

# **FOLFOXIRI plus bevacizumab and atezolizumab as upfront treatment of unresectable metastatic colorectal cancer (mCRC): Updated and overall survival results of the phase II randomized AtezoTRIBE study.**

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**Background:** AtezoTRIBE (NCT03721653) is a phase II randomized trial in which unresectable mCRC pts were randomized 1:2 to 1<sup>st</sup>-line FOLFOXIRI/bev [arm A] or FOLFOXIRI/bev/atezo [arm B]. Adding atezo to FOLFOXIRI/bev was safe and improved PFS (primary endpoint), with a modest benefit also among pts with pMMR tumors. Subgroup analyses suggest that TMB and Immunoscore IC (IS IC) -an IHC biomarker measuring CD8 and PD-L1 cell densities and their proximity- may identify pts with pMMR tumors deriving benefit from adding atezo to FOLFOXIRI/bev. **Methods:** The study had 85% power to detect a HR for PFS (time from randomization to 1<sup>st</sup> PD or death [PD1]) of .66 in favor of arm B with 1-sided  $\alpha$  error of .10. Secondary endpoints included PFS2 (time from randomization to PD on any treatment given after PD1 or death [PD2]), 2<sup>nd</sup> PFS (time from PD1 to PD2), and OS. MMR, TMB, IS IC were correlated to clinical outcome. **Results:** 218 pts (arm A/B: 73/145) were enrolled. Main pts' characteristics were right-sided 44%/45%, RAS mut 71%/74%, BRAF mut 14%/8%, dMMR 7%/6%, high TMB 10%/12%, high IS IC 32%/32%. At a median follow-up of 37.0 mos, 175 (80%, arm A/B: 64/111) PD1, 150 (69%, arm A/B: 53/97) PD2, and 118 (54%, arm A/B: 43/75) OS events were collected. Out of 175 pts with a PD1 event, 135 (77%, arm A/B: 50/85) received a subsequent treatment; among them, 121 pts (arm A/B: 43/78) had a PD2 event. PFS, PFS2, 2<sup>nd</sup> PFS and OS results in the intention-to-treat (ITT) population and the pMMR group are listed in the Table. In the ITT population, significant interactions between treatment and MMR status ( $P_{int}$  .011), TMB ( $P_{int}$  .008), and IS IC ( $P_{int}$  .037) were reported in terms of PFS. Only IS IC was associated with a differential OS benefit ( $P_{int}$  .065), with pts bearing IS IC-high tumors deriving benefit from adding atezo (HR 0.43, 95%CI 0.19-1.00), differently than those with IS IC-low tumors (HR 1.09, 95%CI 0.65-1.83). In the pMMR group, significant interactions between treatment and TMB and IS IC were reported in terms of PFS ( $P_{int}$  .016 and .051, respectively) and OS ( $P_{int}$  .043 and .063, respectively). Pts bearing IS IC-high tumors derived higher OS benefit from adding atezo (HR 0.44, 95%CI 0.19-1.03), than those with IS IC-low tumors (HR 1.15, 95%CI 0.67-1.97). **Conclusions:** Pts with IS IC-high and/or TMB high pMMR mCRC seem to derive a survival benefit from adding atezo to FOLFOXIRI/bev as upfront treatment. These findings deserve confirmation in a properly designed phase III trial. Clinical trial information: NCT03721653. Research Sponsor: GONO Foundation; Roche.

| mos                   | ITT (n = 218) |       |                  |      | pMMR (n = 201) |       |                  |      |
|-----------------------|---------------|-------|------------------|------|----------------|-------|------------------|------|
|                       | Arm A         | Arm B | HR (80% CI)      | P    | Arm A          | Arm B | HR (80% CI)      | P    |
| mPFS                  | 11.5          | 13.1  | 0.71 (0.58-0.87) | .015 | 11.5           | 13.0  | 0.79 (0.64-0.97) | .073 |
| mPFS2                 | 19.9          | 22.6  | 0.85 (0.68-1.05) | .164 | 19.9           | 21.0  | 0.90 (0.72-1.12) | .269 |
| m2 <sup>nd</sup> PFS* | 5.7           | 6.3   | 1.15 (0.90-1.48) | .228 | 5.7            | 6.3   | 1.13 (0.88-1.45) | .270 |
| mOS                   | 27.2          | 33.0  | 0.81 (0.63-1.04) | .136 | 26.9           | 30.8  | 0.83 (0.64-1.07) | .172 |

\*assessed on 135 pts in ITT and 127 pts in pMMR populations.

# Trastuzumab deruxtecan (T-DXd) in patients (pts) with HER2-overexpressing/amplified (HER2+) metastatic colorectal cancer (mCRC): Primary results from the multicenter, randomized, phase 2 DESTINY-CRCO2 study.

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**Background:** T-DXd (6.4 mg/kg, every 3 weeks [Q3W]) demonstrated antitumor activity in pts with HER2+ mCRC in DESTINY-CRCO1 (Siena et al. *Lancet Oncol.* 2021). We present primary results of DESTINY-CRCO2 (NCT04744831), which assessed the efficacy and safety of T-DXd (5.4 and 6.4 mg/kg) in pts with HER2+ mCRC. **Methods:** This was a multicenter phase 2 study. Eligible pts had centrally confirmed HER2+ (immunohistochemistry [IHC] 3+ or IHC 2+/in situ hybridization [ISH]++) mCRC. Pts with RAS wild-type (wt) or mutant (m) mCRC were eligible. Pts had received prior standard therapy, unless contraindicated; prior anti-HER2 therapy was allowed. In stage 1, 80 pts were randomized 1:1 to 5.4 (n = 40) or 6.4 (n = 40) mg/kg T-DXd Q3W. In stage 2, an additional 42 pts received 5.4 mg/kg T-DXd. Primary endpoint was confirmed objective response rate (cORR) by blinded independent central review (BICR). Secondary endpoints included duration of response (DoR), progression-free survival (PFS), overall survival (OS), and safety. **Results:** At data cutoff (Nov 1, 2022), most pts in the 5.4 and 6.4 mg/kg T-DXd arms had HER2 IHC 3+ (78.0% and 85.0%), RAS wt tumors (82.9% and 85.0%), and a median of 3 and 4 prior lines of therapy, respectively. cORR was 37.8% (95% CI, 27.3-49.2%) in the 5.4 mg/kg arm and 27.5% (95% CI, 14.6-43.9%) in the 6.4 mg/kg arm (all partial responses in both arms). Key efficacy data are shown in the Table: Grade ≥3 treatment-emergent adverse events (AEs) were observed in 41/83 pts (49.4%) and 23/39 pts (59.0%) in the 5.4 and 6.4 mg/kg T-DXd arms, respectively. Serious AEs were observed in 20/83 pts (24.1%) and 12/39 pts (30.8%) in the 5.4 and 6.4 mg/kg arms, respectively. Independently adjudicated drug-related interstitial lung disease occurred in 7/83 pts (8.4%) with 5.4 mg/kg T-DXd and 5/39 pts (12.8%) with 6.4 mg/kg T-DXd, and most events were grade 1/2 (1 grade 5 in the 6.4 mg/kg arm). **Conclusions:** T-DXd showed promising antitumor activity in pts with HER2+ mCRC at both 5.4 and 6.4 mg/kg doses. Antitumor efficacy was observed irrespective of RAS mutation status at 5.4 mg/kg T-DXd, and in those with prior anti-HER2 therapy. Overall, safety was consistent with the known safety profile of T-DXd and favored the 5.4 mg/kg dose. Clinical trial information: NCT04744831. Research Sponsor: Daiichi Sankyo, Inc and AstraZeneca.

| Efficacy summary.                                            |                                        |                             |
|--------------------------------------------------------------|----------------------------------------|-----------------------------|
|                                                              | 5.4 mg/kg T-DXd<br>n = 82              | 6.4 mg/kg T-DXd<br>n = 40   |
| Median follow-up, mo (range)                                 | 8.9 (0.5-17.1)                         | 10.3 (0.7-16.4)             |
| Median DoR by BICR, mo (95% CI)                              | 5.5 (4.2-8.1)                          | 5.5 (3.7-non-evaluable)     |
| Median PFS, mo (95% CI)                                      | 5.8 (4.6-7.0)                          | 5.5 (4.2-7.0)               |
| Best overall response by BICR in subgroups, n/N (%) [95% CI] |                                        |                             |
| Prior anti-HER2 therapy                                      | 7/17 (41.2)<br>[18.4-67.1]             | 4/10 (40.0)<br>[12.2-73.8]  |
| HER2 IHC 3+                                                  | 30/64 (46.9)<br>[34.3-59.8]            | 10/34 (29.4)<br>[15.1-47.5] |
| HER2 IHC 2+/ISH+                                             | 1/18 (5.6)<br>[0.1-27.3]               | 1/6 (16.7)<br>[0.1-50.0]    |
| RAS wt                                                       | 27/68 (39.7)<br>[28.0-52.3]            | 11/34 (32.4)<br>[17.4-50.5] |
| RASm                                                         | 4/14 (28.6) <sup>a</sup><br>[8.4-58.1] | 0/6                         |

<sup>a</sup>All RASm responders were IHC 3+.

LBA3503

Oral Abstract Session

**Phase III randomized clinical trial comparing the efficacy of neoadjuvant chemotherapy and standard treatment in patients with locally advanced colon cancer: The NeoCol trial.**

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Oral Abstract Session

**Total neoadjuvant therapy with mFOLFIRINOX versus preoperative chemoradiation in patients with locally advanced rectal cancer: 7-year results of PRODIGE 23 phase III trial, a UNICANCER GI trial.**

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**The full, final text of this abstract will be available at [meetings.asco.org](https://meetings.asco.org) on the day of presentation and in the online supplement to the June 10, 2023, issue of the *Journal of Clinical Oncology*.**

**Long-term outcome of neoadjuvant mFOLFOX6 with or without radiation versus fluorouracil plus radiation for locally advanced rectal cancer: A multicenter, randomized phase III trial.**

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**Background:** Previous results of phase III FOWARC trial demonstrated that mFOLFOX6, with or without radiation, did not significantly improve survival versus fluorouracil with radiation in patients with locally advanced rectal cancer at 3 years. Here, we presented the data of long-term disease free survival (DFS) and overall survival (OS). **Methods:** In this multicenter, phase III trial, patients with stage II/III rectal cancer were randomly assigned (1:1:1) to receive 5 cycles of infusional fluorouracil (leucovorin 400 mg/m<sup>2</sup>, fluorouracil 400 mg/m<sup>2</sup>, and fluorouracil 2.4 g/m<sup>2</sup> over 48 hours) plus radiotherapy (46.0 to 50.4 Gy delivered in 23 to 25 fractions during cycles 2 to 4) followed by surgery and seven cycles of infusional fluorouracil adjuvant treatment, mFOLFOX6 plus radiotherapy, or four to six cycles of mFOLFOX6 followed by surgery and six to eight cycles of mFOLFOX6. **Results:** Totally, 495 patients were enrolled, 165 patients in each group. 445 patients underwent surgery. After a median follow-up of 9.5 years, DFS events were observed in 56, 54, and 55 patients in fluorouracil plus radiotherapy, mFOLFOX6 plus radiotherapy, and mFOLFOX6 groups. The 10-year DFS rate were 55.5%, 63.0% and 62.8% ( $P = 0.934$  by the log-rank test). OS events were reported in 39, 38, and 40 patients in the 3 groups. The 10-year OS rate was 66.2%, 73.2% and 73.0% ( $P = 0.919$  by the log-rank test), respectively. **Conclusions:** With long-term follow up, no significant difference was found in survival outcome between mFOLFOX6, with and without radiation. Comparing with fluorouracil plus radiation, mFOLFOX6 plus radiation also failed to improve long-term survival. Clinical trial information: NCT01211210. Research Sponsor: Supported by National Key Clinical Discipline, China National Natural Science Foundation.

**NeoRAS wild-type metastatic colorectal cancer in the SCRUM-Japan GOZILA study.**

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**Background:** The "NeoRAS" phenomenon refers to *RAS* mutant (MT) metastatic colorectal cancer (mCRC) that becomes *RAS* wild-type (WT) following treatment. This NeoRAS WT population might represent a novel indication for EGFR inhibitors, which are less effective in *RAS* MT mCRC. The incidence and clinicopathological characteristics of NeoRAS WT mCRC using plasma cell-free DNA (cfDNA) next generation sequencing has not been defined. **Methods:** As part of a large-scale nationwide screening platform (SCRUM-Japan GOZILA), 478 patients with an initial diagnosis of *RAS* MT mCRC by tissue analysis (MEBGEN RASKET-B) who received systemic therapy underwent cfDNA testing (Guardant 360) prior to later lines of treatment. Based on the cfDNA results, we evaluated the clinicopathological characteristics of those with NeoRAS WT and *RAS* MT. NeoRAS WT was defined as no *RAS* MT (*KRAS* or *NRAS*) detected in plasma and was assessed in the overall cohort (Cohort A) and in the subgroup with at least one somatic alteration detected in plasma (Cohort B) to exclude those with insufficient tumor DNA shedding. **Results:** Median age at the time of blood sampling was 62.0 years old, and 257 (51.9%) were men. 160 (32.3%) and 319 (64.4%) had right-sided tumors and multi-organ metastases. The lungs were the most frequent site of metastasis (60.2%), followed by liver (57.4%), lymph nodes (28.9%), and peritoneum (28.5%). The prevalence of NeoRAS WT was 19.0% (91/478) in Cohort A and 9.8% (41/429) in Cohort B. The frequency of NeoRAS WT in tumors originally with *KRAS* exon 2 MT tended to be lower than in those with other *RAS* MT (18.1% vs 25.4%,  $P = 0.21$  in Cohort A, 9.0% vs 15.4%,  $P = 0.14$  in Cohort B). There were significant differences in the prevalence of NeoRAS WT between patients with single organ vs multi-organ metastases ( $P < 0.001$  in Cohort A,  $P = 0.004$  in Cohort B), absence vs presence of liver metastasis ( $P < 0.001$  in both Cohort A and B), lymph node metastasis ( $P = 0.006$  in Cohort A), peritoneal metastasis ( $P = 0.002$  in group A), and bone metastasis ( $P = 0.029$  in Cohort A), testing immediately prior to 2<sup>nd</sup> through to 4<sup>th</sup> line treatment vs later lines ( $P = 0.007$  in Cohort A), immediate prior use of regorafenib ( $P = 0.027$  in Cohort A) and any history of vascular endothelial growth factor inhibitors ( $P = 0.003$  in Cohort A,  $P = 0.035$  in Cohort B). In the logistic regression multivariate analysis, absence of liver metastasis (odds ratio [OR], 5.83;  $P < 0.001$  in Cohort A, OR, 2.84;  $P = 0.005$  in Cohort B), absence of lymph node metastasis (OR, 2.18;  $P = 0.034$  in Cohort A) and tissue *RAS* MT other than *KRAS* exon 2 (OR, 2.35;  $P = 0.049$  in Cohort B) were significantly related to the emergence of NeoRAS WT. Among 6 NeoRAS WT patients treated with EGFR inhibitors, one had partial response and another one had stable disease for at least 6 months. **Conclusions:** Liver and lymph node metastasis and *RAS* MT other than those in *KRAS* exon 2 are factors associated with the development of NeoRAS WT mCRC. EGFR inhibitors might be effective treatment. Research Sponsor: SCRUM-Japan Funds.

### Phase III FIRE-4 study (AIO KRK-0114): Influence of baseline liquid biopsy results in first-line treatment efficacy of FOLFIRI/cetuximab in patients with tissue RAS-WT mCRC.

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**Background:** FIRE-4 (AIO KRK-0114) is performed in RAS wild-type (wt) mCRC patients. This randomized study tests the efficacy of early switch maintenance during 1<sup>st</sup>-line therapy (part 1) and re-challenge with cetuximab (part 2) in later-line treatment. In part 1, arm A patients continued FOLFIRI/Cet until progression or intolerable toxicity. In arm B, patients received FOLFIRI/Cet for 8-12 cycles, after which maintenance therapy with 5-FU/FA plus bevacizumab (5-FU/Bev) was applied. The first randomization evaluates the question if an early switch from cetuximab to bevacizumab may prolong PFS. Within the translational protocol, serial baseline liquid biopsy were taken to analyze RAS and BRAF mutations. **Methods:** Within this randomized, controlled, open-label phase-III study, patients received FOLFIRI (irinotecan plus 5-FU/FA) plus cetuximab at the standard dosing schedule. In arm A, FOLFIRI plus cetuximab was continued until progression or intolerable toxicity. In arm B, patients received 8 cycles of FOLFIRI plus cetuximab (in case of tumor response) or 12 cycles (in case of stable disease) followed by maintenance with 5-FU/FA plus bevacizumab (5mg/kg) until disease progression or intolerable toxicity. Overall survival after second randomization (part 2) is evaluated as a primary endpoint. Liquid Biopsies were analyzed by RAS BEAMing and BRAF ddPCR technology. **Results:** From August 2015 to January 2021, 673 patients were randomized, and liquid biopsies of 540 patients were evaluable at baseline. Of those, 70 (13%) were RAS mutant and 38 (7%) BRAFV600E mutant at baseline. Patients with a detectable RASmut had a significant shorter PFS and OS when compared to RASwt patients (PFS: 9.0mo vs. 11.5mo;  $p < 0.001$ ; OS: 22.1mo vs 33.6mo;  $p < 0.001$ ). Whereas, for RASwt patients no difference for both arms with respect to PFS and OS could be observed, RASmut patients ( $n = 70$ ) had a clear trend towards shorter survival in the standard FOLFIRI cetuximab arm (PFS: 6.4mo vs. 10.1mo,  $p = 0.54$ ; OS: 24.9mo vs. 16.3mo,  $p = 0.10$ ). Patients with a BRAF mutation in liquid biopsy had median survival times as expected for BRAF mutant patients (PFS = 5.5mo; OS = 12.0mo). In the standard arm, with a continuous administration of cetuximab, the conversion rate from RASwt to RASmut was significantly higher at progression than in the switch maintenance arm. **Conclusions:** Liquid biopsy detected RAS mutation in 13% of patients deemed RASwt based on tissue analyses. These patients show outcome characteristics expected for RAS mutant patients (median PFS of 9.0 months and median OS of 22 months). The study thus shows the clinical relevance of liquid biopsy in the verification of RAS mutational status. Clinical trial information: NCT02934529. Research Sponsor: Merck.

# Efficacy of panitumumab in patients with left-sided disease, MSS/MSI-L, and *RAS/BRAF* WT: A biomarker study of the phase III PARADIGM trial.

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**Background:** The PARADIGM trial (NCT02394795) showed longer median overall survival (OS) with first-line mFOLFOX6 plus panitumumab (PAN) vs bevacizumab (BEV) in patients (pts) with *RAS* wild type (WT) and left-sided metastatic colorectal cancer (mCRC; 37.9 vs 34.3 months, respectively; hazard ratio [HR], 0.82;  $P=0.031$ ) and similar OS in right-sided pts (HR 1.09; Yoshino T, et al. ASCO 2022 LBA1). Based on current guideline recommendations regarding clinically relevant biomarkers, here we report clinical outcomes in left-sided mCRC pts with microsatellite stable or microsatellite instability low (MSS/MSI-L) and *RAS* (*KRAS/NRAS*)/*BRAF* (*V600E*) WT from PARADIGM. **Methods:** Baseline plasma circulating tumor DNA (ctDNA; >10 ng/mL and >10 nM DNA) from pts enrolled in the biomarker study (NCT02394834) was assessed using a custom panel (PlasmaSELECT-R 91, PGDx). The efficacy (OS, progression-free survival [PFS], response rate [RR], and curative resection rate [RO]) of PAN plus mFOLFOX6 compared with BEV plus mFOLFOX6 according to *RAS*, *BRAF* (*V600E*), and MSI status and primary tumor location was evaluated. **Results:** Among 802 pts in the full analysis set, 733 (91%) had evaluable pretreatment samples for ctDNA analysis. Of these pts, 53 (7.2%) and 78 (10.6%) pts had *RAS* and *BRAF* (*V600E*) mutations, respectively, and 20 (2.7%) pts had MSI high (MSI-H) status. In left-sided mCRC pts with MSS/MSI-L and *RAS/BRAF* WT, OS tended to be longer with PAN vs BEV (40.6 [95% CI, 36.3–44.4] vs 34.8 [95% CI, 31.3–41.2] months, respectively; HR, 0.79 [95% CI, 0.64–0.97]). Although PFS was comparable between PAN (13.6 months [95% CI, 12.6–15.3]) and BEV (12.6 months [95% CI, 11.3–14.1]; HR, 0.95 [95% CI, 0.77–1.17]), RR and RO resection rates were higher with PAN (RR: 83.2% [95% CI, 78.0–87.6]; RO: 18.8% [95% CI: 14.2–24.1]) compared with BEV (RR: 66.4% [95% CI, 60.0–72.3]; RO: 10.0% [95% CI: 6.5–14.5]). OS was similar or inferior to PAN vs BEV regardless of the primary sidedness in pts with MSI-H or *RAS/BRAF* mutations (Table). **Conclusions:** These results support PAN + mFOLFOX6 as a first-line therapy for left-sided pts with MSS/MSI-L and *RAS/BRAF* WT. Clinical trial information: NCT02394795. Research Sponsor: Takeda Pharmaceutical Company Limited, Tokyo, Japan.

| Population             | MSS/MSI-L and <i>RAS/BRAF</i> WT |                  |                         | MSI-H or <i>RAS/BRAF</i> mutation |                  |                         |
|------------------------|----------------------------------|------------------|-------------------------|-----------------------------------|------------------|-------------------------|
|                        | PAN + mFOLFOX6                   | BEV + mFOLFOX6   | Difference; HR (95% CI) | PAN + mFOLFOX6                    | BEV + mFOLFOX6   | Difference; HR (95% CI) |
| Left-sided, n          | 256                              | 241              |                         | 31                                | 26               |                         |
| Median OS, mo (95% CI) | 40.6 (36.3–44.4)                 | 34.8 (31.3–41.2) | 5.8; 0.79 (0.64–0.97)   | 15.4 (9.6–20.9)                   | 25.2 (17.0–30.9) | –9.8; 1.53 (0.84–2.76)  |
| Right-sided, n         | 41                               | 55               |                         | 37                                | 36               |                         |
| Median OS, mo (95% CI) | 37.9 (22.0–42.2)                 | 30.9 (23.2–35.4) | 7.0; 0.94 (0.60–1.48)   | 13.7 (11.3–18.7)                  | 17.9 (11.6–22.4) | –4.2; 1.28 (0.78–2.11)  |
| Overall, n             | 299                              | 299              |                         | 69                                | 66               |                         |
| Median OS, mo (95% CI) | 39.0 (36.3–43.3)                 | 34.1 (31.1–37.6) | 4.9; 0.79 (0.66–0.96)   | 14.6 (11.3–18.8)                  | 19.8 (15.3–25.5) | –5.2; 1.27 (0.88–1.84)  |

