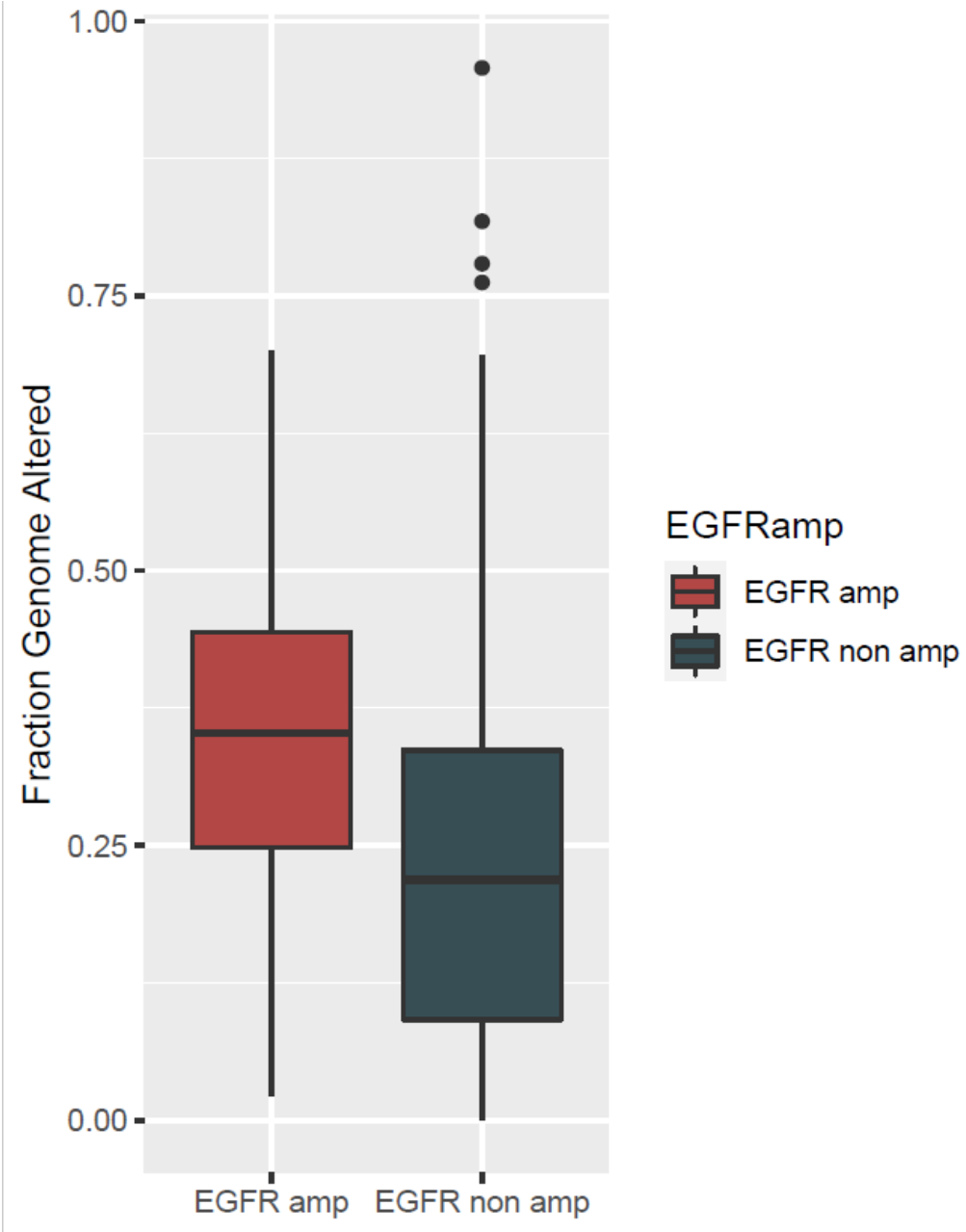
Supplementary Table 2. Response to anti-EGFR-based treatment in patients with *RAS*/*BRAF* wild-type mCRC with *EGFR* amplification.

