Evolution of Mutational Landscape and Tumor Immune-Microenvironment in Liver Oligo-Metastatic Colorectal Cancer

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Supplementary Materials:

**Figure S1.** Inclusion and exclusion criteria flow-chart for patients’ selection.
**Figure S2.** Clinical courses of group A and group B patients.

**Figure S3.** Representative model showing divergent mutational and immunologic dynamics between oligo- and poly-metastatic colorectal cancer.
Table S1. List of genes mutations gained by liver metastases (all coding variants).

| Patient | Mutated genes | Role | AMP/ACMG prioritization | ClinVar ID |
|---------|---------------|------|-------------------------|------------|
| **Group A** | | | | |
| PAT1 | *PRKDC* | p.Gln281His8:g.48855892C>A | DNA repair and recombination | Tier 4 | NR |
| | *ACVR1B* | 12:g.52374752C>T (SAV) | Proliferation, differentiation | Tier 4 | NR |
| | *ALK* | p.Ala96Thr2:g.30143240C>T | Proliferation, differentiation | Tier 3 | 451139 |
| | *CREBBP* | p.Pro1983Thr16:g.3779101G>T | Transcriptional coactivation | Tier 3 | NR |
| | *DNMT3B* | p.Gly76Arg20:g.31372585G>A | Epigenetic modifications | Tier 3 | NR |
| | *FAS* | p.Cys135ValfsTer5210:g.90768707CT>C | Programmed cell death | Tier 4 | NR |
| | *FGF2* | p.Asp179ArgfsTer54:g.123748460A>AC | Proliferation, angiogenesis | Tier 4 | NR |
| | *IFNGR1* | p.Pro431Ser6:g.137519347G>A | Immune response | Tier 4 | NR |
| | *IRF2* | p.Gly270Cys4g.185310154C>A | Transcription factor | Tier 4 | NR |
| | *KEL* | p.Arg180His7:g.142655047C>T | Zinc endopeptidase | Tier 4 | NR |
| | *MDC1* | p.Arg1933Gln6:g.30670948C>T | DNA repair | Tier 4 | NR |
| | *INSR* | p.Ala2Gly19:g.7293898G>C | Glucose homeostasis | Tier 4 | 190228 |
| | *NUP93* | p.Gln813Ter16:g.56878498C>T | Programmed cell death | Tier 4 | NR |
| | *SOX17* | p.Arg142Cys8:g.55371734C>T | Transcription factor | Tier 3 | NR |
| PAT2 | *IFNGR1* | p.Pro431Ser6:g.137519347G>A | Immune response | Tier 4 | NR |
| | *IRF2* | p.Gly270Cys4g.185310154C>A | Transcription factor | Tier 4 | NR |
| | *KEL* | p.Arg180His7:g.142655047C>T | Zinc endopeptidase | Tier 4 | NR |
| | *MDC1* | p.Arg1933Gln6:g.30670948C>T | DNA repair | Tier 4 | NR |
| | *INSR* | p.Ala2Gly19:g.7293898G>C | Glucose homeostasis | Tier 4 | 190228 |
| | *NUP93* | p.Gln813Ter16:g.56878498C>T | Programmed cell death | Tier 4 | NR |
| | *SOX17* | p.Arg142Cys8:g.55371734C>T | Transcription factor | Tier 3 | NR |
| **Group B** | | | | |
| PAT3 | *APC* | p.Glu1309AsfsTer45:g.11217521TAAAG>T | Tumor suppressor gene | Tier 3 | 15855 |
| | *HGF* | p.Pro325Thr7:g.81358988G>T | Proliferation, differentiation, cell motility | Tier 3 | NR |
| | *MLLT3* | p.Ser390_Ser391del9:g.20365693AAAGCTGG>A | Transcription factor | Tier 3 | NR |