**Transcriptional metabolic profiling young onset colorectal cancer (CRC) patients.**

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**Background:** Patients diagnosed with colorectal cancer (CRC) at age < 50 years typically present with more advanced disease, resulting in poor therapeutic response and clinical outcomes. Therefore, there is an unmet need to understand the differences in transcriptional profiles between younger (<50) and older (> 50) CRC patients. **Methods:** Using TCGA (n = 397; > 50 = 349; < 50 = 48) and Oncology Research Information Exchange network (ORIEN) CRC datasets (n = 460; > 50 = 364; < 50 = 96), patients were separated into younger and older populations. Baseline characteristics of the patients in this analysis are provided in the accompanying table. Subsequently, their transcriptional profiles were compared and assessed via differential gene expression analysis (DESeq2), gene set enrichment analysis (GSEA), immune deconvolution (TIMER2.0), and metabolic pathway analysis (MetaPhOR). These pathways were then mapped to assess transcriptional dysregulation, and patterns of predicted metabolic flux. **Results:** Comparisons of older and younger groups revealed a large number of significantly differentially expressed transcripts (n = 2629), enrichment in younger groups of metabolic pathways (amino acids and lipids), oncogenes (MYC targets and NRAS targets), and cellular processes. In the older group we found enrichment of methylation and histone modification, immune response, and other metabolic pathways, like androgens. Metabolic pathway analysis revealed consistent alterations in steroid hormone metabolism and kynurenine metabolism, which were largely upregulated in the over 50 group. Additionally, pathways associated with response to both CTLA4 and PDL1 treatment were largely upregulated in the over 50 group. This transcriptional signature may be associated with a pre-disposition to different clinical outcomes and therapeutic response for agents targeting these pathways. **Conclusions:** Overall, this study has revealed differences in transcriptional metabolic profiles and other drivers of disease, as well as immune profiles, between younger and older CRC populations. This biology should be explored in the future, as new avenues for treatment in younger CRC populations. Research Sponsor: None.

|                | TCGA                  |                     | P     | ORIEN CRC             |                     | P     |
|----------------|-----------------------|---------------------|-------|-----------------------|---------------------|-------|
|                | > 50<br>n = 349 (88%) | ≤50<br>n = 48 (12%) |       | > 50<br>n = 364 (79%) | ≤50<br>n = 96 (21%) |       |
| Race           |                       |                     | 0.371 |                       |                     | 0.621 |
| White          | 165(47)               | 26(54)              |       | 313(86)               | 80(83)              |       |
| Non-white      | 50(14)                | 12(25)              |       | 51(14)                | 16(17)              |       |
| Not reported   | 134(38)               | 10(21)              |       | 0(0)                  | 0(0)                |       |
| Gender         |                       |                     | 0.215 |                       |                     | 0.801 |
| Female         | 159(46)               | 27(56)              |       | 163(45)               | 45(47)              |       |
| Male           | 190(54)               | 21(44)              |       | 201(55)               | 51(53)              |       |
| Tumor stage    |                       |                     | 0.088 |                       |                     | 0.009 |
| I(IA,IB)       | 67(19)                | 4(8)                |       | 21(5)                 | 3(3)                |       |
| II(IIA/B/C)    | 111(32)               | 13(27)              |       | 90(24)                | 10(10)              |       |
| III(IIIA/B/C)+ | 162(46)               | 29(60)              |       | 104(29)               | 34(35)              |       |
| IV(IVA/B/C)+   | 0(0)                  | 0(0)                |       | 87(24)                | 31(32)              |       |
| Not reported   | 9(3)                  | 2(4)                |       | 62(17)                | 18(18)              |       |
| BMI            |                       |                     | 0.169 |                       |                     | 0.624 |
| BMI < 18       | 0(0)                  | 0(0)                |       | 4(1)                  | 1(1)                |       |
| BMI 18-24.9    | 55(15)                | 10(20)              |       | 70(19)                | 24(25)              |       |
| BMI 24.9-30    | 57(16)                | 15(31)              |       | 111(30)               | 25(26)              |       |
| BMI > = 30     | 66(18)                | 7(14)               |       | 109(30)               | 28(29)              |       |
| Not reported   | 171(48)               | 16(33)              |       | 70(19)                | 18(18)              |       |

**Metabolomic differences in young-onset versus average-onset colorectal adenocarcinoma.**

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**Background:** Novel deleterious effects of environmental exposures may play a role in the rising incidence of young-onset colorectal cancer (yoCRC). We used metabolomics to assess differences between yoCRC and average-onset CRC (aoCRC), in comparison to healthy controls, which may provide insight into the pathogenesis and suggest any exposure risks. **Methods:** Patients with stage I-IV CRC and healthy controls were identified from prospective biobanks and categorized based on age < 50 years (yoCRC or young controls) or age > 60 years (aoCRC or older controls). Plasma metabolites were profiled using GC-TOF mass spectrometry. Differential abundance of metabolites was investigated using unadjusted and adjusted logistic regression. Metabolic pathway analysis was performed using Metaboanalyst 5.0. All p-values were adjusted for multiple testing (false-discovery rate, FDR  $p < 0.20$  considered significant). **Results:** The study population comprised 170 CRC patients (66 yoCRC and 104 aoCRC) and 49 healthy controls (34 young and 15 old). Association analyses revealed four differentially abundant metabolites: citrate (FDR  $p = 0.04$ ), cholesterol (0.14), and two unidentified metabolites (UM). Metabolic pathways significantly altered in yoCRC vs. aoCRC included: carbohydrate metabolism (citrate cycle, FDR  $p = 0.04$ ), carbohydrate biosynthesis (glyoxylate and dicarboxylate metabolism, FDR  $p = 0.004$ ), amino-acid metabolism (alanine, aspartate, and glutamate metabolism, FDR  $p = 0.01$ , arginine biosynthesis, FDR  $p = 0.02$ , and amino-acid t-RNA biosynthesis, FDR  $p = 0.03$ ). There were no significant metabolomic differences between young and old controls. Significant associations on survival analysis included: adipic acid with aoCRC (HR = 3.1, 95% CI = , 1.7-5.6, FDR  $p = 0.13$ , unadjusted analysis) suggesting worse survival with higher levels and 4-hydroxyhippuric acid with the whole cohort (HR = 0.4, 95% CI = 0.3-0.7, FDR  $p = 0.05$ , adjusted analyses) indicating better survival with higher levels. **Conclusions:** We identified significant differences in the citrate cycle - a core pathway of cellular metabolism associated with colorectal cancer. Metabolomic differences in pathways of carcinogenic significance (aspartate) and environmental exposures (arginine and dietary red meat) were also noted, suggesting potential relationships with younger age of CRC onset. Research Sponsor: Sondra and Stephen Hardis Chair in Oncology Research.

| Differentially Abundant Metabolite          | OR (95% CI)           | FDR p |
|---------------------------------------------|-----------------------|-------|
| Citrate                                     | 14.54 (4.26-56.35)    | 0.04  |
| Cholesterol                                 | 0.01 (0.001-0.10)     | 0.14  |
| Altered Pathway                             | Pathway Impact Factor | FDR p |
| Glyoxylate and dicarboxylate metabolism     | 0.23                  | 0.005 |
| Citrate (TCA) cycle                         | 0.14                  | 0.04  |
| Alanine, aspartate and glutamate metabolism | 0.28                  | 0.01  |
| Glycine, serine and threonine metabolism    | 0.28                  | 0.14  |
| Aminoacyl-tRNA biosynthesis                 | 0.17                  | 0.01  |
| Arginine biosynthesis                       | 0.13                  | 0.02  |

\*Results for unknown metabolites are not included. Odds Ratio (OR) > 1 indicates higher abundance of metabolites in aoCRC vs. yoCRC.

**Evaluation of genomic alterations in early-onset versus late-onset colorectal cancer.**

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**Background:** The etiology of the rising incidence of early-onset colorectal cancer (EOCRC), defined as CRC in patients aged < 50, remains unknown. In this study, we evaluated tumor genomic differences in patients with EOCRC versus average-onset CRC (AOCRC, age > 60). **Methods:** The cohort included 13,262 patients diagnosed with stages I-III colon or rectal cancer who had whole exome sequencing as part of their ctDNA testing (Signatera, bespoke mPCR NGS assay). Tumor mutational burden (TMB) and microsatellite instability (MSI) status were derived from tumor whole exome sequencing analysis. The prevalence of somatic variants and mutations in known oncogenic pathways was compared between EOCRC and AOCRC groups, stratified by TMB and MSI status. Fisher's exact test was used to test significance between the groups and p-values were adjusted using the FDR method for multiple test correction. **Results:** A total of 3,093 patients with EOCRC (70.8% colon, 27.4% rectal, 1.9% unknown) and 10,169 patients with AOCRC (79.9% colon, 18.3% rectal, 1.7% unknown) were included, where 9.0%/37.3%/53.7% were AJCC stages I, II, and III, respectively. Early-onset patients compared to average-onset patients had fewer cases of stage II CRC (30.7% vs. 39.3%,  $p < 0.01$ ) and more cases of stage III CRC (60.9% vs 51.6%,  $p < 0.01$ ). Patients with EOCRC were less commonly MSI-H compared to patients with AOCRC (10% vs. 17%,  $p < 0.01$ ), or have high tumor mutational burden (15% vs. 19%,  $p < 0.01$ ). The *BRAF*V600E mutation and truncated *RNF43* mutations were less prevalent in EOCRC (3% vs. 15% and 2% vs. 9%,  $p < 0.01$ ), regardless of TMB and MSI status. Molecular alterations of the RTK-RAS pathway were less prevalent in the EOCRC cohort ( $p < 0.01$ ), while TP53 pathway alterations were more frequent in the EOCRC cohort ( $p < 0.01$ ). In the TMB-low/MSS group, *TP53* mutations were more common in EOCRC (8% vs. 5%,  $p < 0.01$ ), but *APC* gene mutations were less common in EOCRC (56% vs. 66%,  $p < 0.01$ ). In the TMB-H/MSI-H group, *BRAF* V600E (4% vs. 60%), *RNF43*G659V (16% vs. 45%), and *WNT1* G619A (6% vs. 20%) mutations were less prevalent in EOCRC ( $p < 0.01$  for all mutations); however, patients with EOCRC had more *PIK3CA* H1047R (22% vs. 9%), *APC*R1468\* (11% vs. 3%), and *KRAS*A146T (7% vs. 2%) variants ( $p < 0.01$  for all mutations). In the TMB-H/MSS group, EOCRC patients were more likely to have driver mutations in the PI3K pathway (74% vs. 56%,  $p < 0.01$ ). The *POLE* P286R mutation was more common in TMB-H/MSS patients with EOCRC (38% vs. 13%,  $p < 0.01$ ), whereas *ACVR2A* K437R was less common (11% vs. 30%,  $p < 0.01$ ). Prevalence of somatic variants and mutated oncogenic pathways did not vary significantly by tumor stage. **Conclusions:** Patients with AOCRC harbored more somatic variants and mutations in established pathways of CRC carcinogenesis. Tumors in EOCRC cases carried unique genomic alterations that varied across the TMB and microsatellite subpopulations. *BRAF* V600E and *RNF43* truncating mutations were more frequent in AOCRC. Research Sponsor: U.S. National Institutes of Health.

**Overall survival results for trifluridine/tipiracil plus bevacizumab vs capecitabine plus bevacizumab: Results from the phase 3 SOLSTICE study.**

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**Background:** SOLSTICE, a phase 3 study, tested trifluridine/tipiracil+bevacizumab (FTD/TPI+bev) vs capecitabine+bevacizumab (cape+bev) in first-line for patients with unresectable metastatic CRC who were ineligible for intensive chemotherapy (full-dose doublet/triplet). As previously reported, the study did not meet the primary endpoint (progression-free survival (PFS): 9.4 months [95% CI, 9.1-10.9] for FTD/TPI+bev vs 9.3 months [95% CI: 8.9-9.8] for cape+bev, HR 0.87 [95% CI: 0.75-1.02]). Here we report on the key secondary endpoint – overall survival (OS) and an update of the safety data. **Methods:** From 21 Mar 2019 to 14 Sep 2020, 856 patients were randomized (1:1) to either FTD/TPI+bev or cape+bev. Stratification factors were: ECOG performance status (0 vs 1 vs 2), reason for non-eligibility for intensive therapy (clinical condition vs non-clinical condition) and tumor localization (right vs left). The primary endpoint was PFS based on investigator assessment according to RECIST 1.1 criteria. The key secondary endpoint was OS, defined as the time from randomization to death from any cause. Other secondary endpoints included overall response rate, disease control rate, quality of life, and safety. OS was analyzed after 578 events to detect a hazard ratio of 0.79 with 80.0% power at one-sided 2.5% level of significance. **Results:** 426 patients were randomly assigned to FTD/TPI+bev and 430 to cape+bev. The median OS was 19.74 months with FTD/TPI+bev and 18.59 months with cape+bev (HR, 1.06; 95% CI, 0.90, 1.25) with survival probabilities for FTD/TPI+bev vs cape+bev comparable at different timepoints. In the multivariate analysis, factors significantly associated with higher OS in the whole population were age < 70 years, left location of the primary disease, surgical resection of the primary tumor, number of metastatic sites (1-2 vs ≥3 sites), absence of liver metastasis, neutrophils lymphocyte ratio < 3, Charlson score 0 vs 1-2, and ECOG PS 1 vs 2. No significant treatment effect was observed after adjustment for the prognosis factors (HR, 1.08; 95% CI, 0.92, 1.28), which is consistent with the main OS analysis. The updated safety data was consistent with those communicated at the time of the primary PFS analysis. No new safety signal was identified. As previously reported, the most common severe emergent adverse events were neutropenia 54% vs 1%, neutrophil count decreased 19% vs 1%, anemia 16% vs 4%, hand-foot syndrome 0% vs 15%, and hypertension 9% vs 11% in FTD/TPI+bev vs cape+bev, respectively. **Conclusions:** This was the largest phase 3 study that explored two treatment regimens in a population ineligible for intensive therapy. FTD/TPI+bev was not superior to cape+bev in terms of PFS and OS. The risk of death was similar in both treatment arms. With a different and manageable safety profile, FTD/TPI+bev represents a feasible alternative to cape+bev in this population. Clinical trial information: NCT03869892. Research Sponsor: Institut de Recherches Internationales Servier.

**Sotorasib (Soto) plus panitumumab (Pmab) and FOLFIRI for previously treated *KRAS* G12C-mutated metastatic colorectal cancer (mCRC): CodeBreaK 101 phase 1b safety and efficacy.**

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**Background:** Soto, a *KRAS*<sup>G12C</sup> inhibitor, had a 9.7% objective response rate (ORR) as monotherapy for chemorefractory patients (pts) with *KRAS* G12C-mutated mCRC. When combined with Pmab, a monoclonal anti-EGFR antibody, ORR increased to 30%, supporting the model that the doublet mitigates Soto-related feedback reactivation of the RAS-MAPK pathway and accumulation of activated EGFR. We hypothesize that Soto plus Pmab and FOLFIRI will further enhance Soto efficacy while maintaining a manageable safety profile. We report the first results for a *KRAS*<sup>G12C</sup> inhibitor combined with an EGFR inhibitor and chemotherapy in pts with prior mCRC treatment. **Methods:** Pts included dose exploration and expansion cohorts from CodeBreaK 101 subprotocol H (NCT04185883) who received Soto (960 mg PO daily) plus Pmab (6 mg/kg IV Q2W) and standard-dose FOLFIRI (IV Q2W). Key eligibility criteria were *KRAS* G12C-mutated mCRC and  $\geq 1$  prior treatment for metastatic disease. Pts in dose expansion were *KRAS*<sup>G12C</sup> inhibitor-naïve. The primary endpoint was safety. Secondary endpoints included efficacy and pharmacokinetics (PK). **Results:** As of November 30, 2022, 33 pts (median age: 53 years; 48% female) were treated (6 in dose exploration, 27 in dose expansion). Median prior lines of systemic therapy was 2 (range: 1-6), with 33% and 67% of pts receiving 1 or  $\geq 2$  prior lines, respectively; 97% had prior fluoropyrimidine and 73% had prior irinotecan. Two pts in dose exploration received prior Soto. None of the 6 pts in dose level 1 of dose exploration had dose limiting toxicities (DLTs) during DLT evaluation (first 28 days), and Soto (960 mg PO daily) plus Pmab (6 mg/kg IV Q2W) and FOLFIRI (IV Q2W) was the recommended phase 2 dose. Treatment-related adverse events (TRAEs) of any grade occurred in 32 (97.0%) pts; 1 pt discontinued the full regimen due to grade 3 ALT increase. Fifteen (45.5%) had grade  $\geq 3$  TRAEs (most commonly dermatologic; n = 5). There were no fatal TRAEs. Safety findings were consistent with known profiles of Soto, Pmab, and FOLFIRI. No clinically meaningful PK interaction was observed between Soto and irinotecan. Of 31 pts evaluable for response, confirmed ORR (all partial responses) was 58.1% (95% CI: 39.1, 75.5). The 2 pts with prior Soto achieved partial response (n = 1) and stable disease (n = 1). Disease control rate was 93.5% (95% CI: 78.6, 99.2). With median follow-up of 5.7 and 7.4 months, respectively, progression-free and overall survival data are not yet mature. Fully enrolled data will be presented. **Conclusions:** In the first ever data set for this novel combination, Soto plus Pmab and FOLFIRI showed promising safety and efficacy in pretreated *KRAS* G12C-mutated mCRC, with a confirmed ORR of 58.1%. Adverse events were manageable and consistent with the expected safety profile of the drugs used, and there was no clinically meaningful Soto and irinotecan PK interaction. Clinical trial information: NCT04185883. Research Sponsor: Amgen, Inc.

### Phase 1 study of WNT pathway Porcupine inhibitor CGX1321 and phase 1b study of CGX1321 + pembrolizumab (pembro) in patients (pts) with advanced gastrointestinal (GI) tumors.

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**Background:** Despite the fundamental role of WNT signaling in GI cancers, especially colorectal cancer (CRC), no drug targeting WNT signaling has been successfully developed. CGX1321, a highly-potent and selective inhibitor of O-acyltransferase Porcupine, blocks WNT ligand secretion. Preclinical studies show that CGX1321 inhibits WNT signaling and growth of tumors with *RSPO* fusions or inactivating *RNF43* mutations. Activation of WNT signaling has been associated with cancer immune-suppression and resistance to immunotherapy. **Methods:** The first-in-human trials (NCT02675946 in U.S., NCT03507998 in China) include phase 1 CGX1321 dose escalation (3 – 18 mg, once daily) and dose expansion (18 mg), and phase 1b CGX1321 + pembro combination (Keynote 596). The primary objectives were safety, tolerability and identification of recommended doses and schedules for further evaluation. Secondary objectives were characterization of the PK profile and PD response and anti-tumor activity. CGX1321 was dosed once daily, orally, for 21 days out of 28-day cycles as single-agent, or for 14 days out of 21-day cycles in combination with pembro. **Results:** As of January 1, 2023, 77 pts were enrolled, including 38 pts with solid tumors in the CGX1321 dose escalation, 17 pts with CRC or small bowel cancer (SBC) carrying *RSPO* or *RNF43* alterations in the CGX1321 expansion, 17 pts with microsatellite stable (MSS) CRC in the CGX1321 + pembro dose escalation and 5 pts with MSS CRC in the CGX1321 + pembro expansion. Six of the 17 pts with MSS CRC or SBC from the CGX1321 single-agent expansion were rolled over to CGX1321 + pembro upon disease progression. Across all cohorts, treatment was well tolerated with the majority of AEs being Grade 1/2 and not related to CGX1321, while Grade ≥ 3 AEs related to CGX1321 were infrequent (~6%). Dysgeusia, a common AE observed with other WNT inhibitors, was mild (mostly Grade 1). Bone resorption, the main on-target AE, was manageable and preventable by prophylactic administration of denosumab or zoledronic acid. PK and PD analyses showed adequate drug exposure and significant inhibition of WNT signaling as measured by reduced hair follicle axin2 expression from 12 mg dose. Twelve of 17 pts (71%) with confirmed tumor genetic alterations in *RSPO* or *RNF43* had SD with median time to progression of 112 days (range: 56 – 392). Of the 6 pts in the roll-over cohort, 3 pts with *RSPO3* fusion tumors achieved PR during CGX1321 + pembro combination therapy. **Conclusions:** CGX1321 has demonstrated potent inhibition of the WNT pathway with manageable side effects. Promising efficacy signals have been observed in pts whose tumors harbor *RSPO* fusion, supporting further development of CGX1321 monotherapy and CGX1321 + anti-PD-1/L1 in a defined patient population that is historically known to be refractory to standard therapies and immune checkpoint inhibitors. Clinical trial information: NCT02675946, NCT03507998. Research Sponsor: Curegenix.

### Postoperative hepatic arterial chemotherapy after resection of colorectal liver metastases in patients at high risk of hepatic recurrence: A multicenter randomized phase II trial (PRODIGE 43 - PACHA-01).