Patient ID	Primary Tumor Location	Anti-EGFR-based Regimen	Line of Anti-EGFR-based Regimen	Best Response	*EGFR* CNV	*EGFR* co-alterations	Individual PFS (months)	OS (months)
MSKCC-03	Left	P	2	PR	7	-	13.1	15.5+
MSKCC-04	Left	P	3	PR	24	-	8.3	15.7
MSKCC-05	Left	P	2	SD	29	Rearrangement c.560-2097_889+126d	4.6	20.8+
MSKCC-07	Left	P	2	PR	6	-	8.5	15.3
MSKCC-09	Rectum	FOLFOX-CET	1	NA	7	-	NA	98.5+
MSKCC-11	Rectum	FOLFIRI-P	2	NA	8	-	NA	68+
MSKCC-12	Rectum	5FU-P	3	CR	12	-	5.4	5.4
MSKCC-14	Rectum	CPT11-P	3	PD	10	-	0.9	2.2
MSKCC-18	Left	CPT11- CET	3	PD	41	chr7:g.55336682del	2.1	6.7
MSKCC-20	Rectum	CPT11- P	3	PD	6	-	3.1	5.8
MSKCC-22	Left	FOLFOX-P	1	PR	17	-	NA	12.6
MSKCC-23	Left	FOLFIRI-P	2	NA	54	-	17.2	17.2
MSKCC-26	Rectum	P	1	PR	11	-	3.6	5.3
MSKCC-27	Rectum	CPT11-CET-BEV	2	PR	43	-	NA	24+
MSKCC-29	Rectum	FOLFOX-P	2	SD	9	-	4.2	20.1+
MSKCC-32	Rectum	P	3	PD	78	-	2.1	5.2+
MSKCC-33	Rectum	CPT11-P	4	SD	49	-	6.2	18.4
MSKCC-34	Rectum	FOLFIRI-CET	3	SD	24	-	15.8	20.2+
VHIO_01	Left	CPT11-CET	2	NA	14.6	*EGFR* G456E*	13	37.6
VHIO_03	Left	FOLFOX-P	1	NA	57	-	12	38.1
VHIO_04	Rectum	FOLFOX-P	1	SD	12	-	22.1	27.8