| | *ESR1* | p.Lys180Arg6:g.152163818A>G | Transcription factor | Tier 3 | NR |
| **Group C** | | | | |
| PAT4 | *H3F3C* | p.Arg18Gly12:g.31945049G>C | Proliferation | Tier 4 | NR |
| | *INSR* | p.Arg399Gln19:g.7172373C>T | Glucose homeostasis | Tier 3 | NR |
| | *KRAS* | p.Gly12Cys12:g.25398285C>A | Proliferation | Tier 2 | 27617 |
| | *PIK3CA* | p.Glu545Lys3:g.178936091G>A | Proliferation, differentiation | Tier 2 | 28694 |
| | *ROS1* | p.Lys1766Tyr6:g.11765059TTT>TATA | Proliferation | Tier 3 | NR |
| PAT5 | *PIK3CA* | p.Met1043Ile3:g.178952074C>T | Proliferation | Tier 2 | 173901 |
| Gene     | Mutation Description                                      | Function                          | Tier | NR   |
|----------|----------------------------------------------------------|-----------------------------------|------|------|
| SMAD4    | p.Gln256Ter18:g.48584593C>T                             | Tumor suppressor gene              | Tier 3 | NR   |
| ARID1A   | p.Pro1619GlnfsTer71:g.27101569TC>T                      | Transcription regulation           | Tier 3 | NR   |
| B2M      | p.Ser16AlafsTer2715:g.45003785CTCTT>C                   | Immune response                    | Tier 4 | NR   |
| BRAF     | p.Pro403LeufsTer87:g.140482926AG>A                      | Proliferation, differentiation     | Tier 3 | NR   |
| CDK12    | p.Gly1461AlafsTer3817:g.37687471TG>T                    | Proliferation                      | Tier 3 | NR   |
| DNMT3B   | p.Leu454SerfsTer13620:g.31384650AG>A                    | Epigenetic modifications           | Tier 3 | 138801|
| EPHA3    | p.Met726CysfsTer53:g.89480334TG>T                       | Proliferation, differentiation     | Tier 3 | NR   |
| ERBB3    | p.Arg1080ValfsTer2212:g.56494876GC>G                    | Proliferation, differentiation     | Tier 3 | NR   |
| FGF2     | p.Ala212Val4:g.123797533C>T                             | Proliferation, angiogenesis        | Tier 4 | NR   |
| GRM3     | p.Arg59Ter7:g.86394636C>T                                | Proliferation                      | Tier 4 | NR   |
| JAK2     | p.Leu309Arg9:g.5054874T>G                               | Immune response                    | Tier 3 | NR   |
| LAMP1    | p.Leu276ArgfsTer2113:g.113974735CTG>C                   | Migration and angiogenesis         | Tier 4 | NR   |
| NAB2     | p.Pro211LeufsTer5812:g.574854499TC>T                    | Transcription regulation           | Tier 4 | NR   |
| NRG1     | p.Asp202Asn8:g.31498104G>A                              | Proliferation, differentiation     | Tier 4 | NR   |
| NOTCH3   | p.Gly2035ValfsTer5019:g.15272336CG>C                    | Proliferation, differentiation     | Tier 3 | NR   |
| PIK3CA   | p.Arg88Gln 3:g.178916876G>A                             | Proliferation                      | Tier 3 | 362928|