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**Background:** Hepatic arterial infusion (HAI) of chemotherapy (CTx) has been proposed as a potential treatment to decrease the risk of hepatic recurrence after resection of colorectal liver metastases (CRLM). Randomized controlled trials (RCTs) led to controversial results and meta-analyses are inconclusive. We conducted a comparative phase II RCT to assess the efficacy of adjuvant HAI oxaliplatin with concomitant intravenous (IV) CTx in pts at high risk of hepatic recurrence ( $\geq 4$  resected CRLM). **Methods:** Pts who underwent curative-intent surgery of at least 4 CRLM after preoperative IV CTx were randomly assigned (1:1) to receive adjuvant IV fluorouracil/leucovorin (LV5FU2) combined with oxaliplatin (85mg/m<sup>2</sup>) by HAI (HAI-IV arm) or IV route (IV arm) every 2 weeks, for at least 3 months to achieve a minimal duration of perioperative CTx of 6 months. The primary endpoint was the hepatic recurrence free survival (h-RFS). Secondary endpoints included the feasibility of delivering at least 4 CTx cycles, toxicity (NCI-CTCAE 4.0) including HAI catheter-related complications, RFS, overall survival (OS) and recurrence pattern. 108 randomized pts were required to detect a gain in 18-month h-RFS from 30% to 50% with a one-sided  $\alpha$  risk of 10% and 95% power. The primary endpoint was analyzed with a Cox regression model, with adjustment for stratification factors (tumor response to preoperative CTx and number of resected CRLM). The study was prematurely stopped after randomization of 99 pts due to recruitment issues and HAI catheters market withdrawal. **Results:** Between Jun 2015 and Dec 2020, 99 pts (median age, 62; median number of CRLM, 5) were randomly assigned in HAI-IV arm (50 pts) or IV arm (49 pts). After a median follow-up of 56 months, the intent-to-treat analysis showed a significantly longer h-RFS in the HAI-IV arm compared to the IV arm (median 25 [16-37] vs 12 months [8-21]; HR 0.598 [95%CI 0.379-0.944],  $p = 0.027$ ). 5-year h-RFS was 32% [19-45] in the HAI-IV arm vs 13% [4-27] in the IV arm, respectively. Median and 5-year OS were 74 months [51-74] and 60% [43-74] in the HAI-IV arm vs 54 months [37-69] and 46% [29-61] in the IV arm (HR 0.551 [0.299-1.015],  $p = 0.056$ ). The median number of postoperative CTx cycles was 6 in both groups with 86% and 78% of patients having received at least 4 cycles in HAI-IV and IV arms ( $p = 0.7$ ), respectively. Grade 3-4 toxicity rate was 58% in the HAI-IV arm vs 31% in the IV arm ( $p = 0.01$ ). Overall, nine pts (18%) experienced HAI-related complications. No toxic death occurred in both arms. **Conclusions:** Despite a higher but acceptable toxicity, combination of HAI oxaliplatin with IV LV5FU2 significantly improved h-RFS after curative resection of CRLM in high-risk patients. This combined treatment should be considered as a valid option for these patients. Clinical trial information: NCT02494973. Research Sponsor: Institut National du Cancer, Programme Hospitalier de Recherche Clinique du Cancer, Ligue Contre le Cancer.

**Adjuvant systemic chemotherapy with or without hepatic arterial infusion of floxuridine in patients following colorectal cancer liver metastases resection (HARVEST): A prospective, randomized controlled trial.**

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**Background:** Several retrospective studies have previously demonstrated that a combination of adjuvant systemic chemotherapy and hepatic arterial infusion (HAI) could benefit patients following colorectal cancer liver metastases (CRLM) resection. This prospective clinical study aimed to determine whether adding HAI to adjuvant systemic chemotherapy could reduce the risk of recurrence following CRLM resection. **Methods:** The HARVEST study is an investigator-initiated, prospective, randomized controlled trial investigating the efficacy and safety of adjuvant intravenous chemotherapy with or without HAI floxuridine (FUDR) in CRLM patients that underwent liver metastasectomy. Patients in the systemic chemotherapy plus HAIC arm (HAI group) received systemic FOLFOX (q2w) plus HAI (FUDR, d1-14, q4w) for up to 6 months, while the systemic chemotherapy group without HAI (non-HAI group) received intravenous FOLFOX only. The primary study endpoint is the relapse-free survival in the modified intention-to-treat (mITT) population. Blood samples at different time points were also collected and circulating tumor DNA (ctDNA) was tested for NPY and SEPT9 methylation. **Results:** The study was prematurely terminated due to FUDR production halt in China. Ninety-two patients were randomized and seventy-seven patients (38 in the HAI group and 39 in the non-HAI group) were eventually included in our mITT analysis. After a median follow-up of 35.8 months, there were 22 (57.9%) and 25 (64.1%) recurrences in the HAI and non-HAI groups, respectively. The median relapse-free survival was 20.0 months in the HAI group and 11.7 months in the non-HAI group ( $p = 0.14$ ; HR 0.65; 95% confidence interval [CI] 0.37 to 1.16). No significant difference was found in terms of overall survival between the two groups ( $p = 0.461$ ). Our subgroup analysis revealed that patients with multiple liver metastases ( $p < 0.01$ ) and RAS/BRAF mutation ( $p < 0.01$ ) could benefit from adjuvant HAI treatment. Based on ctDNA status, patients with positive postoperative ctDNA methylation benefited from adjuvant HAI treatment ( $p < 0.01$ ), while those with negative postoperative ctDNA methylation status ( $p = 0.95$ ) did not. Chemotherapy-related adverse events were comparable between the two groups. **Conclusions:** Adjuvant chemotherapy intensification using HAI did not significantly reduce recurrence following CRLM resection. However, patients with multiple liver metastases, RAS/BRAF mutation and those with positive postoperative ctDNA might benefit from adjuvant HAI treatment. Clinical trial information: NCT03500874. Research Sponsor: None.

### Upfront palliative resection of primary tumor versus no resection in patients with synchronous metastatic colorectal cancer: The randomized phase 3 CAIRO4 study of the Dutch Colorectal Cancer Group (DCCG).

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**Background:** Primary tumor resection (PTR) in patients with synchronous metastatic colorectal cancer (CRC) has been associated with a survival benefit in comparative cohort studies. In the CAIRO4 phase III randomized trial, the potential benefit of upfront PTR followed by systemic therapy versus systemic therapy alone was investigated. **Methods:** Main eligibility criteria included histologically confirmed CRC, unresectable metastases, resectable primary tumor in situ without related severe symptoms, and WHO performance status (PS) 0-2. Eligible patients were randomized to first-line fluoropyrimidine-based chemotherapy plus bevacizumab with or without upfront PTR strategy. Randomization was stratified for number of metastatic sites (1 versus more), institution, WHO PS (0-1 versus 2), serum LDH (normal versus > ULN), and location of the primary tumor (left versus right-sided). The primary endpoint was overall survival (OS), which was analyzed by intention-to-treat (ITT) using the MaxCombo test. The original sample size of 306 patients was amended to 206 participants due to slow accrual. With final primary analysis based on 181 events, the study had 71% power to detect the OS difference of 19 versus 13 months deemed clinically relevant in the original sample size calculation. The trial is registered as NCT01606098. **Results:** Between August 2012 and February 2021, 206 patients were randomized: 103 patients to each arm. Two patients in the upfront PTR arm were excluded due to ineligibility. A total of 204 patients (57% male, median age 65 [IQR 59-71] years, 50% right-sided CRC and 98% WHO PS 0-1) were included with a median follow-up of 63.6 months. In the upfront PTR arm 5% of patients did not undergo PTR and 13% did not receive subsequent systemic therapy. In the arm without upfront PTR, 1% of patients did not receive systemic therapy. The median number of cycles was 9 [IQR 4-15] in the upfront PTR arm versus 11 [6-16] in the arm without upfront PTR. Median OS was 22.2 (95% CI 17.5 – 25.7) months in the upfront PTR arm and 19.5 (95% CI 16.1 – 22.7) months in the arm without upfront PTR ( $p = 0.269$ ). Median PFS was 10.1 (95% CI 8.7-11.7) months in the upfront PTR arm and 10.1 (95% CI 8.6-11.8) months in the arm without upfront PTR ( $p = 0.805$ ). At a later point in their disease course, 1.9% of patients in the arm without upfront PTR underwent a colostomy and 16.5% required PTR for symptom palliation. **Conclusions:** In patients with synchronous metastatic CRC amenable to palliative systemic therapy without severe symptoms related to the primary tumor, upfront PTR did not result in a significant median OS difference compared to immediate start with systemic treatment. Funding: This work was funded by the Dutch Cancer Society (grant KUN 2012-5697) and Hoffmann-La Roche Ltd. Clinical trial information: NCT01606098. Research Sponsor: Hoffmann-La Roche Ltd.; Dutch Cancer Society (grant KUN 2012-5697).

**15-month safety and efficacy data after intraperitoneal treatment with  $^{224}\text{Ra}$ -labelled microparticles after CRS-HIPEC for peritoneal metastasis from colorectal cancer.**

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**Background:** Peritoneal metastasis (PM) from colorectal cancer carries a dismal prognosis. Improved survival can be achieved by extensive cytoreductive surgery (CRS), frequently used together with hyperthermic intraperitoneal chemotherapy (HIPEC), with median time to recurrence around 12 months. Radspherin is a novel treatment principle based on the delivery of short range and cytotoxic alpha particles emitted during the decay of  $^{224}\text{Ra}$ . Alpha particles have high linear energy transfer and a radiation range less than 100  $\mu\text{m}$  (3-10 cell diameters), generating highly localized and effective radiation with non-repairable double-strand DNA breaks in affected cells. Our hypothesis is that Radspherin produces an alpha-particle radiation field exclusively to the surfaces and liquid volumes of the peritoneum, delivering lethal doses to remaining micrometastasis in the peritoneal linings and eradicate free-floating tumor cells after surgical resection, thus assumingly prolonging time to recurrence and potentially overall survival. **Methods:** A phase 1/2a study (EudraCT 2018-002803-33) is ongoing to evaluate safety, tolerability and signal of efficacy of Radspherin injected intraperitoneally two days after CRS-HIPEC. After completion of dose escalation (1-2-4-7 MBq), an activity-dose of 7 MBq was recommended. Assessment of safety and efficacy (diagnostic imaging) was performed every three months. Safety data and progression-free survival (PFS) at 15 months are presented. **Results:** Twenty-three patients (pts) were enrolled across cohorts (safety population), of these 12 pts received the recommended dose of 7MBq, 9 pts single dose and 3 pts split dose (3,5 MBq x2). Twelve pts had synchronous PM and 11 metachronous PM. Median age was 64 years (28-78), 70 % were female and median peritoneal cancer index was 7 (3-19). To date, 271 adverse events (AE) were reported, whereof only 7 (all grade 1-2) were deemed related to Radspherin. Fourteen serious adverse events (SAEs) in 8 pts have been reported, none considered related to Radspherin. At 15 months, 9 out of 23 pts (39 %) had recurred, whereof 4 pts recurred in the peritoneum. In the expansion cohort (7MBq), 3 out of 12 pts (25 %) had recurred and none of these pts had peritoneal recurrences. Median PFS was not reached in the two populations. **Conclusions:** Radspherin was well tolerated with no related SAEs reported. At 15 months median PFS has not been reached and none of the patients at recommended dose had peritoneal recurrences. The results are encouraging and warrant further exploration of Radspherin as a novel treatment principle in clinical trials. Clinical trial information: NCT03732781. Research Sponsor: Oncinvent AS; Innovation Norway.

**Neoadjuvant chemotherapy (NAC) followed by total mesorectal excision (TME) and adjuvant chemotherapy versus TME followed by adjuvant chemotherapy in very low-lying clinical (c) T3 rectal cancer (NAIR): A multicenter, randomized, open-label, phase 2/3 trial.**

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**Background:** Chemoradiotherapy and total neoadjuvant therapy are the standard treatments for locally advanced rectal cancer (LARC). Although radiotherapy (RT) is an integral part of treatment, it negatively impacts anal function after TME for cases of very low-lying LARCs. This trial examined the efficacy, safety, and influence on postoperative anal function of NAC without RT in patients (pts) with very low-lying cT3 LARCs. **Methods:** Pts with cT3N0-2M0 adenocarcinomas located within 5 cm from the anal verge, whose anuses were expected to be preserved, were randomly assigned (1:1) to either the NAC group (preoperative chemotherapy with six cycles of mFOLFOX6 or four cycles of CAPOX followed by TME; then, postoperative identical regimen) or the TME group (upfront TME followed by postoperative chemotherapy with 12 cycles of mFOLFOX6 or 8 cycles of CAPOX), stratified by cN stage, center, and sex. The primary endpoint was the 3-year recurrence-free survival (RFS), which was compared between groups using a stratified log-rank test at a one-sided alpha level of 20%. **Results:** Between February 2013 and March 2019, 130 pts were enrolled and randomly assigned to a treatment group. A total of 127 pts were evaluable (65 in the NAC group and 62 in the TME group). All but one patient with early progression completed preoperative chemotherapy in the NAC group, and all pts in both groups underwent TME. After a median follow-up of 37.4 months (IQR 36.5-40.5), the 3-year RFS was 75.5% (95% CI 62.5-84.5) in the NAC group vs. 70.9% (95%CI 57.2-80.9) in the TME group (hazard ratio 0.67, 95% CI 0.34-1.32;  $P = 0.098$ ), and the primary endpoint was met. There were no differences in the occurrence of grade 3 or higher postoperative complications (20% in the NAC group vs. 21% in the TME group [ $P = 1.000$ ]), chemo-associated grade 3 or higher adverse events (28% in the NAC group vs. 23% in the TME group [ $P = 0.551$ ]), or any grade of peripheral sensory neuropathy lasting for 3 years (22.7% in the NAC group vs. 38.5% in the TME group in pts treated with mFOLFOX6 [ $P = 0.3163$ ], 7.1% in the NAC group vs. 33.3% in the TME group in pts treated with CAPOX [ $P = 0.1038$ ]), between the groups. No treatment-related deaths occurred in either of the groups. At 3 years after randomization, 61 pts in the NAC group (93.8%) and 52 pts in the TME group (83.9%) could defecate via their anuses ( $P = 0.092$ ). The median Wexner incontinence score was 11.0 in the NAC group and 10.0 in the TME group ( $P = 0.3405$ ). **Conclusions:** For pts with very low-lying cT3 LARC, NAC followed by TME and adjuvant chemotherapy achieved significantly better RFS and anus-preserving rates compared to upfront TME followed by adjuvant chemotherapy, without deteriorating postoperative complications or postoperative anal function. Clinical trial information: UMIN000009510. Research Sponsor: National Cancer Center Research and Development Fund (23-A-26).

### Sustained organ preservation in patients with rectal cancer treated with total neoadjuvant therapy: Long-term results of the OPRA trial.

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**Background:** The preliminary results of the OPRA trial demonstrated that a substantial number of patients with locally advanced rectal cancer treated with total neoadjuvant therapy (TNT) could achieve organ preservation. Although most tumor regrowths seem to occur within the first 3 years, longer follow-up is needed to assess the ongoing risk of regrowth. Here, we report the long-term organ preservation rate and oncologic outcomes of the OPRA trial. **Methods:** A prospective, multi-institutional phase II trial, in which patients with stage II or III rectal cancer were randomized to receive either induction chemotherapy followed by chemoradiation (INCT-CRT) or chemoradiation followed by consolidation chemotherapy (CRT-CNCT). Patients underwent reassessment for treatment response 8-12 weeks after TNT. Patients who achieved a complete or near-complete response after finishing TNT were offered a watch and wait approach (WW). Those with incomplete response were recommended total mesorectal excision (TME). We report 5-year disease-free survival (DFS), organ preservation (defined as TME-free survival), local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS) and overall survival (OS) for each treatment group. We also compared DFS between patients who underwent upfront TME after restaging and patients who underwent TME after tumor regrowth. All analyses followed the intention-to-treat principle and groups were compared using the log-rank test. **Results:** Of the 324 patients randomized, 158 were assigned to the INCT-CRT group and 166 to the CRT-CNCT group. Median follow-up was 56 months; 85 DFS events were observed. The rates of 3- and 5-year DFS, TME-free survival, LRFS, DMFS and OS are listed in the Table. In total, 80 of the 225 (36%) patients who started WW developed a regrowth; 94% occurred within 2 years and 99% occurred within 3 years. The rate of TME-free survival at 5 years was significantly higher for CRT-CNCT (54%) than in INCT-CRT (39%). 5-year DFS was similar for patients who underwent TME after restaging (61%) compared to patients who underwent TME after regrowth (62%,  $p = 0.86$ ). **Conclusions:** In patients with rectal cancer treated with TNT and WW, the majority of tumor regrowths occur in the first 2 years, and regrowth after 3 years is vanishingly rare. Salvage TME for tumor regrowth during WW appears to offer similar outcome to immediate TME after incomplete response to TNT. Distant metastases remain the most frequent cause of treatment failure, with similar rates in the two treatment groups. Clinical trial information: NCT02008656. Research Sponsor: U.S. National Institutes of Health.

3- and 5-year rates with 95% CI.

|                   | Induction  |            | Consolidation |            | P    |
|-------------------|------------|------------|---------------|------------|------|
|                   | 3-year, %  | 5-year, %  | 3-year, %     | 5-year, %  |      |
| DFS               | 77 (70-84) | 72 (65-80) | 76 (70-83)    | 71 (63-78) | 0.60 |
| TME-free survival | 41 (34-50) | 39 (32-48) | 55 (48-63)    | 54 (47-63) | 0.01 |
| LRFS              | 95 (91-99) | 94 (90-98) | 94 (90-98)    | 90 (85-95) | 0.27 |
| DMFS              | 83 (78-90) | 82 (75-89) | 83 (77-89)    | 79 (72-86) | 0.66 |
| OS                | 95 (92-99) | 88 (82-94) | 94 (91-98)    | 88 (82-94) | 0.73 |

### Circulating tumor DNA dynamics as an early predictor of recurrence in patients with radically resected colorectal cancer: Updated results from GALAXY study in the CIR-CULATE-Japan.

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**Background:** Postoperative circulating tumor DNA (ctDNA)-based molecular residual disease (MRD) is reported to be associated with a high risk of recurrence. Here, we present an updated analysis and the lead time interval (LTI) of ctDNA positivity to radiographic recurrence in patients (pts) with radically resected colorectal cancer (CRC), stage II-IV in the observational GALAXY study. **Methods:** Serial ctDNA was analyzed using personalized tumor-informed assay (Signatera bespoke multiplex-PCR NGS assay) at 1 (4-week MRD time point), 3, 6, 9, 12, 18, and 24 months after surgery until recurrence, and CT scans were conducted every 6 months. The primary endpoint was disease-free survival (DFS), defined as the time between the date of surgery and date of diagnosis with relapse or death due to any cause. The LTI was defined from the first ctDNA positive date at post-surgery to radiographic recurrence. **Results:** Among 3,615 CRC pts who were enrolled between May 2020 and April 2022 in GALAXY study, 2,083 pts that met the inclusion criteria were analyzed in this report. The median follow-up period was 16.3 months. Of 2,083 pts analyzed, 286 (14%) were ctDNA-positive at 4-weeks MRD time point and 1,797 (86%) were ctDNA-negative. Pts with ctDNA-positivity at 4-weeks MRD timepoint demonstrated an inferior DFS and were 12 times more likely to recur, compared to ctDNA-negative pts (HR: 12, 95CI: 9.1-15%;  $p < 0.0001$ ). We further combined ctDNA status with *BRAF* V600E status at 4-week MRD time point and observed that pts with ctDNA-positivity and *BRAF* V600E mutation showed significantly shorter DFS compared to ctDNA-positive pts with *BRAF* wild-type ( $p < 0.001$ ). Whereas, in pts with ctDNA-negativity no significant difference in DFS was observed ( $p = 0.306$ ). On performing ctDNA dynamics analysis between 4-weeks to 12 weeks, compared to pts who remained ctDNA-negative ( $N = 1529$ ), a significantly shorter DFS was observed for pts who converted from ctDNA-negative to positive ( $N = 112$ , HR: 14.5, 95%CI: 8.8-23.8%,  $p < 0.0001$ ) or who remained positive ( $N = 124$ , HR: 25.4, 95%CI: 18.3-35.3;  $p < 0.0001$ ). Radiographic recurrence was observed in 186 pts (46%). Of these, 121 pts (65%) had CT imaging performed every 6 months, as per the protocol. The median LTI was 142 days (IQR, 43-189 days). **Conclusions:** Our study builds on the existing evidence from the recently published, prospective GALAXY study. ctDNA status at MRD time point post-surgery is prognostic of patient outcomes and is the most significant risk factor regardless of *BRAF* V600E status. ctDNA positivity predicted radiologic recurrence several months ahead of clinical recurrence. Pts with positive postoperative ctDNA should be examined carefully due to a high risk of recurrence. ctDNA-guided adjuvant strategy will further be established by ongoing randomized VEGA and ALTAIR studies in the CIR-CULATE-Japan. Clinical trial information: UMIN000039205. Research Sponsor: Japan Agency for Medical Research and Development (AMED).

### Association of positive ctDNA-based minimal residual disease assays during surveillance and undiagnosed concomitant radiographic recurrences in colorectal cancer (CRC): Results from the MD Anderson INTERCEPT program.

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**Background:** Observational cohorts have shown that detection of circulating tumor DNA (ctDNA)-defined minimal residual disease (MRD) following curative intent therapy has very high specificity and positive predictive value for future radiographic recurrence with a lead time of over 9 months. However, such data have not incorporated rigorous clinical evaluation for concomitant recurrence, nor have they established the clinical utility of MRD monitoring after completion of curative intent therapy. **Methods:** Pts with stages II-IV CRC (6/1/19 - 12/31/22, data cutoff) treated with curative intent at MD Anderson Cancer Center were evaluated with a tumor-informed MRD assay (Signatera, Natera), as part of the institutional INTERCEPT program that aims to integrate MRD-based testing into CRC clinical care. Surveillance visits including scans and tumor markers were performed per established guidelines. ctDNA was recommended post-operatively and q3m with each surveillance visit. Pts and providers were informed of the results and subsequent clinical courses including additional radiologic testing for ctDNA+ MRD tests were tracked. **Results:** 1259 pts were included in the INTERCEPT program (median 57y, [21-93]; 55% male; stage% I-III/IV 69/31; colon/rectum% 61/39), with 1049 pts tested after curative intent surgery. Of these, 159/1049 pts (15%) had ctDNA+; distribution of pts, % (+ve/total) from time of such surgery in m was: 0 - 3 (54.6/43.8); 3-6 (21.3/30); 6-12 (24/33.2). Of the pts with ctDNA+ after surgery, 49 pts (32%) were ctDNA+ prior to or during adjuvant therapy and 86 (57%) during surveillance. Of the 86 pts who were ctDNA+ during surveillance, imaging revealed concomitant new metastases in 46 (53%); i.e. only 40 (47%) were true MRD+. A total of 191 imaging studies were done (range 1-4) within 90d of the initial ctDNA+ including 99 as routine surveillance concurrent with ctDNA testing and 92 as additional follow up based on results. Of 40 pts with true MRD, majority (27 pts, 67.5%) were enrolled onto ongoing MRD trials (<https://crcmr.com/>). **Conclusions:** Our experience provides support for the feasibility of incorporation of MRD testing as part of routine surveillance. ctDNA+ results trigger a high rate of reflex imaging and, as a result, 53% of ctDNA+ patients have concomitant new radiographic findings. While clinical trials are feasible in true MRD+ pts, eligibility criteria for these trials need to be carefully specified about adequate radiographic evaluation. Research Sponsor: None.