SMC-02	Rectum	FOLFIRI-CET	1	PR	10	-	22.6	49.9+
SMC-04	Rectum	FOLFIRI-CET	1	PR	8.9	-	11.1	14.8
SMC-07	Rectum	FOLFOX-CET	1	CR	5.1	-	17.8+	17.8+
SMC-09	Left	FOLFIRI-CET	1	CR	193.5	-	15.5+	15.5+
SMC-10	Left	FOLFOX-CET	1	PR	6	-	34.1	58.6+
SMC-11	Rectum	FOLFOX-CET	1	PR	8.9	-	5.1	5.8
PICCOLO-01	Left	CPT11-P	2	NA	6	NA	27.6	27.6
PICCOLO-03	Right	CPT11-P	2	NA	6	NA	28.2	47.1
INT-02	Rectum	FOLFOX-P	2	CR	80	-	28.2	44.3+
INT-03	Rectum	P	3	PR	6	*EGFR* S464L*	10	13.8+
INT-04	Left	FOLFIRI-P	2	PR	280	*LANCL2-EGFR* *EGFR* S464L*	8.1	16.6
INT-05	Rectum	5FU-P	1	SD	6	-	13.4	16.8+
VALENTINO-01	Left	FOLFOX-P	1	PR	6	-	17.2	33+

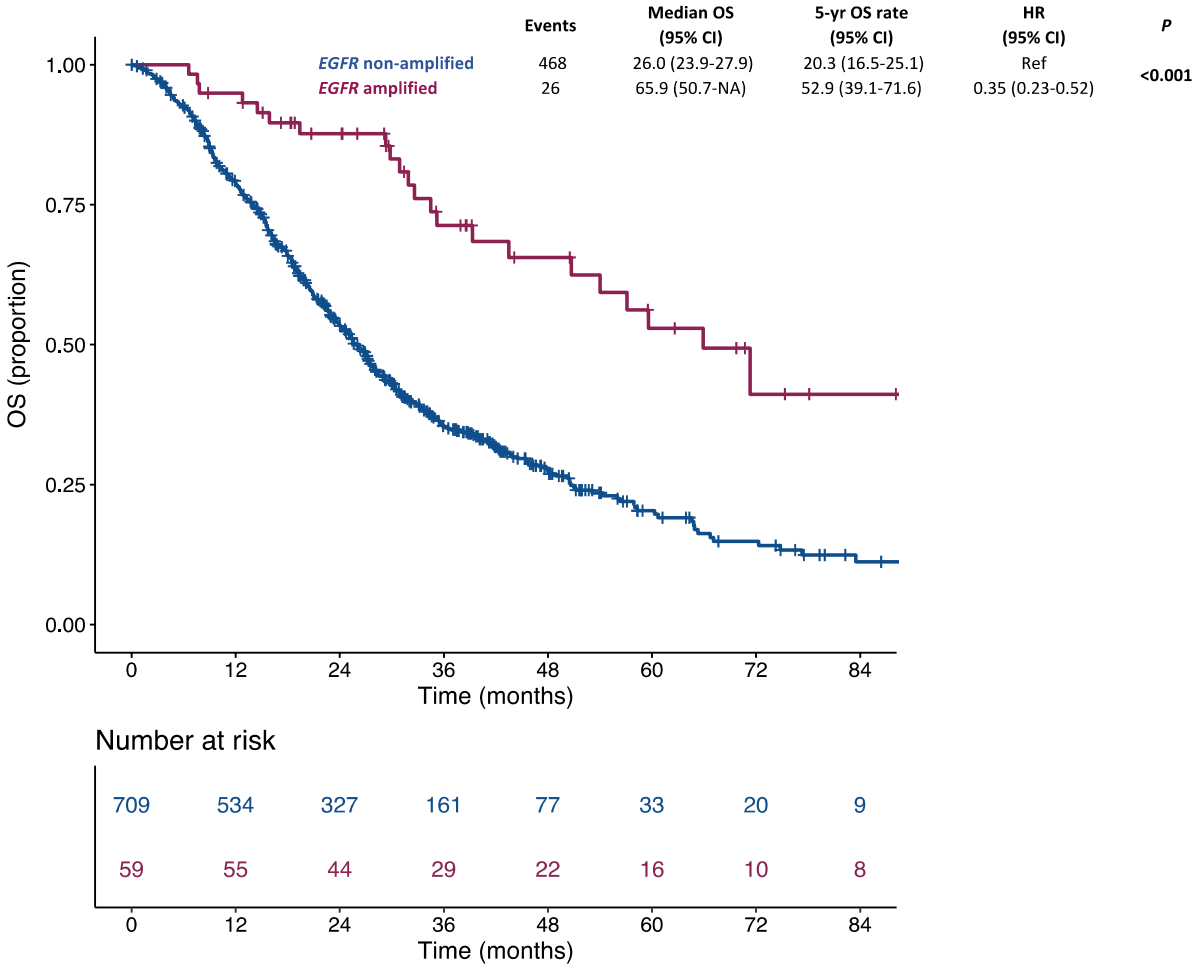
* *EGFR* extracellular-domain mutation was detected by means of liquid biopsy at onset to resistance to anti-EGFR-based therapy.

Abbreviations. 5FU: 5-fluorouracil. BEV: bevacizumab. CET: cetuximab. CPT11: irinotecan. CNV: copy number variations. CR: complete response. mCRC: metastatic colorectal cancer. OS: overall survival. P: panitumumab. PD: progressive disease. PFS: progression-free survival. PR: partial response. SD: stable disease.