SAV: splice acceptor variant
Table S2. Results of studies reporting mutational evolution of matched primary/secondary lesions in poly-metastatic CRC. *when the data were not clearly reported they were derived from Venn Diagrams or descriptive tables.

| Author         | Year | No. of Paired Samples (PT/MT) | Patients’ Characteristics at Diagnosis | Site of Metastases       | NGS Platform | Genetic Sharing PT/MT (Global Concordance) | Four Most Frequent and Shared Mutations | Unshared Altered Genes in PT (Found in Primary only) | Unshared Altered Genes in MT (Found in Metastasis only) | TMB |
|----------------|------|-------------------------------|---------------------------------------|--------------------------|---------------|-------------------------------------------|----------------------------------------|----------------------------------------------------------|-----------------------------------------------------------|-----|
| Brannon AR et al. | 2014 | 69                            | Four pts stage II, 3 stage III, 62 stage IV. Seventy-five percent of metastases were synchronous. Allowed multiple chemotherapeutic lines. Thirty pts were chemonaive. | Liver (only two ovary). | Illumina, HiSeq 2000. | 79% | APC, ASXL1, BAP1, CARD11, CBL, CEBPA, EPHA3, EPHA6, EPHA7, EPHB1, ERBB2, ERBB4, FLT1, FOXL2, GRIN2A, KDM6A, KDR, LGR6, MDM4, MIF, NFkB2, NOTCH3, PBRM1, PDGFRB, PIK3CA, PIK3CD, PIK3CG, SMAD4, STK11, TET1, TP53, TSHR. | ALK, APC, ASXL1, BAP1, CARD11, CBL, CEBPA, EPHA3, EPHA6, EPHA7, EPHB1, ERBB2, ERBB4, FLT1, FOXL2, GRIN2A, KDM6A, KDR, LGR6, MDM4, MIF, NFkB2, NOTCH3, PBRM1, PDGFRB, PIK3CA, PIK3CD, PIK3CG, SMAD4, STK11, TET1, TP53, TSHR. | Not reported |
| Lee SY et al.    | 2014 | 15                            | Stage IV. 6 pts had single liver metastasis. Allowed multiple chemotherapeutic lines. | Liver. | Illumina, HiSeq 2000. | *Mutational concordance showed only for each genes: APC: 100% TP53: 70% KRAS: 100% SMAD4: 75%. | APC, TP53, KRAS, SMAD4, APC and KRAS mutations were ever concordant between PT and MT. | BRAF, CTNNB1, FBXW7, PIK3R1, TP53, SOX9. | ATR, BRAF, CDC42BPG, FBXW7, FLT4, KDR, PIK3CG, RBI, SMAD4, SOX9. | Not reported |
| Author          | Year | Stage | Metastases    | Genes Mutated                                                                 | Concordance | Genes Not Reported |
|-----------------|------|-------|---------------|-------------------------------------------------------------------------------|-------------|--------------------|
| Kim R et al.    | 2015 | IV    | Liver, lungs, lymphnodes, ovary | APC and TP53 found concordant in 10/19 pairs. Kras ever concordant (9/19 pts). PI3K ever concordant (3/19 pts). | 93.5%       | ABCA3, ADAMTS20, APC, BRCA2, CX3CR1, DGBK, ERBB4, FGFR3, GNA11, HSP90AB1, ITGA10, ITGAL, JAK1, LRP1B, MACF1, MAP5K, MAGI2, MARK1, NTRK2, PARP14, PIK3CG, RASA1, ROBO1, SMAD2, SMAD3, SMAD4, TEX14, TNKS, TP53, TTN, WNT2, ZNF217, ZNF831. |
| Vignot S et al. | 2015 | IV    | Multiple sites. Only local (1 pt), only peritoneum (1 pt). | APC, TP53, Kras, and SMAD4 were the most frequent mutated genes. Mutated APC had a concordance of 100%. ALK, BRCA2, GNAS, NF1, PIK3CG, RICTOR, STK11, TNKS. | 78%         | BRCA2, CDH2, CDKN2A, EP HB1, GLUCY1A2, PIK3CG, RB1, RET, SMO. |
| Kovaleva V et al.| 2016 | IV    | Liver and lungs. | TP53, APC, Kras, SMAD4. ABL1, ATM, BRAF, EGFR, ERBB4, FBXW7, FGFR3, GNA11, ABL2, AKT1, ALK, ATM, BRAF, CDH1, CDK2A2, CSF1R, CTNNB1.| *From 0 to 100% (median 8.5%). | Not reported |
metastases. Allowed multiple chemotherapeutic lines.

| MiSeq (Illumina) | GNAQ, HRAS, JAK3, KDR, KIT, MET, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RB1, RET, SMAD4, STK11, TP53, VHL. | EGFR, ERBB2, ERBB4, FBXW7, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL. |