# Predictive models of recurrence from transcriptomic signatures of the tumor microenvironment and cell cycle in stage III colon cancer from PETACC-8 and IDEA France trials.

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**Background:** The objective of this work was to establish predictive models of the risk of recurrence in stage III colon cancer (CC) based on transcriptomic signatures of the tumor microenvironment (TME) and cell cycle from the PETACC-8 (training set) and IDEA- France (validation set) trials. **Methods:** 3'RNAseq was performed in 1733 patients from the PETACC-8 trial (85%) and 1263 patients from the IDEA-France trial (63%). 4 transcriptomic signatures were analyzed: a signature reflecting T-cells infiltration named "Immunoscore-Like", a signature reflecting M2 macrophage infiltration named "M2-like", the expression of CXCL13 (reflecting B cells infiltration) and a score based on the Oncotype DX® Colon Cancer RS using the same formula from the stromal score and the cell cycle score, named "Oncotype DX Like". In the 1<sup>st</sup> multivariate model, we analyzed the 4 signatures separately with a dichotomization into "high" and "low" with the best cut point value for the prediction of time to recurrence (TTR) in PETACC-8 trial. In the 2<sup>nd</sup> multivariate model, we defined a score named "IPS" (Immune Proliferative Stromal), corresponding to the number of deleterious signatures ("high" or "low" depending on the signatures), ranging from 0 to 4. **Results:** The 1<sup>st</sup> multivariate model, built from PETACC-8 trial, showed that these 4 signatures were significantly associated with TTR with a protective effect of Immunoscore Like and CXCL13 "high" (HR: 0.66, p = 0.003 and 0.60, p < 0.001 respectively) and a deleterious effect of M2 Like and Oncotype Like "high" (HR: 1.28, p = 0.05 and HR: 1.37, p = 0.01, respectively), independently of known prognostic factors, with a C-index of 0.73. This model was applied to the IDEA-France cohort by calculating a predictive score for each patient, with TTR significantly different depending on the quartile of this score with a 3-year TTR ranging from 55% for the lowest quartile to 90% for the highest quartile (p < 0.001). In the 2<sup>nd</sup> model, in the 2 cohorts, IPS score was independently associated with TTR with a HR increasing with the IPS score, independently of T and N stage and intra-tumoral CMS heterogeneity (Table). **Conclusions:** Using transcriptomic data of patients with stage III CC from 2 large-scale adjuvant trials, two predictive models based on signatures of the TME and the cell cycle, provide important information in addition to known prognostic factors for patient stratification on risk of recurrence. Beyond T and N stage, for the decision of adjuvant chemotherapy in stage III CC, the combination of these different variables could be exploited in the future for personalized care (de-escalation, intensification). Research Sponsor: ARCAD.

|                                | PETACC-8 |        | IDEA-France |        |
|--------------------------------|----------|--------|-------------|--------|
|                                | HR       | p      | HR          | p      |
| T4                             | 2.0      | < 0.01 | 1.6         | < 0.01 |
| N2                             | 2.2      | < 0.01 | 2.4         | < 0.01 |
| IPS                            |          |        |             |        |
| 1                              | 1.8      | 0.2    | 2.3         | 0.2    |
| 2                              | 3.4      | < 0.01 | 3.9         | 0.058  |
| 3                              | 4.4      | < 0.01 | 4.6         | 0.03   |
| 4                              | 6.7      | < 0.01 | 5.7         | 0.02   |
| Dismal CMS combination         | 1.6      | < 0.01 | 1.1         | 0.5    |
| Treatment duration<br>3 months |          |        | 1.5         | < 0.01 |

**Circulating tumor DNA (ctDNA) to assess response in patients with anal cancer treated with definitive chemoradiation.**

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**Background:** We hypothesize that circulating tumor DNA (ctDNA) dynamics can provide an early response indicator in patients with anal squamous cell carcinoma (ASCC) undergoing definitive chemoradiotherapy (CRT). **Methods:** Since 2021, patients with ASCC undergoing CRT at 2 institutions were offered ctDNA monitoring with the Natera Signatera assay, a commercial tumor-bespoke, multiplex PCR assay. All patients provided written informed consent for ctDNA testing. Patients were clinically restaged 3-4 months after CRT by clinical exam, endoscopy, and/or MRI, as well as annually with a chest, abdomen, and pelvis CT. Complete clinical response (cCR) was defined as having no tumor observed by digital exam, endoscopy, and/or MRI. Molecular ctDNA response was described according to cCR, tumor recurrence, and survival. **Results:** From January 2021 to October 2022, 41 patients with ASCC treated with CRT underwent ctDNA response assessment. Most patients (66%) had stage III disease. Patients were treated to a median radiation dose of 54 Gy in 27 fractions — with combinatorial mitomycin and fluoropyrimidine-based chemotherapy in 88% of patients, and fluoropyrimidine-based chemotherapy alone in 12%. The median follow-up was 22 weeks (range 0-89 weeks). ctDNA testing was performed in 36 patients at baseline, 31 patients during CRT, 27 patients within 40 days after CRT, 23 patients 3-6 months post-CRT, 23 patients 6-12 months post CRT, and 10 patients > 12 months post CRT. At baseline, 89% of patients had detectable ctDNA. Patients with stage III, as compared to stage I-II, disease had numerically higher baseline ctDNA levels (29 vs. 2.9 mean tumor molecules per milliliter (MTM/mL),  $p = 0.04$ ). ctDNA levels decreased with treatment (24 vs. 2.1 MTM/mL,  $p = 0.005$ ) among the 24 patients with detectable baseline ctDNA and ctDNA tested during CRT. Fifty eight percent of patients converted from ctDNA positive to ctDNA negative during CRT. Similarly, post-CRT ctDNA levels decreased (23 vs. 0.01 MTM/mL,  $p = 0.01$ ), with 95% of patients converting from ctDNA positive to ctDNA negative. The time to ctDNA clearance was significantly shorter than the time to cCR (median 31 vs. 131 days,  $p < 0.0001$ ). In follow up 2 patients reverted from ctDNA negative to ctDNA positive at 113 -155 days post-CRT. Currently all patients are clinically and radiographically without evidence of disease. **Conclusions:** Surveillance ctDNA monitoring provides an earlier response assessment for patients with ASCC undergoing CRT. However, longer term follow-up is required to determine if ctDNA response correlates with long-term recurrence free survival. Prospective trials are needed to assess the clinical utility of integrating molecular ctDNA response into therapeutic response surveillance. Research Sponsor: U.S. National Institutes of Health.

**HPV integration as a prognostic biomarker for metastatic anal cancer: A next-generation sequencing ctDNA-based approach.**

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**Background:** Anal cancer is an HPV-associated malignancy with increasing prevalence in the United States. Immune checkpoint blockade demonstrates limited efficacy in patients with metastatic squamous cell cancer of the anal canal (mSCCA), and no prognostic or predictive biomarkers have demonstrated clinical utility in this population. Available circulating tumor DNA (ctDNA) assays have utilized ddPCR methodologies to report only the most common HPV types (HPV-16 and HPV-18) and HPV copy numbers. To extend the utility of an HPV ctDNA assay, we created a novel next-generation sequencing HPV ctDNA assay to analyze HPV integration as a prognostic biomarker for patients with mSCCA. **Methods:** Using an IRB-approved protocol, ctDNA isolated from the plasma of patients with mSCCA was sequenced using a 1.4Mb hybrid-capture target-enrichment panel covering the whole genome sequences of all 193 HPV types. HPV type, HPV copy number, and HPV integration status/locus were determined using a bioinformatic pipeline developed in-house. A Fisher's exact test was used to compare radiographic and ctDNA changes in response to systemic treatment. Unpaired t-tests were used to compare demographics between patients with HPV integration (I) or no integration (NI) status in the ctDNA. Median survival was estimated (Kaplan Meier) and compared according to integration status using a log-rank test. **Results:** 68 plasma samples from 27 patients with mSCCA were analyzed. While HPV-16 was detected in ctDNA of all 27 patients, five patients had additional oncogenic HPV types [HPV-18 (2), HPV-45 (2), HPV-73 (1)]. Radiographic changes in metastatic disease volume were concordant with HPV copy number changes in the ctDNA (odds ratio 12.2, 95% confidence interval (CI) 2.0-75;  $p = .007$ ). HPV integration events were detected in the ctDNA for 16 (59%) patients (median 2, range 1-7 unique events per patient). For the 23 patients with mSCCA who received anti-PD1 immunotherapy (15 I, 8 NI), there were no differences in age (60 vs 59 years,  $p = .88$ ) or prior lines of treatment (3.0 vs 3.5,  $p = .72$ ). There was a trend towards improved median progression-free survival for NI ctDNA status (5.8 vs 3.2 months, hazard ratio (HR) 1.8, 95% CI .71-4.5). Relative to NI status, I status was associated with worse overall survival rates (OS) (22.5 vs 44.9 months, HR 5.0, 95% CI 1.5-17,  $p = .005$ ). **Conclusions:** Detection of HPV integration status for all HPV types is feasible using a ctDNA-based approach for patients with SCCA. HPV integration was prognostic for worse OS in patients with mSCCA. Identification of HPV integration as a novel prognostic biomarker for survival outcomes in mSCCA warrants further evaluation in larger clinical trials. Research Sponsor: MD Anderson HPV Moonshot; Anonymous donor.

### Variation in FOLFOX, FOLFIRI, and FOLFOXIRI effects on CD8+ T cell and PDL1 levels in MSS CRC patients.

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**Background:** Colorectal cancer (CRC) is a heterogeneous disease treated with FOLFOX, FOLFIRI, and FOLFOXIRI chemotherapy regimens. About 85% of CRC patients have microsatellite stable (MSS) tumors that resist immune checkpoint blockade (ICB). Some studies suggest that chemotherapy modulates the MSS CRC tumor microenvironment (TME) to enhance CD8+ T cell infiltration, but ICB remains ineffective. We evaluated the TME of MSS CRC patients to investigate chemotherapy impact on immune markers. **Methods:** CRC patient samples (n = 16,827) underwent DNA (592-gene or whole exome)/RNA (whole transcriptome) sequencing at Caris Life Sciences. Immune cell fractions within TMEs were estimated from RNA deconvolution (quanTIseq; Finotello, 2019; n = 11,109). PDL1 expression was assessed by IHC (SP142;  $\geq 2+/5\%$ ). Patients who received FOLFOX (n = 425), FOLFIRI (n = 88), or FOLFOXIRI (n = 19) < 1 year prior to tumor collection or who didn't receive these treatments > 4000 days before tumor collection (untreated, n = 6,608) were analyzed. Statistical significance was determined using chi-square, Fisher's exact, and Mann-Whitney U tests, where appropriate. **Results:** FOLFOX-treated CRC (n = 213) had a higher CD8+ T cell fraction vs untreated (n = 3,449, FC = 1.9, p < 0.01) CRC and there was no difference among FOLFIRI-treated (n = 37, FC = 1.1, p = 0.13) or FOLFOXIRI-treated (n = 9, FC = 2.2, p = 0.49) CRC. Survival outcomes were similar in FOLFOX-treated CD8+ T cell-high (n = 82) vs low (n = 161, HR = 1.3, p = 0.12) and in FOLFOX-treated PDL1+ (n = 13) vs PDL1- (n = 338) CRC (HR = 1.6, p = 0.14). The CD8+ T cell fraction was similar in untreated KRAS wild-type (WT, n = 1714) vs untreated KRAS-mutated (mut, n = 1718) CRC (FC = 1.3, p < 0.01). FOLFOX-treated KRAS-mut CRC had a higher CD8+ T cell fraction (n = 1718 untreated, 96 treated, FC = 2.3, p < 0.01) and CD69 expression (n = 1724 untreated, 97 treated, FC = 1.7, p < 0.01) vs untreated KRAS-mut CRC. This FOLFOX-dependent effect on CD8+ T cells was lower in WT KRAS CRC (n = 114 FOLFOX-treated, n = 1714 untreated, FC = 1.6, p < 0.01) but survival outcomes were similar in FOLFOX-treated WT (n = 210) vs KRAS-mut (n = 210) CRC (HR = 1.0, p = 0.82). The CD8+ T cell fraction was similar in untreated right- (R; n = 803) vs left-sided CRC (L; n = 927, FC = 1.3, p < 0.01) but PDL1+ IHC rates were significantly higher in R-CRC (n = 1340 L, 1124 R, FC = 2.2, p < 0.01). The CD8+ T cell fraction was similar in untreated WT (n = 3144) vs BRAF-mut CRC (n = 248, FC = 0.8, p < 0.01), but BRAF-mut were more frequently PDL1+ (n = 4622 WT, 340 mut, FC = 4.2, p < 0.01). PDL1+ rates were similar in untreated (n = 1124) vs FOLFIRI-treated R-CRC (n = 12, FC = 2.0, p = 0.46) but were higher in FOLFIRI-treated (n = 9) vs untreated (n = 1340) L-CRC (n = 9, FC = 6.0, p = 0.04). **Conclusions:** We describe an association between FOLFOX and subsequent infiltration by CD8+ T-cells and high PDL1+ rates in R-CRC, BRAF-mut CRC, and FOLFIRI-treated L-CRC. The findings provide insights for future therapeutic strategies. Research Sponsor: U.S. National Institutes of Health.

### Pathological analysis of tumor microenvironmental features using deep learning to predict survival of colon carcinomas.

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**Background:** Advances in deep learning may improve the ability to evaluate and quantify pathological features in solid tumors to improve prediction of patient disease-free survival (DFS). We used an artificial intelligence (AI) algorithm to quantify morphological features in the tumor microenvironment of stage III colon cancers. **Methods:** We analyzed digitized stage III colon carcinomas (N = 402; 382 met QC) from participants in a phase III trial of FOLFOX-based adjuvant chemotherapy that included all available tumors with dMMR and a randomly selected cohort of pMMR tumors (median follow-up 60 months) [NCCTG N0147; Alliance for Clinical Trials in Oncology]. Fifteen morphological features were extracted using the QuantCRC algorithm that segments images into carcinoma (low-grade, high-grade, signet ring cell), stroma (immature, mature, and inflammatory), mucin, tumor/stroma ratio, tumor budding/poorly differentiated clusters (TB/PDC), necrosis, smooth muscle, fat, and tumor-infiltrating lymphocytes (TILs) and tumor area (mucin, epithelium and TB/PDC). Analysis of AI-derived features with clinical variables, molecular alterations (*BRAF/KRAS*), and DFS were examined using Kaplan-Meier methodology and multivariable Cox regression. **Results:** Among the 15 AI-derived morphological features, the following differed significantly between dMMR (n = 191) and pMMR (n = 189) whereby dMMR tumors had lower mature stroma, but higher inflammatory stroma and tumor, higher tumor grade and increased mucin, TB/PDCs, signet ring cells, and TILs (all  $p < 0.05$ ). Among dMMR tumors, multivariable analysis revealed that tumor area [mucin, epithelium and TB/PDC] ( $HR_{adj}$  0.45, 95%CI (0.24, 0.84),  $p:0.01$ , [Q2/3 vs Q1]) and N stage were the only significant and independent prognostic variables. Among pMMR tumors, multivariable analysis identified that tumor budding/poorly differentiated clusters (TB/PDCs) ( $HR_{adj}$  0.20, 95%CI (0.06, 0.61),  $p:0.01$ , [Q1 vs Q4]) was the strongest prognostic variable and the only morphological feature that was significantly associated with DFS along with age, N stage and T stage. **Conclusions:** Using AI, we can extract and quantify distinct morphological features in tumor sections that differ between dMMR and pMMR and multivariately, can significantly and robustly enhance prognostication within each MMR group. Among pMMR tumors, tumor budding/poorly differentiated clusters was the strongest predictor of DFS. Support: NIH U10CA180821, U10CA180882, U24CA196171, R01 CA210509 (to FAS). Study NCCTG N0147 received funds from Sanofi <https://acknowledgments.alliancefound.org>. Clinical-Trials.gov Identifier: NCT00079274 Key words Artificial Intelligence; Tumor Microenvironment; Colonic Neoplasms; Disease-free survival. Research Sponsor: U.S. National Institutes of Health; Sanofi; NIH U10CA180821, U10CA180882, U24CA196171, R01 CA210509 (to FAS).

## HER2 testing in the MOUNTAINEER trial: Analysis of treatment response based on central HER2 assessment using IHC/ISH and NGS.

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**Background:** Tucatinib (TUC) is a highly selective HER2-directed TKI approved by the FDA in combination w/trastuzumab (Tras) for treatment (tx) of pts w/ RAS wild-type HER2+ unresectable or metastatic colorectal cancer (mCRC) that has progressed following tx w/ fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy. There are currently no established best practices for HER2 testing/interpretation in mCRC. Here we present results of central HER2 testing across multiple platforms and response to tx for MOUNTAINEER pts treated w/ TUC + Tras based on central HER2 status. **Methods:** MOUNTAINEER (NCT03043313) enrolled pts w/ local HER2+ mCRC using  $\geq 1$  method: IHC, ISH, and/or NGS testing; retrospective central assessment of HER2 status was performed on multiple platforms. Pts in cohorts A+B were treated w/ TUC + Tras; pts in cohort C were treated w/ TUC monotherapy. Confirmed objective response rates (cORRs) using RECIST v1.1 per BICR, DOR, and PFS were calculated for pts treated w/ TUC + Tras based on each central testing method. **Results:** 114 pts were enrolled in cohorts A (n = 45), B (n = 39), and C (n = 30) w/ HER2+ tumors per  $\geq 1$  local testing method. Of samples submitted for central testing for Cohorts A and B, 70 per IHC/FISH, 50 per tissue NGS, and 71 per blood NGS had evaluable results. In all cohorts, there was 81.0% (95% CI, 68.6-90.1) agreement between blood and tissue NGS, 92.6% (95% CI, 83.7-97.6) between IHC/FISH and tissue NGS, and 79.5% (95% CI, 69.2-87.6) between IHC/FISH and blood NGS. In cohorts A and B, pts w/ HER2+ tumors by central IHC/ISH had a mDOR of 16.4 months (95% CI: 10.6-25.5) and mPFS of 10.1 months (95% CI: 4.2-15.2). cORR was 41.1% to 47.7% for the 3 assays. Detailed HER2 results are presented in the Table. **Conclusions:** Percent agreement of HER2 status was highest w/ tissue-based platforms. Detection of HER2 amplification by ctNDA NGS is useful; however, pts w/o HER2 amplification should be confirmed w/ a tissue-based assay. HER2 status by all 3 platforms predicted tx response to TUC + Tras. cORR in IHC2+/ISH+ was numerically lower but remained clinically relevant. These data support use of the above methods to identify HER2+ mCRC patients that may benefit from TUC + Tras. Clinical trial information: NCT03043313. Research Sponsor: Seagen Inc.

| Response                    | Central IHC + FISH<br>(n = 70)  |                                          |                      | PGDx tissue NGS<br>(n = 50) |                     | Guardant ctDNA<br>(n = 71) |                      |
|-----------------------------|---------------------------------|------------------------------------------|----------------------|-----------------------------|---------------------|----------------------------|----------------------|
|                             | Positive<br>(IHC3+)<br>(n = 45) | Positive<br>(IHC2+/<br>ISH+)<br>(n = 15) | Negative<br>(n = 10) | Positive<br>(n = 44)        | Negative<br>(n = 6) | Positive<br>(n = 56)       | Negative<br>(n = 15) |
| CR                          | 3                               | 0                                        | 0                    | 1                           | 0                   | 1                          | 1                    |
| PR                          | 18                              | 3                                        | 1                    | 20                          | 0                   | 22                         | 2                    |
| SD <sup>a</sup>             | 17                              | 5                                        | 4                    | 16                          | 2                   | 18                         | 7                    |
| PD                          | 7                               | 6                                        | 5                    | 7                           | 4                   | 14                         | 4                    |
| NA                          | 0                               | 1                                        | 0                    | 0                           | 0                   | 1                          | 1                    |
| cORR, n, (%)                | 21, (46.7%)                     | 3, (20.0%)                               | 1, (10.0%)           | 21, (47.7%)                 | 0, (0%)             | 23, (41.1%)                | 3, (20.0%)           |
| (95% CI)                    | (31.7-<br>62.1)                 | (4.3-<br>48.1)                           | (0.3-<br>44.5)       | (32.5-<br>63.3)             | (0-<br>45.9)        | (28.1-55)                  | (4.3-48.1)           |
| mDOR,<br>months<br>(95% CI) | 16.4<br>(10.6, 25.5)            | -                                        | -                    | 15.3<br>(8.9, 25.5)         | -                   | 12.4<br>(6.2, 38.3)        | -                    |
| mPFS, months<br>(95% CI)    | 10.1<br>(4.2, 15.2)             | 2.8<br>(1.2, 6.3)                        | 10.9<br>(7.0, 20.7)  | 2.1<br>(1.3, -)             | 8.1<br>(3.1, 10.2)  | 10.9<br>(2.0, 18.4)        | -                    |

<sup>a</sup>Includes non-CR/non-PD.

# A prospective, cross-sectional, multicentre study to evaluate the clinical performance of the ColoSTAT in vitro diagnostic for the detection of biomarkers associated with colorectal cancer.