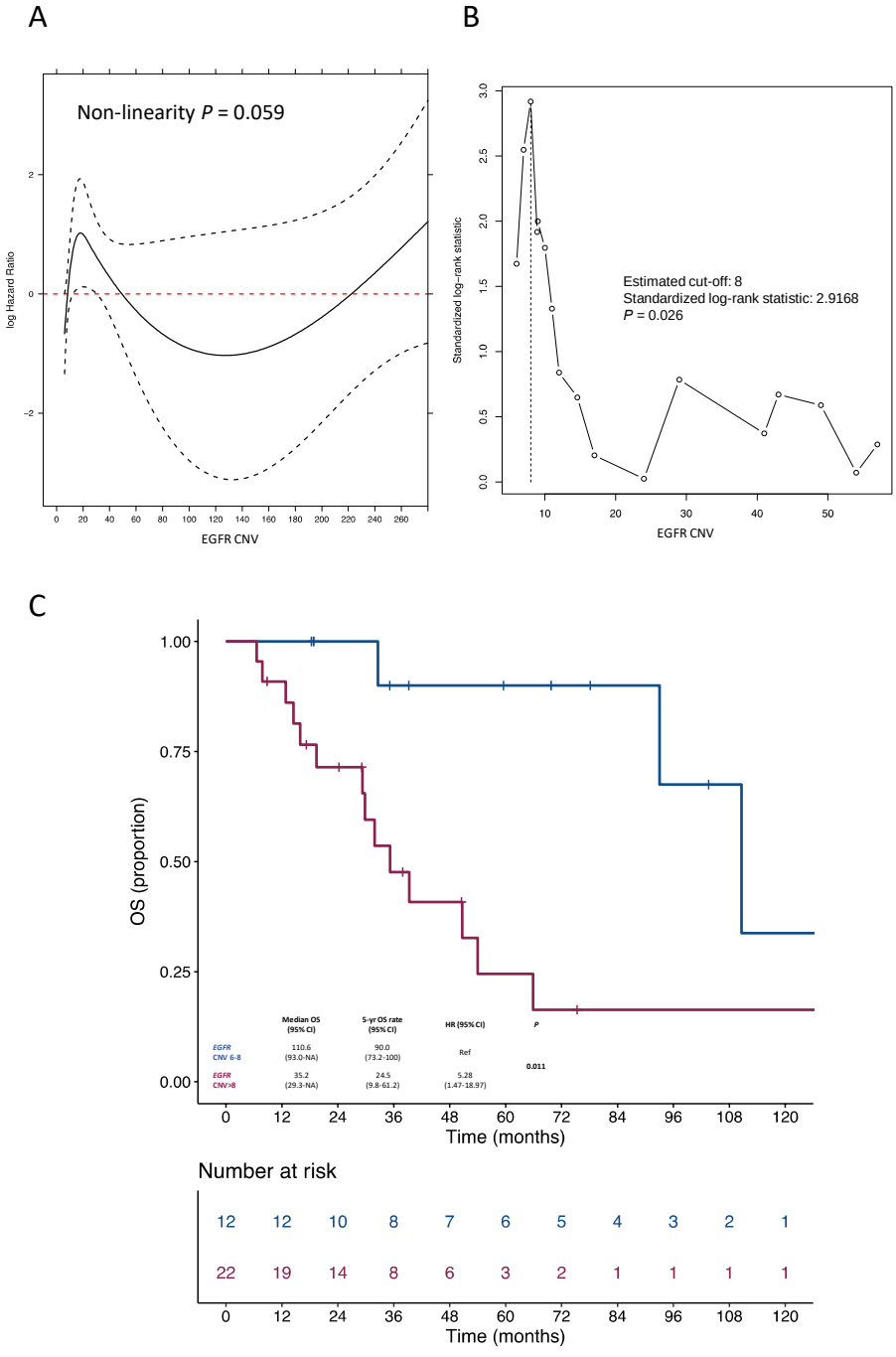
Supplementary Figure 1. Fraction of genome altered for *EGFR*-amplified (N=35) and *EGFR* non-amplified samples (N=439).



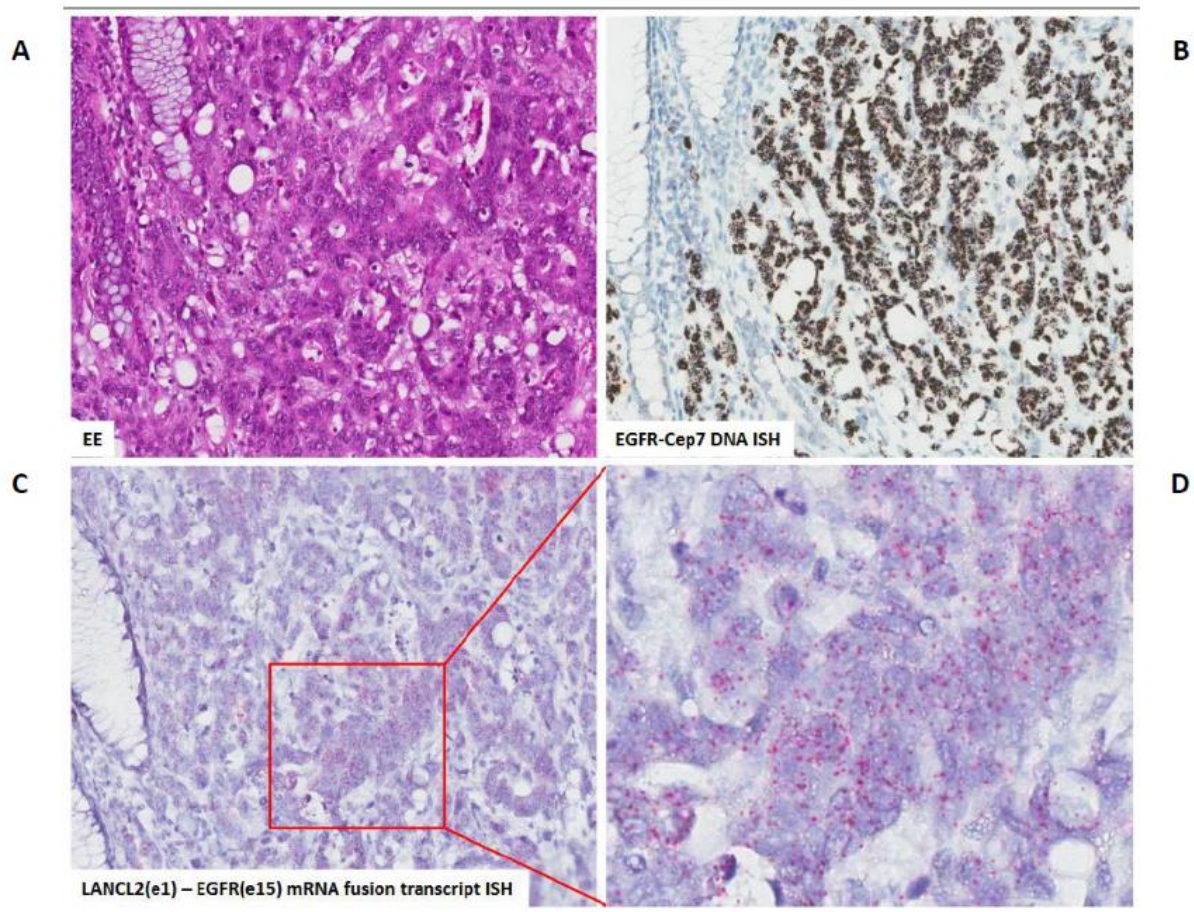
Supplementary Figure 2. Kaplan-Meier estimates of overall survival according to the presence of *EGFR* amplification in the subgroup of patients with *RAS*/*BRAF* wild-type mCRC (N=768). Blue lines indicate patients with *EGFR* non-amplified mCRC (N=709), whereas violet lines indicate patients with *EGFR*-amplified mCRC (N=59). In line with the results observed in the entire study population, patients with *EGFR*-amplified mCRC showed a better overall survival compared to patients with *EGFR* non-amplified mCRC.



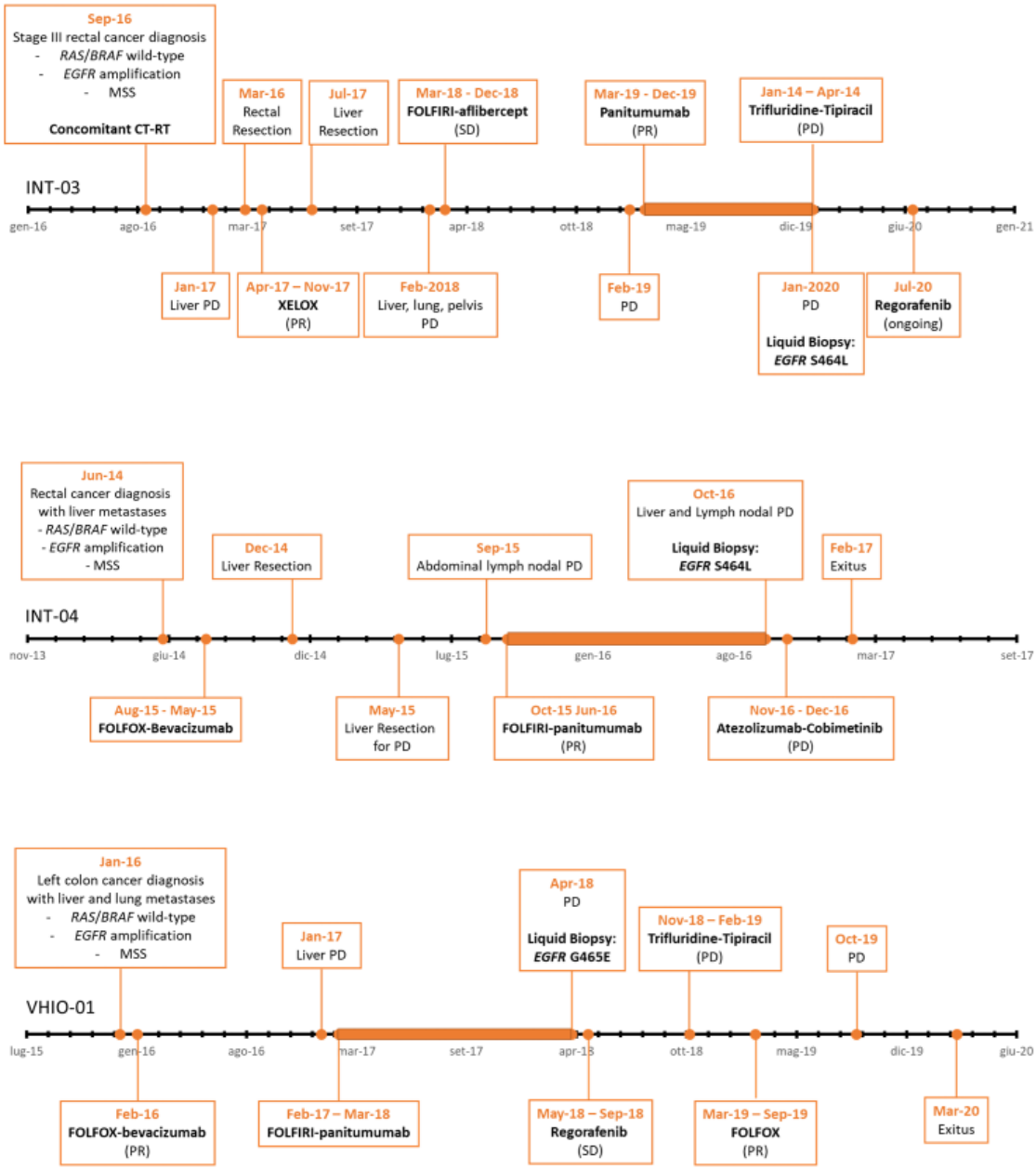
Supplementary Figure 3. Identification of an *EGFR* copy number variations (CNV) prognostic cut-off in patients with *EGFR*-amplified, *RAS*/*BRAF* wild-type mCRC treated with anti-EGFR agents (N=34). Panel A shows the effect plot depicting a non-linear continuous relationship (log Relative Hazard ±95%CI) between *EGFR* CNV (modeled by means of 3-knots natural cubic splines) and overall survival. Panel B shows the dot plot depicting the distribution of standardized log-rank test statistics for overall survival according to *EGFR* CNV. The best cut-off value for *EGFR* CNV was 8. Panel C shows the Kaplan-Meier estimates of overall survival according to *EGFR* CNV. Blue lines indicate patients whose tumor had an *EGFR* CNV 6-8 (N=12), whereas violet lines indicate patients whose tumor had an *EGFR* CNV >8 (N=22). Patients whose tumor had an *EGFR* CNV 6-8 showed a better overall survival compared to patients whose tumor had an *EGFR* CNV >8.



Supplementary Figure 4. Histological section of *EGFR*-amplified tumor with 280 *EGFR* gene copy number and *LANCL2-EGFR* fusion. (A). Hematoxylin & Eosin. (B) *EGFR*/chr.7 dual color bright field silver in situ hybridization (SISH). (C-D). LANCL2-EGFR mRNA fusion transcript by RNAScope® ISH. To identify the fusion partner, we performed RNA sequencing analysis and found EGFR expression was at 693 fragments per kilobase of exon model per million reads mapped (FPKM), which was the 3rd most highly expressed gene in the sample (out of 18,000 genes). Neighboring genes, *LANCL2* and *VOPP1* were also very highly expressed (in the top 100 of all genes). EGFR expression was consistently high across all exons suggesting that the full-length transcript was expressed. Interestingly, fusion analysis identified an in-frame *LANCL2(e1)-EGFR(e15)* fusion transcript. To morphologically detect such fusion transcript, we designed a RNAScope® ISH probe from the sequence 20bp on either side of the fusion breakpoint (CTTTTCATCAGGACGGGAAGGGACCAGACAACTGTATCCA). As shown in **panels C-D**, dot-like expression of the fusion transcript was identified.



Supplementary Figure 5. Disease course of patients with *EGFR*-amplified, *RAS*/*BRAF* wild-type metastatic colorectal cancer with acquired resistance to anti-EGFR therapy and emergence of *EGFR* extracellular-domain mutations detected by means of liquid biopsy.



Abbreviations. CT-RT (chemotherapy – radiotherapy). MSS: microsatellite stability. PD: progressive disease. PR: partial response.