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**Background:** Colorectal cancer (CRC) survival rates could be improved if more cancers were detected early. The ColoSTAT blood test and algorithm combines concentrations of 5 protein biomarkers with age and sex to provide an alternative to current CRC screening methods like the faecal immunochemical test (FIT). We compared the performance of ColoSTAT to colonoscopy in detecting CRC. **Methods:** Patients in this Australian study were either recently diagnosed with CRC using colonoscopy and progressing to surgery or neoadjuvant treatment (Cohort 1) or had no CRC history and were scheduled for colonoscopy (Cohort 2). Due to COVID-19 pandemic-related recruitment delays, the samples from Cohort 1 were supplemented with bio-banked blood samples (BBS) from patients with clinically confirmed CRC. Patients provided a 17 mL blood sample and were followed until start of cancer treatment or colonoscopy. All blood samples were de-identified prior to testing by an independent laboratory. The primary endpoints were ColoSTAT sensitivity of  $\geq 73\%$  (lower 95% confidence limit [LCL]  $> 60\%$ ), and specificity  $\geq 90\%$  (LCL  $> 80\%$ ). Sensitivity by TNM stage was an exploratory endpoint (ACTRN12619000301167). **Results:** Cohort 1 enrolled 29 patients, Cohort 2 enrolled 768 patients and 192 BBS were included. Patient demographic characteristics were similar in Cohorts 1, 2 and BBS. Overall, the median age of the patients ( $n = 989$ ) was 64 years (range 40 to 88) and 53.4% were female. Definitive ColoSTAT results were obtained for 22 patients in Cohort 1, 554 in Cohort 2 and 81 in BBS. Overall, the estimated sensitivity of ColoSTAT for detection of CRC compared with colonoscopy was 81.3% (95%CL 73.0%-87.4%) and estimated specificity 91.0% (95%CL 87.7%-93.5%) (Table). **Conclusions:** The ColoSTAT test met the primary endpoints of performance based on sensitivity and specificity in detecting CRC compared to colonoscopy. ColoSTAT sensitivity and specificity for CRC were comparable with published performance parameters for FIT which range from 74-93% (sensitivity) and 85-96% (specificity).<sup>1</sup> Clinical trial information: ACTRN12619000301167. Research Sponsor: This study was solely funded by Vision Tech Bio Pty Ltd (subsidiary of Rhythm Biosciences Limited). S. Gibb, WriteSource Medical Pty Ltd provided medical writing services funded by Rhythm Biosciences.

## Sensitivity and specificity of ColoSTAT vs colonoscopy.

|                                                                         | All participants/BBS (N = 989)                                                                   |
|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Definitive ColoSTAT result (indeterminate, invalid, no test)            | 657 (97, 208, 27)                                                                                |
| Colonoscopy result available (no colonoscopy)                           | 911 (78)                                                                                         |
| Definitive ColoSTAT and colonoscopy                                     | 603                                                                                              |
| ColoSTAT True +ve (A), False +ve (B), False -ve (C), True -ve (D)       | 91, 35*, 21, 354*,                                                                               |
| Sensitivity (95%CI) (A/(A+C))                                           | 81.3% (73.0%-87.4%)                                                                              |
| Specificity (95%CI) (D/(B+D))                                           | 91.0% (87.7%-93.5%)                                                                              |
| ColoSTAT sensitivity by CRC stage (I, II, III, IV) <sup>#</sup> (95%CI) | I: 87.5% (64.0%-96.5%), II: 91.3% (73.2%-97.6%), III: 92.3% (66.7%-98.6%), IV: 100% (87.9%-100%) |

\*Specificity calculated using the prospective cohorts <sup>#</sup>Exploratory endpoint; staging data available for BBS only <sup>1</sup>Switalski J et al, Cancers 2022, 14, 4391.

### Comprehensive study of the intratumoral microbiome in early- vs. late-onset colorectal cancer: Final analysis of COSMO CRC.

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**Background:** Although colorectal cancer (CRC) incidence has declined overall, CRC in individuals under age 45 (early-onset CRC, EOCRC) has risen dramatically. Obesity and diabetes may partially explain this epidemiologic shift; however, many patients (pts) with EOCRC are neither obese nor diabetic. Certain bacteria disrupt colonic luminal integrity and promote inflammation, leading to oncogenic mutations in colonic epithelial cells. *Fusobacterium nucleatum* (*F. nuc*) promotes CRC by suppressing immune response within the tumor microenvironment, activating the  $\beta$ -catenin pathway, and causing chemoresistance due to autophagy. The intratumoral microbiome (MB) in pts with EOCRC may differ from pts with late-onset CRC (LOCRC). **Methods:** We compared the intratumoral MB in pts with CRC diagnosed before age 45 (EOCRC) and after age 65 (LOCRC) in a prospective/retrospective study between 2017 and 2022. Primary and metastatic tumors were included. DNA was extracted from tumors and analyzed using 16S ribosomal gene sequencing. We compared the frequency of *F. nuc* and other bacterial and fungal DNA in tumors EOCRC vs. LOCRC pts. Next-generation tumor sequencing and diet questionnaire data were available for some pts. **Results:** Tumors from 36 EOCRC pts (median age 38 years) and 27 LOCRC pts (median age 72 years) were analyzed. In total, 917 unique bacterial and fungal species were detected. *F. nuc* was found in 30.6% of EOCRC and 29.6% of LOCRC ( $p = 0.94$ ). *Cladosporium* sp. was seen more commonly in EOCRC (30.6% vs. 11%,  $p = 0.04$ ), whereas *Pseudomonas luteola* (2.8% vs. 22.2%), *Ralstonia* sp. (22.2% vs. 48.1%), and *Moraxella osloensis* (19.4% vs. 44.4%) were seen more commonly in LOCRC ( $p < 0.05$ ). *Clostridium perfringens* (11.1%), *Escherichia coli* (11.1%), *Leptotrichia hofstadii* (11.1%), *Mycosphaerella* sp. (11.1%), *Neodevriesia modesta* (11.1%), *Penicillium* sp. (11.1%), and *Leptosphaeria* sp. (11.1%) were seen exclusively in LOCRC ( $p < 0.05$ ). There was no significant difference in median MB diversity in EOCRC vs. LOCRC (43 vs. 45 organisms per pt). Median follow up from time of diagnosis was 39.6 months (mo). Twenty-three EOCRC pts (64%) and 19 LOCRC pts (70%) are alive. Median overall survival (mOS) was 75.5 mo (95 CI: 40 - NA) in EOCRC and 60 mo (95 CI: 50.5 - NA) in LOCRC. There was no significant difference in OS in LOCRC vs. EOCRC (Log-rank test  $p = 0.85$ ). Pts with *F. nuc* positive tumors ( $N = 17$ ) had a mOS of 75.5 mo (95 CI: 23 - NA), whereas pts with *F. nuc* negative tumors ( $N = 46$ ) had a mOS of 60 mo (95 CI: 50.5 - NA). There was no significant difference in OS in *F. nuc* positive vs. *F. nuc* negative groups (Log-rank test  $p = 0.87$ ). **Conclusions:** There are significant differences in the intratumoral microbiome in EOCRC and LOCRC. These findings warrant larger, prospective studies to elucidate the role the intratumoral microbiome plays in the carcinogenesis, the tumor immune microenvironment, and responsiveness to specific therapies. Research Sponsor: Colorectal Cancer Alliance; Victoria Casey and Peter Teeley Foundation.

### Chemotherapeutic sensitivity in colorectal cancer expressing low RNA of wild type homologous recombination genes.

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**Background:** Homologous recombination deficient (HRD) colorectal cancer (CRC) has improved overall survival (OS) when exposed to DNA damaging agents (DDA) oxaliplatin (OX) and irinotecan (IR). However, this OS benefit is only observed in HRD mutated CRC. Low expression of wild type (WT) *BRCA1* RNA displays prolonged OS in ovarian cancer; this finding has not been investigated in CRC or outside *BRCA*. We investigate if low expression of WT HR genes is associated with prolonged OS in response to DDA. **Methods:** A total of 12,860 CRC patients tumor biopsies were analyzed by next-generation sequencing (592, NextSeq; WES, WTS NovaSeq). Patients with HRD mutations and MSI-H were excluded (N = 935). 11,925 patients were included in the analysis. A total of 11 core (*BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *MRE11*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*) and 7 related (*BAP1*, *WRN*, *DNMT3A*, *ERCC1*, *FANCA*, *FANCF*, *RECQL4*) HR genes were analyzed. Samples were classified by RNA expression percentiles within the studied cohort. Real world OS was extracted from insurance claims and calculated using Kaplan-Meier estimates for molecularly defined cohorts from first of OX or IR to last contact. **Results:** OS benefit is seen in low RNA expression (bottom 10%) compared to high expression (top 10%) of all core WT HR genes except *RAD51B*, *BARD1* and *BRCA2* in response to IR. We observe a progressively longer OS with lower associated RNA expression in response to DDA. OS of nearly 7 years was observed in low expressing *BRCA1* and *RAD51* following IR exposure and 4.5-year OS benefit when compared to high expression of these genes. OS benefit in response to OX was less robust but significantly prolonged in *BRCA1* and *BRIP1* (Table). Patients harboring HRD mutations had similar OS to WT HR RNA low (bottom 10%) in response to OX (HR = 0.75; p = 0.1) and IR (HR = 0.82; p = 0.36). **Conclusions:** Here we report a novel subclass of CRC defined as patients with low RNA expressing WT HR genes that exhibit differential sensitivity to DDA. Significantly longer survival is noted in CRC with low expression *BRCA1*, *RAD51* and *BLM*, while post-OX survival was significantly prolonged with low expression of *BRCA1*, *BRIP1* and *FANCA*. Further characterization of sensitive HR genes will better predict DDA sensitivity and impact treatment sequencing. Research Sponsor: None.

| Gene –<br>DDA        | HR   | Top<br>10%<br>RNA      | Bottom<br>10% RNA   | p-val   | Gene-<br>DDA         | HR   | Top<br>10%<br>RNA      | Bottom<br>10% RNA   | p-val |
|----------------------|------|------------------------|---------------------|---------|----------------------|------|------------------------|---------------------|-------|
| <i>BRCA1</i> -<br>IR | 0.62 | 25.9 m<br>(N =<br>232) | 80.5 m<br>(N = 197) | 0.009   | <i>BRCA1</i> -<br>OX | 0.66 | 40.3 m<br>(N =<br>362) | 61.5 m<br>(N = 358) | 0.005 |
| <i>RAD51</i> -<br>IR | 0.55 | 26.1 m<br>(N =<br>255) | 80.5 m<br>(N = 231) | < 0.001 | <i>RAD51</i> -<br>OX | 0.69 | 61.8 m<br>(N =<br>406) | 64.7 m<br>(N = 420) | 0.008 |
| <i>BLM</i> -IR       | 0.50 | 26.6 m<br>(N =<br>232) | 53.3 m<br>(N = 205) | < 0.001 | <i>BLM</i> -<br>OX   | 0.66 | 50.5 m<br>(N =<br>348) | 61.8 m<br>(N = 389) | 0.008 |
| <i>BRIP1</i> -<br>IR | 0.60 | 26.6 m<br>(N =<br>234) | 45.0 m<br>(N = 211) | 0.007   | <i>BRIP1</i> -<br>OX | 0.67 | 47.3 m<br>(N =<br>336) | 74.9 m<br>(N = 381) | 0.009 |
| <i>FANCA</i> -<br>IR | 0.63 | 25.0 m<br>(N =<br>224) | 35.3 m<br>(N = 194) | 0.01    | <i>FANCA</i> -<br>OX | 0.64 | 41.4 m<br>(N =<br>327) | 60.9 m<br>(N = 357) | 0.004 |

**Circulating tumor DNA in plasma as an early predictor for pathological lymph node involvement and (neo-)adjuvant chemotherapy in patients with colon cancer.**

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**Background:** Postoperative chemotherapy is standard for colon cancer patients with pathological lymph node involvement (pN+). If the presence of pN+ could be accurately predicted prior to surgery, the initiation of neoadjuvant chemotherapy could potentially reduce the presence of micrometastasis and enhance survival. The objective of this study was to assess the utility of preoperative methylated circulating tumor DNA (meth-ctDNA) as a predictive marker for pN+ and its clinical value as a marker for the initiation of neoadjuvant chemotherapy. **Methods:** The clinical data of 203 colon cancer patients (stage I-III) was collected from the Colorectal Cancer Database established at Danish Colorectal Cancer Center South, Vejle Hospital, Denmark. Patients were randomized into a discovery and a validation cohort. Plasma collected preoperatively was analyzed for three different meth-ctDNAs (Neuropeptide Y, Galactose-3-O-Sulfotransferase 3, and KN Motif and Ankyrin Repeat Domains 1) by droplet digital PCR (ddPCR). **Results:** Based on analysis of the discovery cohort, samples were considered positive if two or more of the three methylation markers had at least three positive droplets in the ddPCR analysis. 40% of patients in the discovery cohort and 46% of patients in the validation cohort were considered positive. When using meth-ctDNA as a diagnostic tool to predict pN+, the sensitivity and specificity in the discovery cohort were 42% and 61% respectively. In the validation cohort the values were 54% and 59%, respectively. AUC was < 60% in both cohorts. Meth-ctDNA was associated to clinical tumor (T)- and node (N)-category in both cohorts ( $p \leq 0.01$ ). Four-year disease free survival (DFS) in patients with and without detectable meth-ctDNA was 60% and 90% in the discovery cohort ( $p = 0.01$ ), and 62% and 78% in the validation cohort ( $p = 0.04$ ). Meth-ctDNA remained the strongest prognostic factor of DFS in the multivariate analysis compared to clinical T- or N-category, gender and age ( $p = 0.065$ , HR 2.46, 95% CI 1.03-5.88), however not statistically significant. Four-year overall survival (OS) in patients with and without detectable meth-ctDNA was 75% and 95% in the discovery cohort ( $p = 0.006$ ), and 78% and 91% in the validation cohort ( $p = 0.02$ ). In the multivariate analysis, meth-ctDNA was the strongest prognostic factor of OS compared to clinical T- or N-category, gender and age ( $p = 0.016$ , HR 4.54, 95% CI 1.32-15.6). **Conclusions:** The preoperative measurement of meth-ctDNA was not found to be a clinically significant predictor of pN+ or a reliable indicator for the initiation of neoadjuvant chemotherapy. Nonetheless, the results showed that preoperative ctDNA is a powerful prognostic indicator, suggesting that randomized controlled trials should be conducted to determine the efficacy of neoadjuvant chemotherapy in colon cancer patients with preoperative detectable ctDNA. Research Sponsor: Moltum, Grønbeck-Olsen and Højmosgaard.

### Correlation of single-cell cytokine secretion with clinical outcome in patients (pts) with metastatic colorectal cancer (mCRC) treated with capecitabine (C), bevacizumab (B), ± atezolizumab (A).

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**Background:** The BACCI trial (NCT02873195) randomized 133 mCRC pts to C+B+A (investigational) or C+B+placebo. Longer progression free survival (PFS) was observed in the investigational vs placebo arm. To investigate T-cell functional heterogeneity, we performed single-cell proteomic analyses on pts with either long or short PFS and evaluated T-cell function as a prognostic and predictive biomarker. **Methods:** Isolated CD8+ and CD4+ cells were analyzed at baseline and first restaging from pts with long (> 6 mo, N = 14) or short (< 2 mo, N = 13) PFS. Single-cell, functional proteomic analyses were conducted using the IsoLight system (Isoplexis), which assessed secretion of 32 cytokines from an average of 786 cells per sample. The cumulative readout was polyfunctional strength index (PSI), a composite score of frequency and magnitude of secreted cytokines. Cytokines are organized into effector (E), stimulatory, chemoattractive (CA), regulatory, and inflammatory (I) clusters. Differential expression analyses were done using Wilcoxon rank-sum tests. Potential predictive effects were assessed using logistic regressions with interaction between treatment and biomarkers. No multiple comparison control was done due to the exploratory nature of analyses. **Results:** Baseline CD8+ and CD4+ cells were stimulated *ex vivo* and single-cell cytokine analyses performed. Prognostic analyses indicated that CD8+ cells from pts with long PFS had ~3-fold higher PSI compared to pts with short PFS (p = 0.005). Further analysis identified that the E (p = 0.005) and CA clusters (p = 0.005) most impacted PSI, significant individual cytokines are listed in the table. Baseline CD8+ cell function was not predictive of outcome. Baseline CD4+ cell function was not prognostic or predictive of outcome. Analysis of CD8+ cells after treatment identified that changes in CA cluster was prognostic of long PFS (p = 0.024). Cytokine changes from CD4+ cells were not prognostic; however, early changes in the CD4+ CA cluster were predictive of outcome (p-int = 0.055). Similarly, early changes in the CD8+ I cluster were predictive of outcome (p-int = 0.073). **Conclusions:** These results reveal pts with long PFS had more functional CD8+ T-cells with a higher PSI than pts with short PFS. Predictive analyses suggested that early changes in the CD4+ CA and CD8+ I clusters were associated with benefit from immunotherapy. These preliminary results highlight the importance of single-cell functional proteomic analyses. Research Sponsor: Mario Family Foundation.

| Effect     | Cell-type | Timepoint       | Cytokine (p-value) - Cluster                                                                                                       |
|------------|-----------|-----------------|------------------------------------------------------------------------------------------------------------------------------------|
| Prognostic | CD8+      | Baseline        | TNF- $\alpha$ (p = 0.033) - E<br>IFN- $\gamma$ (p = 0.036) - E<br>MIP-1 $\alpha$ (p = 0.014) - E<br>MIP-1 $\beta$ (p = 0.005) - CA |
| Prognostic | CD8+      | On-study change | MIP-1 $\alpha$ (p = 0.007) - E<br>MIP-1 $\beta$ (p = 0.027) - CA                                                                   |
| Predictive | CD4+      | On-study change | MIP-1 $\alpha$ (p = 0.033) - E<br>MIP-1 $\beta$ (p = 0.029) - CA                                                                   |
| Predictive | CD8+      | On-study change | TNF- $\alpha$ (p = 0.084) - E                                                                                                      |

### Gut microbiome composition as predictor of the efficacy of adding atezolizumab to first-line FOLFOXIRI plus bevacizumab in metastatic colorectal cancer: A translational analysis of the AtezoTRIBE study.

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**Background:** Gut microbiome has emerged as a biomarker of clinical benefit to immune-checkpoint inhibitors (ICI) but no data are available in metastatic colorectal cancer (mCRC). The AtezoTRIBE study demonstrated that the addition of atezolizumab (atezo) to FOLFOXIRI plus bevacizumab (bev) prolongs progression-free survival (PFS), but this benefit is limited for patients with proficient mismatch repair (pMMR) tumors. Here, we aimed at investigating the potential predictive role of microbiome in identifying mCRC patients able to achieve benefit from ICI. **Methods:** AtezoTRIBE was a phase II trial in which 218 mCRC patients, unselected for MMR status, were randomized 1:2 to receive first-line FOLFOXIRI/bev (arm A) or FOLFOXIRI/bev/atezo (arm B). Stools were prospectively collected. Metagenomic (MG) data from whole genome sequencing (WGS) at level of species genome bins (SGBs) were analysed by linear models corrected for clinical and tumor-related parameters and fold-ratios. We defined as responders (R) those patients who experienced a PFS  $\geq$  12 months. **Results:** Stool samples were collected at baseline for 171 (78%) patients (55 in arm A and 116 in arm B) but only 163 were available for MG. Patients with deficient MMR (dMMR) tumors (N = 10) showed significantly lower MG diversity than pMMR ones (N = 148) harboring oral bacteria and pathobionts whose intrinsic immunogenicity has not been demonstrated. Regarding pMMR mCRC patients, baseline microbiome composition was not significantly different according to the treatment arm. The microbiome diversity was not significantly different between R and not-R in both arms, but specific immunogenic SGBs (Lachnospiraceae family members) were over-represented in R treated in arm B. Veillonellaceae and pathobionts were associated with poor prognosis and/or differential benefit from the addition of atezo. *Fusobacterium nucleatum* was associated with a poor prognosis, also in arm B. **Conclusions:** This is the largest prospective analysis showing that SGBs may be useful as a biomarker of potential benefit or detrimental effect from atezo in pMMR mCRC patients. Our results prompt the design of microbiota-centered diagnostic tests to identify pMMR mCRC patients more likely to benefit from ICI-based therapeutic strategies. Clinical trial information: NCT03721653. Research Sponsor: GONO Foundation.

### Consensus molecular subtyping of colorectal cancer to demonstrate cetuximab benefit in right-sided CMS2 tumors, and pembrolizumab benefit in MSS CMS1 tumors.

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**Background:** Consensus Molecular Subtypes (CMS) of colorectal cancer (CRC) were first developed in 2015 using microarray-based assays but are not widely used clinically. We developed a Caris CMS classifier on whole transcriptome sequencing data (WTS) with high concordance with the previously established CMS pipeline (Guinney et al 2015), and applied to a large clinic-genomic database of CRC to investigate the utility of CMS classification in identifying patients that may respond well to therapies commonly used in CRC. **Methods:** Next-generation sequencing (NGS) of DNA (592-gene or whole exome) and WTS was tested on CRC patient samples (n = 12,788) at a CLIA-certified lab (Caris Life Sciences, Phoenix, AZ). Caris CMS classifier was trained against the original CMS datasets using a classic SVM model and cross-validated for optimization of the SVM parameters. Possible overtraining was evaluated by predicting CMS from an independent blinded dataset (TCGA, N = 512) with an accuracy of 88.3%. Real-world overall survival was obtained from insurance claims and calculated from tissue collection to last contact (OS); time on treatment (TOT) was from first to last of treatment time. Kaplan-Meier estimates were calculated for molecularly defined cohorts. Significance was determined as p of < 0.05. **Results:** Among all patients, CMS1 was seen in 16%, CMS2 in 32%, CMS3 in 17% and CMS4 in 35%. MSI-H/MMRd (31%) and BRAF mut (33%) were most prevalent in CMS1 with KRAS mt the highest in CMS3 (66%). CMS2 was associated with the longest mOS (33m; 95% CI: 31m-35m), followed by CMS4 (29m; 28-31m), CMS3 (27m; 25-29m) and CMS1 (22m; 20-23m). In the microsatellite stable (MSS) tumors treated with pembrolizumab, CMS1 (N = 22, mTOT: 4.2m; 2.8-9.1m) had longer TOT than CMS2 (N = 45, mTOT: 2.1m; 1.4-3.1m), CMS3 (N = 21, 2.1m; 1.4m-3.0m) and CMS4 (N = 40; 2.1m; 1.4m-2.8m); CMS1 vs. CMS2-4 in MSS (HR: 0.58; CI: 0.34-0.97, p = 0.035). When investigating cetuximab, CMS2 had the longest mTOT (N = 189, mTOT: 6.3m; 5.4m-7.7m) among the four groups. Interestingly, although among all Ras WT tumors left-sided CRC showed longer mTOT on cetuximab (n = 457, mTOT: 5.4m; 4.7m-6.3m) than right-sided (n = 151, mTOT: 3.8m; 3.3m-4.5m), CMS2 showed similar mTOT from the left (n = 128) and the right (n = 16, mTOT: 6.3m vs. 5.6m, HR = 0.896, 95% CI: 0.501-1.604, p = 0.722). Notably, CMS2 comprises 49% of left- and 15% of right-sided tumors, potentially underlying the TOT difference seen between left and right. **Conclusions:** A WTS based CMS classifier allows for investigation in a large real-world clinic-genomic database. We found that MSS CMS1 CRC's may derive benefit from immunotherapy. Additionally, CMS2 subgroup of right-sided tumors may derive benefit from cetuximab. Routine CMS subgrouping of CRC provides important treatment associations that should be further investigated. Research Sponsor: None.

**Genomic landscapes of early-onset versus average-onset colorectal cancer populations.**

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**Background:** Early-onset colorectal cancer (eoCRC, initial CRC diagnosis at age < 50 years) has been increasing in the past two decades especially in Western countries. This study evaluates somatic and germline profiles in eoCRC compared to average-onset CRC (aoCRC, initial CRC diagnosis at age ≥ 50 years). **Methods:** This is a retrospective, cross-sectional study utilizing data from de-identified records of colorectal cancer patients tested with the Tempus xT assay from 2017 to 2022. Briefly, the assay is a targeted panel that detects single nucleotide variants, insertions and/or deletions, and copy number variants in 598-648 genes, as well as chromosomal rearrangements in 22 genes with high sensitivity and specificity. Baseline characteristics and immunologic markers were compared between eoCRC and aoCRC by Wilcoxon Rank Sum or Chi-squared test (reporting p-values). Somatic and germline mutations were compared between two age groups with false discover rate adjustments (reporting q-values). **Results:** In this study, 2,379 eoCRC and 8,627 aoCRC patients were included, with the majority diagnosed with stage IV disease. Additionally, germline alterations were assessed on a subset of 6,311 patients with tumor/normal match testing (eoCRC = 1,413 and aoCRC = 4,898). Left-sided primaries were more common in eoCRC (85% left/rectum in eoCRC vs. 75% left/rectum in aoCRC ( $p < 0.001$ ), and rates of microsatellite instability-high (MSI-H) or deficient mismatch repair (dMMR) were lower compared to aoCRC (4.2% vs. 6.8%,  $p < 0.001$ ). eoCRC has a unique somatic mutation profile compared to aoCRC. A higher prevalence of germline mutations was observed in eoCRC overall (6.9% vs. 5%,  $p = 0.006$ ); however, no statistically significant differences were observed in individual germline genes compared to aoCRC, likely due to relatively small numbers. **Conclusions:** eoCRC has a unique mutational profile and presence of germline mutations in 6.9% of eoCRC, indicating a potential role for universal germline testing in CRC. Research Sponsor: TEMPUS Labs.

|                                      | eoCRC (n = 2,379) | aoCRC (n = 8,627) | p-value/q-value |
|--------------------------------------|-------------------|-------------------|-----------------|
| <b>Baseline characteristics</b>      |                   |                   |                 |
| Median age at diagnosis (IQR)        | 43 (38, 47)       | 64 (57, 72)       | < 0.001         |
| Gender: male                         | 1,275 (54%)       | 4,967 (58%)       | < 0.001         |
| Stage IV                             | 1,389 (81%)       | 5,278 (80%)       | 0.030           |
| Left sided/rectal primary *          | 840 (85%)         | 2,530 (75%)       | < 0.001         |
| <b>Immunologic markers</b>           |                   |                   |                 |
| TMB ≥ 10                             | 124 (5.7%)        | 614 (7.6%)        | 0.002           |
| MSI-H                                | 97 (4.2%)         | 564 (6.7%)        | < 0.001         |
| dMMR                                 | 25 (2.6%)         | 253 (6.9%)        | < 0.001         |
| <b>Somatic mutations profiles **</b> |                   |                   |                 |
| BRAF                                 | 111 (4.7%)        | 845 (9.8%)        | < 0.001         |
| BRAF <sup>V600E</sup> /MSI-H         | 1 (1.0%)          | 262 (46%)         |                 |
| RNF43                                | 68 (2.9%)         | 515 (6.0%)        | < 0.001         |
| AMER1                                | 83 (3.5%)         | 503 (5.8%)        | < 0.001         |
| ZNRF3                                | 24 (1.0%)         | 192 (2.2%)        | 0.008           |
| <b>Germline mutation profiles **</b> |                   |                   |                 |
|                                      | eoCRC (N = 1,413) | aoCRC (N = 4,898) |                 |
| Overall prevalence                   | 6.9%              | 5.0%              | 0.006           |
| TP53                                 | 5 (0.4%)          | 2 (< 0.1%)        | 0.2             |
| APC                                  | 9 (0.6%)          | 11 (0.2%)         | 0.4             |
| ATM                                  | 11 (0.8%)         | 19 (0.4%)         | 0.4             |
| RAD51C                               | 4 (0.3%)          | 3 (< 0.1%)        | 0.4             |

\* Include patients with CRC tissue sequenced. \*\* list first 4 mutations based on the lowest q values in order of prevalence.

**Deep learning tumor heterogeneity metric from histopathology images vs next generation sequencing-derived scores for colon cancer prognostication.**

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**Background:** Tumor heterogeneity is as an important determinant of clinical behavior in many cancer types, with increased heterogeneity thought to confer inferior clinical outcome. Sequencing based assessment of colon cancer has been used to quantify tumor heterogeneity and correlate it with survival, but is sensitive to the mutation calling algorithm utilized. Digitization of histopathology slides allows application of deep learning methods for image analysis. Automated slide annotation and feature extraction provides numeric representations of underlying phenotype from which tumor heterogeneity can be estimated. Here we compare the ability of a deep-learning tumor heterogeneity score (THS) to estimate overall survival in colon cancer patients to estimates produced by bulk sequencing derived mutant-allele tumor heterogeneity (MATH) and copy number variation (CNV) event scores. **Methods:** Digitized whole slide images (WSIs) from The Cancer Genome Atlas (TCGA) colon adenocarcinoma dataset are processed in a computational pipeline for tissue detection, numerical feature extraction, and cell type classification. WSIs are tiled and local THS calculated from imaging features. Corresponding MATH and CNV derived heterogeneity metrics are calculated from published sequencing data. Kaplan Meier curves are used to compare patient survival stratified by tumor heterogeneity as calculated by the three independent methods. Automated regional annotation identifies intra-tumoral and tumor-stromal boundary tiles and tumor THS is correlated to distance from the boundary. **Results:** Images from 379 patients (209 right-sided, 152 left-sided tumors) yielded 575,762 tiles. Automated annotation resulted in 272,791 intra-tumoral and 11,340 tumor-stromal boundary tiles. Stratification by imaging derived THS provided significant separation of overall survival curves ( $p < .01$ ) in contrast to MATH and CNV methods ( $p > .05$  for both). THS significantly separated the curves in right- but not left-sided cancers ( $p = .03$  and  $.44$ , respectively). Evaluating THS as a function of distance from the tumor-stromal boundary, right-sided tumors showed significant decrease in THS with increasing distance from tumor edge. **Conclusions:** Our novel pipeline produced spatially resolved imaging data informed by underlying tumor phenotype without need for pathologist annotation. The resultant THS correlated with outcome and outperformed sequencing-based prognostication methods. The spatial information identified higher heterogeneity at the tumor edge than interior regions of right-sided, but not left-sided tumors, capturing known but poorly understood differences in colon tumors by location. Deep learning image analysis provided reproducible and cost-effective data with great potential both in clinical biomarker discovery and as a research tool. Research Sponsor: The Jackson Laboratory for Genomic Medicine; Hartford Healthcare.

# Evaluation of microsatellite instability status, a definitive predictive biomarker for immune checkpoint inhibitors (ICI), in underrepresented minorities (URM) with gastrointestinal (GI) cancers.

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**Background:** Mismatch repair deficiency (dMMR) results in MSI-H state and is the first tumor type-agnostic biomarker predictive of ICI response. Among GI cancers, MSI-H is most frequent in colorectal cancer (CRC 15%), gastroesophageal (GEC, 5%) and other (small bowel, hepatopancreatobiliary; 1%). For CRC, MSI-H can be attributed to germline mutation (Lynch syndrome, 3%) or somatic inactivation (sporadic, 12%) of foundational MMR genes. Studies evaluating ICI efficacy in dMMR cancers focus primarily on non-Hispanic White (NHW) patients (pts). We present prevalence, tumor genomic features, and outcomes in pts from a large cohort at Memorial Sloan Kettering (MSK). **Methods:** Retrospective analysis of MSI-H GI cancers from MSK-IMPACT database. Pts were grouped by self-reported race and ethnicity into 4 study arms: NHW, Asian, non-Hispanic Black (NHB), and Hispanic. Age, tumor type, tumor mutation burden (TMB), and MMR genes were analyzed. Overall survival (OS) estimated with Kaplan-Meier. **Results:** Of 776 pts with MSI-H GI cancers: 623 (80.3 %) NHW, 60 (7.7 %) Hispanic, 50 Asian (6.5 %), and 43 (5.5 %) NHB. CRC (76%), GEC (14%), other cancers (10%). We present initial evaluation of CRC and GEC: Median age, TMB, and most frequently altered MMR genes (MMR gene FA) are in table. Median OS (mOS) in NHW/URM by receipt of ICI in MSI-H CRC were 38.5m/25.3m (p 0.07) in no-ICI group, 34.2m/28.7m (p 0.64) in +ICI group; MSI-H GEC 43.4m/30m (p 0.44) in no-ICI group, 28.8m/26.7m in +ICI group. **Conclusions:** Number of URM MSI-H CRC/GEC pts is 7 to 15-fold less than NHW, with no such difference in % MSI-H/MSS between groups; reflecting significant undertesting in URM pts. In MSI-H CRC, median age (m-Age) at sequencing was younger in URM compared to NHW; pronounced in Asian and Hispanic patients, who were 10+ years younger than NHW. No such age difference seen in GEC. No difference in mOS detected between NHW and URM, however a non-significant trend towards worse mOS in URM was observed in the no-ICI group. Next steps include validation of clinico-genomics of MSI-H GI cancers in other large cohorts, including TEMPUS (N = 768) which is ongoing. Research Sponsor: None.

|                          | NHW   CRC            | NHB   CRC          | Asian   CRC        | Hispanic   CRC      |
|--------------------------|----------------------|--------------------|--------------------|---------------------|
| MSI-H/MSS % (MSI-H, N)   | 13% (3679)           | 10% (349)          | 8% (430)           | 14% (331)           |
| m-Age I range (p vs NHW) | 67   19-60 (1)       | 61   28-78 (0.025) | 57   26-89 (0.023) | 55   25-87 (0.048)  |
| m-TMB I range (p vs NHW) | 58   3-369 (1)       | 54   21-201 (0.43) | 50   26-219 (0.06) | 61   32-400 (0.95)  |
| MMR gene FA (%   N)      | MSH6 (27%   492)     | MLH1 (26%   34)    | MSH2 (26%   34)    | MSH6 (36%   45)     |
|                          | NHW   GEC            | NHB   GEC          | Asian   GEC        | Hispanic   GEC      |
| MSI-H/MSS % (MSI-H, N)   | 5% (1314)            | 7% (83)            | 5% (184)           | 7% (124)            |
| m-Age I range (p vs NHW) | 71   45-90 (1)       | 70   55-81 (0.3)   | 68   49-85 (0.55)  | 68   35-88 (0.53)   |
| m-TMB I range (p vs NHW) | 50   2-172 (1)       | 75   30-87 (0.34)  | 49   18-72 (0.59)  | 44   31-62 (0.3)    |
| MMR gene FA (%   N)      | MSH6/MLH1 (13%   76) | MLH1 (33%   6)     | MSH6 (30%   10)    | MSH2/MSH6 (11%   9) |

# Prognostic and predictive significance of pre- and post-operative plasma tumor markers in patients with appendiceal adenocarcinoma undergoing cytoreductive surgery: Insights from a single-center study.

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**Background:** Appendiceal adenocarcinomas (AA) are both rare and heterogeneous diseases. Cytoreductive (CRS) surgery followed by heated intraperitoneal chemotherapy (HIPEC) remains the best treatment for AA patients with peritoneal metastasis. This study seeks to investigate the clinical utility of pre- and post-operative plasma tumor markers (TM, CEA, CA19-9, CA-125) by association with survival outcomes. **Methods:** Under an approved IRB protocol the Palantir Foundry software system was used to query MD Anderson internal database to identify patients with AA who underwent CRS between 2016 to 2022. Elevation of TM was defined as above the laboratory upper limit of normal (CEA > 3 ng/mL, CA 19-9 > 37 U/mL, and CA-125 > 37 U/mL). Relationship between normal and elevated serum TM and progression-free survival (PFS) and overall survival (OS) was evaluated using univariate and multivariate Cox-proportional hazards regression analysis, considering multiple clinicopathologic variables. **Results:** 296 patients were identified; preoperative CEA, CA19-9 and CA125 was elevated in 60%, 29% and 28% of patients, respectively. Preoperative elevation of any TM was associated with higher PCI score (22 vs. 15,  $p < 0.0001$ ). Moreover, elevated preoperative TM was associated with incompleteness of cytoreduction (OR = 6.5,  $p < 0.0001$ ). Compared to preoperative measurements, all TM levels dropped after surgery (Mean score 31 vs 11 for CEA, 136 vs 32 for CA19-9 and 36 vs 17 for CA125). Preoperative elevation of any TM (HR = 1.5,  $p = 0.0288$ ) and post-op elevation of any TM were with poor PFS on univariate analysis, however only post-op elevated TM levels (HR = 2.3,  $p = 0.0001$ ) were associated with poor PFS on multivariate analysis. Interestingly, elevated TM was not associated with OS. Post-op TM levels were analyzed to evaluate the positive and negative predictive value in prediction of relapse in 6 months from surgery, PPV was 45% and NPV was 84%. **Conclusions:** Preoperative elevation of TM can identify patients at higher risk for incomplete cytoreduction and relapse after surgery. Postoperative elevation of TM is a risk factor for relapse. Research Sponsor: Conquer Cancer Foundation of the American Society of Clinical Oncology; CCSG; Col. Daniel Connelly Memorial Fund; U.S. National Institutes of Health; Cancer Prevention & Research Institute of Texas.

| Clinical Characteristics           | Univariate Analysis |          |           | Multivariate Analysis |         |         |
|------------------------------------|---------------------|----------|-----------|-----------------------|---------|---------|
|                                    | HR                  | P value  | 95 CI%    | HR                    | P value | 95 CI%  |
| Black or African American          | 2.506               | 0.0039   | 1.34 4.68 | 1.7                   | 0.13    | 0.9 3.5 |
| Goblet/Signet Tumor Histopathology | 1.69                | 0.0047   | 1.18 2.43 | 1.8                   | 0.0059  | 1.2 2.8 |
| High Grade Tumor                   | 1.705               | 0.0026   | 1.20 2.41 | 1.3                   | 0.28    | 0.8 2   |
| Incomplete CRS (CCR 2/3)           | 3.912               | 1.78E-11 | 2.63 5.82 | 3.1                   | 0.0001  | 1.8 5.5 |
| Preop elevation of TM              | 1.513               | 0.0288   | 1.04 2.19 | 0.97                  | 0.89    | 0.6 1.5 |
| Postop elevation of TM             | 2.572               | 4.73E-08 | 1.83 3.61 | 2.3                   | 0.0001  | 1.5 3.5 |
| Utilization of Neoadjuvant         | 2.062               | 0        | 1.47 2.90 | 1.3                   | 0.22    | 0.9 2   |
| Utilization of Adjuvant            | 2.835               | 1.75E-06 | 1.85 4.35 | 1.6                   | 0.065   | 1.0 2.7 |
| PCI score more than 25             | 1.684               | 0.0038   | 1.18 2.40 | 1                     | 0.96    | 0.6 1.7 |

**Does serial circulating tumor DNA (ctDNA) monitoring identify additional acquired actionable alterations in metastatic colorectal cancer (mCRC)?**

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**Background:** Traditionally, biomarker ascertainment occurred once for patients (pts) with mCRC. An advantage of ctDNA is the ease of repeated assessments. However, real-world evidence about the value of serial ctDNA in revealing actionable alterations is needed. **Methods:** We retrospectively evaluated 3350 pts with mCRC and a Guardant360 ctDNA assay (Guardant Health) revealing  $\geq 1$  somatic alteration who underwent  $\geq 1$  subsequent Guardant360 test to compare detection of mutations (mut: single nucleotide variants (SNVs) and indels), amplifications (amps), fusions, microsatellite instability (MSI), and blood tumor mutational burden (bTMB). Variants were filtered to pt-specific levels of detection by limiting variants across assays to those above a 0.1% mutant allele frequency (MAF) threshold on the serial test with the lowest somatic MAF for that pt. Clonal muts were defined as those at  $\geq 10\%$  of max MAF per sample. **Results:** A total of 9130 assays (mean of 2.7 assays/pt) occurring a median of 165 days apart were evaluated. Among 1476 pts initially with no alteration in MAPK pathway genes (*RAS* and *EGFR* SNV or indel, *BRAF*V600E SNV, or *ERBB2/MET* amplifications), 382 (25.8%) acquired a MAPK alteration on their second test. More gains were clonal than subclonal (231:151, 60.5%:39.5%). In pts with a subsequent assay after an acquisition (N = 80), 12 (15.0%) clonal and 15 (18.8%) subclonal acquisitions disappeared in the third assay, a median of 150 days later. Among alterations with a therapy, *KRAS*G12C/D, *BRAF*V600E, and *ERBB2* amps acquisitions occurred at any later assay in 84/1476 (5.7%), 29/1476 (2%), and 21/1476 (1.4%) pts without an initial MAPK alteration, respectively. Of these, 84/134 (62.6%) emerged without another concurrent MAPK alteration, 61/84 (72.6%) of which are subclonal. Of 92 fusions noted in 86 pts, 87/92 (94.5%) were subclonal and only 28/92 (30.4%) were initially present. The majority of fusions were acquired de-novo subclonal fusions (58/64, 90.6%) but 2 pre-existing subclonal fusions subsequently became clonal. Among pts evaluable for MSI, 56/3030 (1.8%) were initially MSI-H and 30 (1%) subsequently had MSI detected on a future assay. New MSI detection was more common in pts with DNA repair muts (*BRCA1/BRCA2/ATM/CHEK2/MLH1/RAD51D*) on an initial assay (OR 7.52, 95% CI 3.39-16.69,  $P < 0.0001$ ). Among pts evaluable for bTMB, 60/1387 (4.3%) initially had bTMB  $\geq 20$  muts/Mb, and 256/1327 (19.3%) subsequently rose above 20 muts/Mb on a future assay, a median of 417 days after initial assay. Rising bTMB associated with rising max somatic MAF (Spearman rho = 0.50,  $P < 0.0001$ ). Pts with DNA repair muts on an initial assay were more likely to have bTMB rise to  $\geq 20$  muts/Mb (OR 2.58, 95% CI 1.81-3.68,  $P < 0.001$ ). **Conclusions:** In this large mCRC cohort, serial ctDNA appears to be a feasible approach to identify acquired alterations with therapeutic implications. Research Sponsor: Guardant Health.

**Racial and genetic ancestry associations with gene expression patterns in a real-world cohort of colorectal cancer patients.**

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**Background:** There is a growing incidence of colorectal cancer (CRC) among young adults and persistent disparities in outcomes by race/ethnicity across all ages. Gene expression signatures, as well as consensus molecular subtypes (CMS) derived from these, have been proposed to predict prognosis and therapy response in CRC. However, it is unclear whether gene expression or CMS are associated with racial disparities observed in CRC. We assessed whether race or genetic ancestry are associated with CMS or gene expression patterns in a deidentified cohort of 1,768 CRC patients. **Methods:** Patients' tumors underwent tumor profiling with the Tempus xT NGS 648-gene assay as well as full-transcriptome RNA sequencing. We used a set of 654 ancestry-informative markers to infer genetic ancestry likelihoods for Africa (AFR), America (AMR), East Asia (EAS), Europe (EUR), and South Asia (SAS). Race/ethnicity labels, often missing in real-world data, were imputed using ancestry proportions from the literature and adjusted based on observed metadata. Gene expression data was used to assign CMS to all patients (CMS1, 2, 3, 4 and indeterminate) using CMScaller, and multinomial logistic regression was used to assess associations with race/ethnicity imputed labels and ancestry proportions. We then assessed differential expression (DE) in the MSigDB hallmark and C2 BioCarta gene sets using four separate workflows. The first two workflows used limma-voom followed by ROAST to assess DE among the imputed labels and then among the ancestry proportions (isometric log ratio transformed). The second two workflows used GSVA followed by limma. **Results:** Among 1,768 patients, 240 were imputed non-Hispanic (NH) Black, 94 NH Asian, 261 Hispanic/Latino/Native American (HLN), and 1,173 NH White. NH Black patients had higher odds of CMS3 vs CMS1 (OR = 2.66,  $p < 0.001$ ) and HLN patients had higher odds of indeterminate CMS vs CMS1 (OR = 1.90,  $p = 0.020$ ), compared to NH White. AFR ancestry was significantly associated with CMS3 (OR = 1.05 per doubling in AFR proportion,  $p = 0.047$ ) and indeterminate CMS (OR = 1.07,  $p = 0.023$ ). In the gene set analysis, NH Black race/ethnicity was associated with over-expression of the BioCarta *WNT* pathway gene set. Both AFR ancestry and NH Black race/ethnicity were associated with under-expression of the MSigDB hallmark coagulation and BioCarta alternative complement gene sets and over-expression of the *PITX2* pathway gene set (all significant with both ROAST and GSVA). **Conclusions:** We found that NH Black patients and AFR ancestry were associated with higher rates of CMS3, which is associated with *KRAS* mutation and was previously reported to be more common among Black patients. Indeterminate CMS associations with AFR ancestry and HLN highlights the need to use diverse patient cohorts when training unsupervised learning models to improve prognosis prediction in non-White patients. Research Sponsor: Dedman Family Scholarship.

### Artificial intelligence–derived immune phenotypes for prediction of prognosis in patients with stage III colon cancer (NCCTG N0147; Alliance).

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**Background:** Solid tumors can be characterized by distinct immune phenotypes based on the level of T cell infiltration in the tumor microenvironment (TME) which may provide prognostic information and also inform strategies to restore the anti-tumor immune response. Immune phenotypes were determined using an artificial intelligence (AI) algorithm in digitized whole-slide images (WSI) of tumors with deficient (d) mismatch repair (MMR) vs proficient (p) MMR. **Methods:** Stage III colon carcinomas (N = 401; 393 met QC) from participants in a phase III trial of FOLFOX-based adjuvant chemotherapy were analyzed including all available tumors with dMMR (n = 196) and a randomly selected cohort of pMMR tumors (n = 195). Using an AI algorithm (Lunit SCOPE) previously trained on solid tumors, digitized tumors were categorized into three immune phenotypes based on epithelial and stromal TIL data-driven cutpoints in dMMR and pMMR (inflamed: TIL high in epithelium (> upper 25%); excluded: TIL high in stroma (>lower 25%), low in epithelium; and desert: TIL low in epithelium and stroma). Phenotypes were then examined in relationship to disease-free survival (DFS) using Kaplan-Meier methodology. **Results:** Of the 3 immune phenotypes in dMMR tumors, 25% were inflamed, 50% were immune-excluded, and 25% were immune-desert. Based on univariate results, multivariable modeling was performed incorporating immune phenotype in addition to age, histological grade, N stage, T stage, performance status, treatment arm, *BRAF* and *KRAS* status and identified immune phenotypes and N stage to be significantly associated with DFS. Among dMMR tumors, immune desert phenotype was associated with the poorest DFS (Desert: HR<sub>adj</sub> 1.95, 95%CI (1.01, 3.80); Excluded: HR<sub>adj</sub> 0.89, 95% CI (0.47, 1.69); Inflamed: ref; *p*:0.01). When compared to immune desert tumors, immune-excluded tumors had significantly better DFS (HR<sub>adj</sub> 0.49, 95%CI (0.27, 0.88), *p*:0.01). At a median follow-up of 60 months, 3-year DFS of patients with dMMR was 71.2% with Immune inflamed, 78.5% with Excluded, and 54.5% with Desert phenotypes. In contrast to dMMR, univariate analyses of data-driven cutpoints in pMMR tumors was not prognostic. Using a different data-driven cutpoint (15%), the revised immune phenotypes remained non-significant for DFS within pMMR tumors. **Conclusions:** Distinct AI-derived immune phenotypes in the TME were identified that were significantly prognostic in patients with dMMR, but not pMMR colon cancers. A data-driven immune-desert phenotype was identified in dMMR tumors that was associated with significantly poorer survival. Further investigation of the potential predictive utility of these phenotypes for immunotherapy are planned. Support: <https://acknowledgments.alliancefound.org>. ClinicalTrials.gov Identifier: NCT00079274. Research Sponsor: U.S. National Institutes of Health; Sanofi; U10CA180821, U10CA180882, U24CA196171, R01 CA210509 (to FAS).

### A deep learning model for the prediction of microsatellite instability and pathogenic POLE mutations in colorectal cancer using histopathologic images.

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**Background:** Immunotherapy has brought about a landmark change in anti-tumor treatment in the past years. High microsatellite instability (MSI-H) is now the only clinically approved biomarker predicting response to immunotherapy in CRC. Increasing evidence suggests that POLE mutations in the exonuclease domain could drive an ultra-mutational phenotype and improve the treatment outcomes of ICI in solid tumors. In this study, we set out to apply a deep learning model using H&E-stained, formalin-fixed, paraffin-embedded (FFPE) whole slide images (WSIs) of CRC primary tumors. **Methods:** The deep learning model is developed and validated through five-fold cross-validation using WSI of primary tumors from 506 CRC patients and externally validated using 52 WSIs from a prospective cohort. The microsatellite status, tumor mutation burden (TMB) and POLE genotype were determined by next-generation sequencing (NGS). Patients with MSS status and a low TMB (< 20Mutations/Mb) were admitted to the MSS group, and CRCs with a POLE mutation which was defined as an oncogenic mutation referring to the POLE functional mutation list at OncoKB(POLE (oncokb.org) were admitted to the POLE mutant group. Clustering-constrained-attention multiple-instance learning (CLAM) model is employed as the base model, and we conduct the model ensemble by performing a large-scale hyper-parameter search, selecting five models with the highest value in one of the performance metrics, including the AUROC, accuracy, precision, recall, and f1 score, and finally averaging the predictions of the five models. **Results:** The internal dataset included 237 MSS, 142 MSI-H, and 127 POLE mutant CRC. The three groups had significant differences in primary location ( $p < 0.0001$ ), histology ( $p < 0.0001$ ), tumor differentiation ( $p = 0.002$ ), tumor stage ( $p < 0.0001$ ), Crohn's-like reaction ( $p < 0.0001$ ) and tumor growth pattern ( $p = 0.001$ ). The cross-validation performance of the ensemble model ( $M_E$ ) in the internal dataset achieves an AUROC of 0.944 for three-way classification task (POLE vs. MSI-H vs. MSS) and 0.940 for two-way classification task (POLE & MSI-H vs. MSS) which were superior to the performance of each single CLAM model. To demonstrate the generalizability of the deep learning model, a domestic perspective cohort consisting of 20 MSS, 17 MSI-H, and 15 POLE mutant CRC H&E images were used to validate the external performance. And the  $M_E$  retained robust performance on the external dataset, with an AUROC of 0.904 for three-way classification task and 0.836 for two-way classification task. **Conclusions:** A CLAM-based deep learning model could directly predict the MSI-H and POLE mutation from histological images that could be used to stratify CRC patients for immunotherapy with faster turnaround time and lower costs compared with traditional sequencing methods. Research Sponsor: None.

**Prognostic role of TP53 variants in the phase III study of FOLFIRI/cetuximab versus FOLFIRI/cetuximab followed by cetuximab (Cet) alone in first-line therapy of patients with RAS and BRAF wild-type (wt) metastatic colorectal cancer (mCRC) (ERMES study).**

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**Background:** The ERMES study explored the optimal intensity of anti-EGFR-based first line therapy for RAS/BRAF wt mCRC once achieved disease control. Although the study did not demonstrate non-inferiority of maintenance with Cet alone versus standard treatment, preliminary results suggested that a strategy of de-escalation treatment with only Cet might be effective in selected patients. Conflicting results have been previously reported for the predictive and prognostic role of TP53 variants in mCRC patients receiving anti-EGFR monoclonal antibody therapy. Here we describe the role of TP53 mutations within the ERMES study. **Methods:** Patients with untreated RAS/BRAF wt mCRC were randomly assigned (1:1) to receive either FOLFIRI/Cet until PD/toxicity (arm A) or FOLFIRI/Cet for 8 cycles followed by Cet alone (arm B). Tumor tissue samples were tested using the OncoPrint Solid Tumor DNA kit covering hot spot mutations in 22 genes, including KRAS, NRAS, BRAF and TP53. Variant calling was performed using the Variant Caller Ion Torrent suite 5.16 software. The prognostic value of TP53 variants was assessed in the intention-to-treat (ITT) patients' population with available sequencing data. **Results:** Tumor tissue specimens were available for 418/593 (70.5%) patients of the ITT population. Testing failed in 29 cases, whereas KRAS/NRAS/BRAF V600E variants were found in 36/389 (9.2%) patients, who were enrolled on the basis of local testing. Progression free survival (PFS) data were not available for 4 cases. Therefore, the final population in analysis was 349 cases. TP53 variants were detected in 165/349 (47.3%) patients, of which 86/165 (52.1%) in arm A and 79/165 (47.9%) in arm B. The median PFS (mPFS) of TP53 wild type patients in the overall population was 9.4 months versus 10.4 months in the TP53 mutant subgroup (HR 0.8439; 95%CI, 0.673-1.058;  $p = 0.142$ ). In arm A, the mPFS of TP53 wild type patients was 10.8 months versus 10.7 months in TP53 mutant patients, with an HR 1.053 (95%CI, 0.7582-1.462;  $p = 0.76$ ). The mPFS of TP53 wild type patients in arm B was 8.3 months versus 10.2 months in the TP53 mutant cohort, with an HR 0.7 (95% CI, 0.5087-0.9657;  $p = 0.0298$ ). No significant difference was observed for the overall response rate (ORR) in both arms between TP53 mutant and wild type patients. **Conclusions:** These preliminary data suggest a possible prognostic role of TP53 variants in RAS/BRAF wild type mCRC patients receiving first line FOLFIRI/cetuximab followed by Cet alone. Comprehensive genomic profiling is ongoing to identify genomic signatures associated with sensitivity to Cet. Research Sponsor: This study was financially supported by Merck Serono S.p.A., Rome, Italy, an affiliate of Merck KGaA, Darmstadt, Germany (CrossRef Funder ID: 10.13039/100009945).

### Plasma arginine as a candidate predictive biomarker for response to immune checkpoint inhibition (ICI) in metastatic colorectal cancer (mCRC): Analysis of the CCTG CO.26 trial.

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**Background:** Nutritional stress is one of the mechanisms used by tumour cells to evade the immune system. Arginine (ARG), an amino acid involved in several cellular functions including immunomodulation, is important in regulating T-lymphocyte cell activity and the anti-tumour response. ARG deficiency in the tumour microenvironment has been shown to impair T-cell response while ARG supplementation may promote anti-tumour immune activity. In this exploratory post-hoc analysis of the Phase II CO.26 trial (NCT02870920), we investigated the role of plasma ARG in predicting response to ICI in patients (pts) with refractory mCRC. **Methods:** CO.26 was a phase II trial which randomized pts with refractory mCRC to durvalumab plus tremelimumab (D+T) versus best supportive care (BSC). Plasma ARG concentrations were determined from blood samples pre-treatment using HPLC-tandem mass spectrometry. The median plasma ARG value was used as a cut-off stratifying pts into ARG-high ( $\geq 10650$  ng/ml) versus ARG-low ( $< 10650$  ng/ml) groups. Progression-free (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method and compared between groups using the log-rank test. Cox proportional hazard models were used to analyze prognostic and predictive impacts of ARG on PFS and OS. **Results:** Of 180 pts enrolled in CO.26, 162 pts (N = 115 treated with D+T and 47 BSC) had pre-treatment blood samples for baseline ARG analysis. There were no significant differences in baseline characteristics between pts included in this analysis and the total study pts, or between ARG-high and ARG-low pts. In pts treated with D+T, ARG-high was associated with more favourable prognosis (ARG-high median OS 7.62 months vs. ARG-low 5.49 months, multivariable hazard ratio [HR] 0.60, 95% confidence interval [CI] 0.40-0.91,  $p = 0.016$ ). In ARG-high pts, D+T significantly improved OS (median OS 7.62 months with D+T vs 3.61 months BSC; HR 0.61, 95% CI 0.37-0.99,  $p = 0.04$ ). In ARG-low pts there was no OS benefit with D+T (median OS 5.49 months D+T vs 4.27 months BSC; HR 0.84, 95% CI 0.50-1.41,  $p = 0.51$ . Interaction  $p = 0.037$ ). Baseline ARG values had no association with PFS or disease control rate. **Conclusions:** Baseline plasma ARG was prognostic in pts with mCRC treated with D+T, and high ARG was predictive of improved OS with ICI. Prospective studies should be done to validate ARG as a biomarker identifying mCRC pts likely to derive benefit from ICI. Therapeutic approaches targeting the ARG pathway should be investigated in future studies. Research Sponsor: The Canadian Cancer Trials Group (CCTG) is funded by the Canadian Cancer Society.; AstraZeneca provided durvalumab and tremelimumab and contributed partial funding for the CO.26 trial.

### A phase 1 dose escalation study of GCC19CART: A novel coupled CAR therapy for patients with metastatic colorectal cancer.

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**Background:** GCC19CART, the first clinical candidate from the CoupledCAR solid tumor platform, is designed to overcome the limitations of conventional CAR T-cells in solid tumor malignancies by pairing solid tumor CAR T-cells with CD19 targeting CAR T-cells to amplify the proliferation and activation of the solid tumor CAR T component. GCC19CART targets guanylate cyclase-C (GCC), which is expressed in the metastatic lesions of 70%-80% of subjects with colorectal cancers and largely restricted to the intestinal tract. A Phase 1 investigator-initiated clinical trial is underway in China for patients with relapsed or refractory metastatic colorectal cancer who have received at least 2 prior lines of therapy. As of a data cutoff on Jan 28, 2023 21 subjects have been enrolled in 2 dose escalation groups at 5 hospitals in China. **Methods:** Subjects are screened for GCC expression by immunohistochemistry. Eligible subjects undergo leukapheresis, a single dose of lymphodepleting chemotherapy (fludarabine 30mg/m<sup>2</sup> and cyclophosphamide 300mg/m<sup>2</sup>) 3 days prior to infusion, and then administration of a single infusion of GCC19CART at one of two preassigned doses: 1x10<sup>6</sup> or 2x10<sup>6</sup> CAR T-cells/kg. All responses were confirmed by an independent third-party imaging contract research organization. **Results:** 13 subjects have been enrolled to dose level 1 (1x10<sup>6</sup> cells/kg) and 8 subjects have been enrolled to dose level 2 (2x10<sup>6</sup> cells/kg). The most common adverse events were cytokine release syndrome (CRS) in 21/21 subjects (Grade 1 19/21 (90.48%) or Grade 2 2/21 (9.52%)) and diarrhea in 21/21 subjects (Grade 1 6/21 (28.57%) Grade 2 5/21 (23.81%) Grade 3 9/21 (42.86%) or Grade 4 1/21 (4.76%)). All patients with grade 3 and higher side effects were well managed. Immune effector cell-associated neurotoxicity syndrome (ICANS) was observed in 2/21 (9.52%) subjects at Grade 3 or 4 and resolved with corticosteroids. The combined overall response rate (ORR) for both dose levels was 28.6% (6/21). For dose level 1, overall response rate (ORR) was 15.4% (2/13). Two subjects demonstrated a partial response (PR) while 3 additional subjects had partial metabolic response (PMR) on PET/CT with stable disease (SD) or progressive disease (PD) per RECIST 1.1. For dose level 2, The ORR was 50% (4/8). 4 subjects demonstrated a PR and 2 additional subjects had PMR on PET/CT with SD per RECIST 1.1. The median PFS was 1.9 months in the dose 1 group and 6.3 months in the dose 2 group. The median overall survival was 13.3 months in the dose 1 group and 18.3 months in the dose 2 group. **Conclusions:** Preliminary results demonstrate that GCC19CART has meaningful dose-dependent clinical activity and an acceptable safety profile in relapsed or refractory metastatic colorectal cancer. This trial is ongoing and updated data will be presented. A Phase 1 trial of GCC19CART in the US has opened for accrual and is expected to enroll patients in mid-2022. Clinical trial information: ChiCTR2000040645. Research Sponsor: Innovative Cellular Therapeutics.

### A prospective study of FOLFIRI plus aflibercept as second-line treatment after failure of FOLFOXIRI plus bevacizumab in patients with unresectable/metastatic colorectal cancer (CRC): EFFORT study.

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**Background:** FOLFOXIRI plus bevacizumab (BEV) is a first-line treatment option for patients with unresectable or metastatic CRC. However, there are no clear recommendations for second-line therapy after failure of FOLFOXIRI plus BEV. The EFFORT study investigated whether FOLFIRI plus aflibercept is active following FOLFOXIRI plus BEV in unresectable/metastatic CRC. **Methods:** EFFORT was an open-label, multicenter, single arm phase II study. Patients with unresectable/metastatic CRC who failed FOLFOXIRI plus BEV as a first-line therapy received aflibercept plus FOLFIRI (aflibercept 4 mg/kg, irinotecan 150 mg/m<sup>2</sup> IV over 90 min, with leovorinate 200 mg/m<sup>2</sup> IV over 2 hours, followed by fluorouracil 400 mg/m<sup>2</sup> bolus and fluorouracil 2400 mg/m<sup>2</sup> continuous infusion over 46 hours) every 2 weeks on day 1 of each cycle. The primary endpoint was progression-free survival (PFS) in the full analysis set (FAS). To achieve 80% power to show a significant benefit with a one-sided alpha level of 0.10, assuming a threshold PFS of 3 months and an expected value of at least 5.4 months, 32 patients needed to be enrolled. Major secondary endpoints included overall survival (OS), overall response rate (ORR) and safety. **Results:** From April 2019 to May 2021, 35 patients were enrolled and FAS included 34 patients (one patient who did not met eligibility criteria was excluded). Of them, 18 were males, median age was 63 years (range: 32-78) and ECOG Performance Status was either 0 (n = 28) or 1 (n = 6). The primary tumor was left-sided in most patients (23/34) and 27 patients had liver metastases. And 23 patients had RAS mutation. The primary endpoint was met with a median PFS of 4.3 months [80% CI: 3.7-5.1]. The median OS was 15.2 months [95% CI: 8.9-22.7]. Objective tumor responses were CR (n = 1), PR (n = 4), SD (n = 21) or PD (n = 8). ORR was 14.7% (5/34) [95% CI: 5.0-31.1], and disease control rate was 76.5% (26/34) [95% CI: 58.8-89.3]. Main grade 3 or 4 adverse events were neutropenia (7/35, 20.0%), thrombopenia (3/35, 8.6%), leucopenia (2/35, 5.7%), and hypertension (3/35, 8.6%). No severe proteinuria and no treatment-related death were reported. **Conclusions:** Aflibercept plus FOLFIRI given after failure of FOLFOXIRI plus BEV is active and shows a manageable safety profile. This regimen maybe a useful as second-line treatment option in such patients. Clinical trial information: jRCTs071190003. Research Sponsor: Sanofi Pharmaceutical Co., Ltd.

# First-line (1L) nivolumab (NIVO) + ipilimumab (IPI) in patients (pts) with microsatellite instability-high/mismatch repair deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC): 64-month (mo) follow-up from CheckMate 142.

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**Background:** NIVO + IPI demonstrated robust, durable clinical benefit, and was well tolerated as a 1L therapy in pts with MSI-H/dMMR mCRC in the phase 2 CheckMate 142 study (NCT02060188), leading to the inclusion of NIVO + IPI in the NCCN guidelines as an initial therapy option for these pts. At 52-mo median follow-up, 1L NIVO + IPI continued to demonstrate durable clinical benefit, and no new safety signals were identified. Here we report longer follow-up results. **Methods:** Pts with MSI-H/dMMR mCRC and no prior treatment for metastatic disease received NIVO 3 mg/kg Q2W + IPI 1 mg/kg Q6W until disease progression or unacceptable toxicity. The primary endpoint was objective response rate (ORR) by investigator assessment (INV) per RECIST v1.1. Other key endpoints were disease control rate (DCR), duration of response (DOR), progression-free survival (PFS), all by INV; overall survival (OS); and safety. **Results:** In total, 45 pts received 1L NIVO + IPI. With median follow-up of 64.2 mo (range, 59.4–68.9 mo), ORR by INV was 71% (95% CI, 56–84%). The proportion of pts with a best overall response of complete response (CR) was 20%, partial response (PR) was 51%, stable disease (SD) was 13%, and progressive disease was 16%. Median DOR (mDOR) was not reached, and the 60-mo DOR rate was 72%. Median PFS (mPFS) by INV and median OS (mOS) were not reached, with 60-mo PFS and OS rates of 55% and 67%, respectively (Table). Among pts alive at the data cutoff (n = 31), 30 remained treatment-free after initial study treatment without receiving any subsequent systemic therapy, with a median treatment-free interval of 34.7 mo (range, 1.6–61.4 mo). Exploratory analysis by tumor mutational burden status will be presented. Safety data are shown in the Table. **Conclusions:** At 64-mo follow-up, NIVO + IPI continued to demonstrate clinically meaningful survival and durable responses, with mPFS, mOS, and mDOR still not reached, suggesting the potential for long-term clinical benefit. Safety remained consistent with previous data. These findings further support current recommendations for NIVO + IPI as a 1L treatment for pts with MSI-H/dMMR mCRC. Clinical trial information: NCT02060188. Research Sponsor: Bristol Myers Squibb.

| Efficacy                                   | 1L NIVO + IPI<br>(N = 45) |
|--------------------------------------------|---------------------------|
| ORR <sup>a,b</sup> (95% CI), %             | 71 (56–84)                |
| DCR <sup>a,c</sup> (95% CI), %             | 84 (71–94)                |
| mPFS <sup>a</sup> (95% CI), mo             | NR (28.8–NE)              |
| 60-mo PFS rate (95% CI), %                 | 55 (38–69)                |
| mOS (95% CI), mo                           | NR (NE)                   |
| 60-mo OS rate (95% CI), %                  | 67 (51–79)                |
| Safety, n (%)                              |                           |
| Any-grade/grade 3 or 4 TRAEs               | 36 (80)/9 (20)            |
| Any-grade TRAEs leading to discontinuation | 7 (16)                    |

<sup>a</sup>Per INV; <sup>b</sup>Pts with CR or PR divided by the number of treated pts; <sup>c</sup>Pts with CR, PR, or SD (for ≥ 12 weeks) divided by the number of treated pts. NE, not estimable; NR, not reached; TRAE, treatment-related adverse event.

# Impact of number and size of colorectal metastases (CRM) on survival in patients with RAS wild-type metastatic colorectal cancer treated within the PanaMa trial (AIO KRK 0212).

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**Background:** In this exploratory analysis, we aim to evaluate surrogates of metastatic burden, namely number of CRMs and size of largest CRM, for their prognostic and predictive value on efficacy of maintenance therapy with 5-fluorouracil/leucovorin (FU/FA) plus panitumumab (pmab) or FU/FA alone in RAS wildtype mCRC patients treated within the PanaMa trial. **Methods:** Number of CRMs and size of largest CRM at baseline were determined taking into account target and non-target lesions measured by central radiological review. Median number of metastases was set as threshold ( $n \leq / > 10$ ) to assess the prognostic and predictive value of CRM count on PFS and OS of maintenance therapy. A threshold of  $\leq / > 50\text{mm}$  was set to evaluate the impact of the largest CRM on the above time-to-event endpoints. PFS and OS were expressed by Kaplan-Meier method and compared by log-rank testing. Hazard ratios (HR) with 95% CIs were estimated using Cox regression models. **Results:** Out of 248 patients receiving maintenance therapy, CT and MRI scans of 211 patients were centrally evaluable (FU/FA+ Pmab,  $n = 106$ ; FU/FA alone,  $n = 105$ ). At baseline, 50.1% of the patients were diagnosed with  $> 10$  CRM. Median size of the largest CRM was 45 mm. Number of CRMs  $n \leq 10$  was associated with favorable PFS, while both, number  $n \leq 10$ , and size of largest CRM  $\leq 50\text{mm}$  correlated with favorable OS compared to  $n > 10$  and size of largest CRM  $> 50\text{mm}$ , respectively. In patients with  $> 10$  CRMs, and those with largest CRM sized  $> 50\text{mm}$ , PFS of maintenance therapy was significantly superior with FU/FA+ Pmab compared to FU/FA alone (Table). **Conclusions:** Metastatic burden was found to be prognostic for PFS and OS of maintenance therapy. Additionally, number and largest size of CRMs proved predictive of PFS of maintenance therapy with Pmab, the primary endpoint of the trial. Patients with high metastatic burden at baseline appear to benefit particularly from Pmab-containing maintenance therapy. Clinical trial information: NCT01991873. Research Sponsor: Amgen Inc.

Metastatic burden according to number and size of colorectal metastases (CRM) and treatment arms.

|                             | $\leq 10$ CRMs               |            | Largest CRM $\leq 50\text{mm}$ |            |
|-----------------------------|------------------------------|------------|--------------------------------|------------|
|                             | A (n = 52)                   | B (n = 52) | A (n = 64)                     | B (n = 60) |
| Median PFS, months          | 10.3                         | 6.0        | 8.4                            | 6.5        |
| HR (95% CI),<br>P(log-rank) | 0.74 (0.49–1.13)<br>P= 0.164 |            | 0.90 (0.61–1.33)<br>P= 0.586   |            |
|                             | $> 10$ CRMs                  |            | Largest CRM $> 50\text{mm}$    |            |
|                             | A (n = 54)                   | B (n = 53) | A (n = 42)                     | B (n = 45) |
| Median PFS, months          | 8.1                          | 5.6        | 9.2                            | 5.0        |
| HR (95% CI),<br>P(log-rank) | 0.63 (0.42–0.95)<br>P= 0.028 |            | 0.48 (0.31–0.75)<br>P= 0.001   |            |

[A] FU/FA+ Pmab; [B] FU/FA alone.

**Alternating short-course oxaliplatin-based chemotherapy and nivolumab as first-line treatment of patients with abdominal metastases from microsatellite-stable (MSS) colorectal cancer (CRC): A randomized phase 2 trial.**

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**Background:** The CRC incidence increases sharply from the age of 60. Most patients harbor primarily non-immunogenic MSS disease and abdominal metastases are in particular considered unresponsive to immune checkpoint blockade (ICB). The METIMMOX trial explored if MSS-CRC can be transformed into an immunogenic malignancy by short-course oxaliplatin-based therapy and if followed by ICB (without concomitant chemotherapy that might compromise an invoked tumor-defeating immunity) may result in durable clinical response for patients with abdominal metastases. **Methods:** Patients had MSS-CRC with infradiaphragmatic metastases deemed unresectable, ECOG performance status 0-1, and were eligible for first-line oxaliplatin-based therapy. They were randomly assigned to FLOX (oxaliplatin 85 mg/m<sup>2</sup> day 1, bolus 5-fluorouracil 500 mg/m<sup>2</sup> and folinic acid 100 mg days 1-2) Q2W (control arm) or alternating cycles of 2 FLOX Q2W and 2 nivolumab (240 mg) Q2W (experimental (exp) arm) with break periods at prespecified intervals. Response assessment per i/RECIST by blinded central review was done every 8 weeks with progression-free survival (PFS) as primary endpoint. Sample size of 40 in each arm with 1:1 randomization would detect exp arm doubling of median PFS. Associations between PFS and relevant patient and disease variables were estimated by Cox proportional-hazards regression models. Prespecified correlative analyses included circulating tumor DNA (ctDNA) by droplet digital PCR and circulating immune cell composition by high-dimensional single-cell mass cytometry. **Results:** Of 80 enrolled subjects (05/2018-10/2021), 76 intention-to-treat (ITT) cases were equally allocated to the study arms and followed to censoring at 10/2022 with identical median PFS of 9.3 months (95% CI, 6.4-12.9 (control arm) and 4.6-15.2 (exp arm)). The adjusted Cox model revealed interaction between age and study arm, as patients ≥60 years had significantly lowered risk of progression when receiving exp therapy (median PFS 13.6 months (95% CI, 8.5-18.8);  $p = 0.022$ ). No unexpected adverse events were recorded; specifically, no grade ≥4 immune-mediated adverse event occurred. Of note, 17% of exp arm patients had durable complete response, among whom all *BRAF*-mutant cases ( $n = 3$ ) with PFS 21-35 months and rapid clearance of baseline *BRAF*-mutant ctDNA (control arm *BRAF*-mutant cases ( $n = 10$ ), median PFS 3.6 months). Responding exp arm patients showed distinct subsets of circulating immune cells with more homogenous profiles than ICB-unresponsive subjects. **Conclusions:** With equal primary endpoint for the ITT cases, alternating short-course oxaliplatin-based therapy and nivolumab significantly improved PFS compared to standard first-line chemotherapy in MSS-CRC patients ≥60 years with abdominal metastases. Clinical trial information: NCT03388190. Research Sponsor: Norwegian Cancer Society, including its Umbrella Foundation for Cancer Research, Grants 182496 and 215613; Bristol-Myers Squibb.

### Size-related heterogeneity of colorectal liver metastases (CRLM) in patients with advanced *RAS* wild-type metastatic colorectal cancer (mCRC) treated in the PanaMa trial: Implication for treatment decisions.

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**Background:** In patients with mCRC, the liver represents the primary site of metastasis. In patients with primarily unresectable metastatic disease, primary tumor location and molecular subtypes mainly determine the therapeutic approach. In addition, image-based characterization of hepatic metastatic pattern may provide additional information concerning prognosis and treatment efficacy. Therefore, CRLM were characterized according to size-based heterogeneity in the PanaMa trial (maintenance therapy with 5-fluoruracil/leucovorin (FU/FA) plus panitumumab (Pmab) vs. FU/FA alone following induction therapy with six cycles of FU/FA, oxaliplatin and Pmab). **Methods:** Assessments were performed in patients with at least two lesions, considering target and non-target lesions, within a central radiological review of the trial. Variance in size expressed as ratio between the smallest and largest lesion measured ( $\leq$  /  $>$  threefold difference in size) was evaluated for its prognostic and predictive impact on progression-free (PFS) and overall (OS) survival of maintenance therapy. Time-to-event endpoints were expressed by Kaplan-Meier method and compared by log-rank tests. Cox regressions were used to indicate Hazard ratios (HR) with 95% CIs. **Results:** Imaging data of 211/248 (85.1%) patients receiving maintenance therapy were evaluable for central radiological review (FU/FA+ Pmab, n = 106; FU/FA, n = 105). Of those, 165/211 patients (78.2%) had at least two CRLM. Size of CRLM ranged between 5–180mm, with the smallest metastasis in the individual assessments measuring a median of 12mm and the largest a median of 53mm. Large heterogeneity with more than a threefold difference in size between the smallest and largest CRLM was observed in 49.7% of patients, particularly pronounced in patients with polymetastatic disease ( $>$  5 lesions). Homogeneous metastasis in terms of lesion size ( $\leq$  threefold difference) was associated with favorable OS (HR 0.63; 95% CI, 0.42–0.95; P = 0.027) - irrespective of study arm- compared to heterogeneous lesion disease. Maintenance therapy with FU/FA+ Pmab compared with FU/FA alone seemed favourably active in both patients with heterogeneous metastases (HR 0.53; 95% CI; 0.34–0.85; P = 0.008) as well as to a numerically lesser extent in patients with homogeneous metastases (HR 0.68; 95% CI, 0.43–1.10; P= 0.116). **Conclusions:** Imaging-based characterization of CRLM focusing on size-related heterogeneity was associated with favorable prognosis potentially indicating more aggressive underlying tumor dynamics in case of heterogeneous lesion size. In addition, the efficacy of Pmab during maintenance was evident in both patient groups (homogeneous and heterogeneous disease lesions) with a numerically greater effect in patients with heterogeneous lesions. Clinical trial information: NCT01991873. Research Sponsor: Amgen Inc.

## Prognostic value of liver metastases in colorectal cancer treated by systemic therapy: An ARCAD pooled analysis.

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**Background:** Approximately 30% of patients (pts) diagnosed with colorectal cancer (CRC) develop liver metastases (LM). Liver is the most common organ of metastasis of CRC. The ARCAD database contains individual patient data of randomized trials that included CRC pts with initially unresectable metastases treated with systemic therapy. The aim of this study was to assess the response and survival outcomes in non-LM (NLM) vs LM across different lines of treatment. **Methods:** We analyzed survival outcomes of mCRC pts with either single site (SS) or multiple sites (MS) according to LM status in the following treatment groups: A: chemotherapy (CT) alone, B: CT + VEGF-antibodies, C: CT + EGFR-antibodies in KRAS wild-type tumors, within first-line (1L) and second line (2L) of therapy and D: pts enrolled on third line ( $\geq 3L$ ) trials treated with trifluridine/tipiracil or regorafenib and placebo. The primary and secondary endpoints were overall survival (OS) and progression-free survival (PFS) which were assessed using Kaplan-Meier estimates and adjusted Cox models on ECOG PS, age, and gender. **Results:** We included 26 trials with 17924 pts. 14066 pts had LM. Pts with LM had a higher rate of colon vs rectum as primary tumor (72 vs 62%;  $P < .001$ ) and less SS (31 vs 47%;  $P < .001$ ) than those with NLM. OS and PFS results in subgroups are reported in the table. In groups A and B, we found better OS and PFS outcomes in NLM pts as either SS or MS in 1L and 2L. In group C from 1L, we found better survival outcomes in pts with SS LM. In pts with MS, NLM superiority was observed in OS but not in PFS. However, these results were influenced by primary tumor sidedness. In group D, better OS and PFS was observed in pts without LM than those with LM whether in pts with SS or MS. Response rates were higher in LM than in NLM in most 1L and 2L subgroups. **Conclusions:** LM is a poor prognostic factor for mCRC increasing from the 1L to  $\geq 3L$ . Survival with CT alone and CT + anti-VEGF according to LM and NLM differs significantly in 1L and 2L but not with CT + anti-EGFR. This data justifies using LM as a stratification factor at least in  $\geq 3L$  trials. Research Sponsor: ARCAD Foundation.

| NLM vs LM<br>(Reference) | 1L            |               | 2L            |               | $\geq 3L$     |               |
|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                          | OS            | PFS           | OS            | PFS           | OS            | PFS           |
| <b>Group A</b> All pts   | .76 (.68-.84) | .84 (.77-.92) | .64 (.58-.71) | .72 (.66-.79) | .36 (.29-.44) | .52 (.44-.60) |
| SS                       | .79 (.67-.94) | .93 (.80-1.1) | .58 (.49-.69) | .67 (.57-.78) | .33 (.21-.51) | .43 (.31-.59) |
| MS                       | .76 (.67-.86) | .81 (.72-.91) | .71 (.63-.81) | .77 (.69-.87) | .39 (.30-.51) | .58 (.48-.71) |
| <b>Group B</b> All pts   | .81 (.74-.89) | .91 (.84-.98) | .62 (.55-.71) | .75 (.67-.84) | .40 (.32-.52) | .60 (.49-.73) |
| SS                       | .88 (.77-1.0) | .99 (.89-1.1) | .55 (.44-.68) | .76 (.63-.91) | .14 (.07-.28) | .43 (.25-.73) |
| MS                       | .84 (.73-.96) | .88 (.79-.99) | .81 (.68-.96) | .79 (.68-.91) | .51 (.39-.68) | .62 (.50-.79) |
| <b>Group C</b> All pts   | .97 (.86-1.1) | 1.0 (.91-1.2) | 1.1 (.84-1.5) | 1.2 (.93-1.6) |               |               |
| SS                       | 1.2 (1.0-1.5) | 1.1 (.95-1.3) | 1.1 (.44-2.8) | 1.9 (.88-3.9) |               |               |
| MS                       | .85 (.72-1.0) | 1.1 (.90-1.3) | 1.1 (.80-1.5) | 1.1 (.82-1.5) |               |               |

**A phase Ib/II study to evaluate surufatinib combined with camrelizumab and chemotherapy in the second-line treatment of advanced colorectal cancer: Phase Ib results.**

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**Background:** Surufatinib is a novel small-molecule kinase inhibitor targeting VEGFR1-3, FGFR and CSF-1R with confirmed efficacy in epNET and pNET. Preclinical studies have demonstrated that surufatinib combined with immune checkpoint inhibitors (ICIs) have synergistic antitumor effects by modulating tumor immune microenvironment. Additionally, surufatinib plus toripalimab (anti-PD-1) has showed preliminary anti-tumor activity in multiple solid tumors, including colorectal cancer (CRC). This study aimed to evaluate the efficacy and safety of surufatinib plus camrelizumab (anti-PD-1), irinotecan and GM-CSF as second-line treatment in advanced CRC. Here we reported the results of the phase Ib study. **Methods:** This ongoing phase Ib/II, open-label trial (NCT04929652) enrolled patients (pts) aged 18-75 years old with pathologically confirmed locally advanced or metastatic CRC who have progressed on/been intolerant to the standard first-line therapy. In the Ib dose escalation/de-escalation phase, pts received surufatinib at 250mg once daily (QD) as starting dose, in combination with fixed dose of camrelizumab (200 mg, d1), irinotecan (200 mg/m<sup>2</sup>, d1) and GM-CSF (5ug/kg, d2-d7), every 3 weeks (Q3W). Based on DLTs, the dose of surufatinib should escalate to 300 mg or de-escalate to 200 mg. The primary objective of the phase Ib study was to determine the safety and DLT in first treatment cycle defining the recommended phase 2 dose (RP2D). Additional 36 pts were enrolled in the phase II dose expansion stage using RP2D. Primary endpoint of phase II was ORR as per RECIST 1.1. Secondary endpoints included PFS, DCR, OS and safety. **Results:** Enrollment opened in Nov 2021 and data cutoff in Dec 2022. 12 pts (median age 54) were treated in Phase Ib study (surufatinib 250 mg: N = 6; surufatinib 300 mg: N = 6). 75% were male and 17% had ECOG PS 1. 11/12 (92%) pts were positive for KRAS, NRAS or BRAF mutations. No DLT was observed in the starting dose cohort (surufatinib 250 mg), and one patient in the surufatinib 300 mg cohort experienced DLT (Grade [G] 3 vomiting). Surufatinib 300 mg QD was defined as the RP2D. Other G3 treatment related adverse events (TRAEs) were hypertension (1/12; 8.3%) and diarrhea (1/12; 8.3%). All other TRAEs were grade 1-2, and the most common ( $\geq 40\%$ ) were diarrhea (75%), fatigue (58%), vomiting (58%), nausea (50%), hypertension (42%), proteinuria (42%) and alopecia (42%). Of the 12 pts evaluable for tumor response, 3 pts achieved PR, 9 pts achieved SD. The ORR was 25% (3/12), the DCR was 100% (12/12) and median PFS was 7.2 months (95%CI 3.7-10.7). **Conclusions:** Surufatinib plus camrelizumab, irinotecan and GM-CSF was well tolerated, with a manageable safety profile, and showed preliminary anti-tumor activity in patients with advanced CRC. The dose-expansion phase is ongoing. Clinical trial information: NCT04929652. Research Sponsor: None.

### Trifluridine/tipiracil (FTD/TPI) in extensively pre-treated metastatic colorectal cancer (mCRC) patients: Evaluation of prognostic subgroups of the TALLISUR study.

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**Background:** Compared to placebo, FTD/TPI significantly improved overall survival (OS) in patients (pts) with pre-treated mCRC in the pivotal phase III RECURSE trial. Subgroup analyses indicated that all subgroups benefitted from FTD/TPI. Of note, FTD/TPI prolonged survival even more in pts  $\geq 65$  years old. To evaluate these observations with data from daily clinical practice, we performed a subgroup analysis of the TALLISUR study. **Methods:** In this prospective, multi-center, German, open-label, phase IV study, pts with extensively pre-treated mCRC chose between best supportive care (BSC) or oral FTD/TPI (35 mg/m<sup>2</sup> bid on days 1 – 5 and 8 – 12 of each 28-day cycle). Duration of treatment and OS were analyzed for various subgroups. Based on a post-hoc analysis of the RECURSE trial, 3 subgroups were defined according to: best, good and poor prognostic characteristics (BPC, GPC, PPC). Pts with  $< 3$  metastatic sites at inclusion and  $\geq 18$  months from diagnosis to inclusion were considered to have GPC. GPC pts without liver metastasis at inclusion were considered to have BPC. All remaining pts were considered to have PPC. **Results:** Of 195 eligible pts, 186 pts chose treatment with FTD/TPI, while 9 pts decided to receive BSC only. Median OS was 6.9 (95% CI 6.1 – 8.3) months. Mean duration of treatment with FTD/TPI was 14.6 (range 0.1 – 102.7) weeks. Results of the subgroup analysis are summarized in the table. Pts  $\geq 65$  years old presented a longer median OS (7.2 vs 6.2 months). **Conclusions:** When given the choice between treatment and BSC in late-stage CRC, the vast majority of pts opted for treatment. Low metastatic burden and indolent disease were factors of good prognosis for FTD/TPI therapy regarding OS. Independent of baseline characteristics such as age, sidedness, and time of onset of metastases, virtually all pts benefitted from therapy with FTD/TPI. Albeit not statistically significant, elderly pts tend to have improved survival with FTD/TPI, consistent with results from RECURSE. Clinical trial registration number: EudraCT No 2017-000292-83. Research Sponsor: Servier.

| Subgroup analysis – survival and duration of treatment with FTD/TPI. |                             |                                            |
|----------------------------------------------------------------------|-----------------------------|--------------------------------------------|
| Subgroup                                                             | Median OS (95% CI) [months] | Mean duration of treatment (range) [weeks] |
| BPC (n = 20)                                                         | 12.2 (6.0 – 18.2)           | 16.3 (0.4 – 83.7)                          |
| GPC (n = 65)                                                         | 7.9 (6.2 – 13.3)            | 15.1 (0.4 – 102.7)                         |
| PPC (n = 121)                                                        | 6.8 (5.4 – 8.1)             | 14.3 (0.1 – 102.7)                         |
| Age < 65 years (n = 74)                                              | 6.2 (4.8 – 8.6)             | 13.7 (0.1 – 102.7)                         |
| Age $\geq 65$ years (n = 112)                                        | 7.2 (6.3 – 11.1)            | 15.2 (0.7 – 102.7)                         |
| Synchronous metastases (n = 102)                                     | 6.9 (6.2 – 10.1)            | 12.4 (0.1 – 72.7)                          |
| Metachronous metastases (n = 63)                                     | 7.3 (5.3 – 12.4)            | 16.8 (1.3 – 102.7)                         |
| Left sided tumor (n = 138)                                           | 6.8 (6.0 – 8.3)             | 13.9 (0.1 – 102.7)                         |
| Right sided tumor (n = 42)                                           | 8.1 (5.9 – 12.8)            | 16.9 (0.4 – 72.7)                          |

**Preliminary results from ERAS-007 plus encorafenib and cetuximab (EC) in patients (pts) with metastatic BRAF V600E mutated colorectal cancer (CRC) in HERKULES-3 study: A phase 1b/2 study of agents targeting the mitogen-activated protein kinase (MAPK) pathway in pts with advanced gastrointestinal malignancies (GI cancers).**

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**Background:** The RAS/MAPK pathway (including BRAF) is dysregulated in a broad range of cancers including CRC, resulting in downstream activation of ERK1/2. Metastatic CRC with BRAF V600E mutation (BRAF V600E mCRC) has dramatically worse survival than non-BRAF V600E mutated CRCs, and novel therapies are needed. ERAS-007 is a novel, potent, and orally bioavailable inhibitor of ERK. The combination of a BRAF plus EGFR inhibitor (EC) is approved for the treatment of pts with BRAF V600E mCRC; however, only 20% of pts experience an objective response. ERAS-007 alone or in combination with EC showed promising in vitro and in vivo activity in BRAF V600E CRC models to support the combinatorial clinical benefit of ERAS-007+EC in BRAF V600E mCRC. **Methods:** HERKULES-3 is a Phase 1b/2 study to assess the safety, tolerability, PK, and preliminary clinical activity of ERAS-007 combinations targeting the MAPK pathway in pts with advanced GI cancers. Within this study, we are currently evaluating the safety and tolerability of escalating doses of ERAS-007 + EC in pts with BRAF V600E CRC. Prior BRAF inhibitor and EGFR inhibitor treatment is neither required nor excluded to be enrolled in this study. **Results:** As of 30 November 2022, 12 patients were dosed with ERAS-007 twice daily-once a week (BID-QW) (75 and 100 mg; n = 10) or once daily once a week (QW) (150 mg; n = 2) in combination with EC (300 mg oral daily + 500 mg/m<sup>2</sup> intravenous infusion once every 2 weeks). The treatment-emergent AEs (TEAEs) occurring in ≥20% of pts were fatigue (50%), headache (42%), constipation, diarrhea, nausea, dermatitis acneiform (33% each), and vomiting (25%). No TEAEs led to ERAS-007 discontinuation or death. Grade ≥3 TEAEs were reported in 3 pts (25%). Grade ≥3 treatment-related AEs reported in ≥ 2 patients (17%) include hypertension, headache, confusional state, and skin toxicity. Three pts (25%) died due to disease progression. No DLTs were reported. Out of 4 efficacy evaluable EC naïve pts, one confirmed partial response (PR) and one unconfirmed PR were observed. Evaluation of PK is ongoing and preliminary data will be presented. **Conclusions:** ERAS-007+EC in pts with BRAF V600E CRC shows acceptable preliminary safety/tolerability and evidence of clinical activity. The highest dose of ERAS-007 evaluated and cleared by the safety review committee to date is 100 mg BID-QW when combined with EC. Observed PK, toxicity, and preliminary activity support continued evaluation of this combination in pts with BRAF V600E CRC. Clinical trial information: NCT05039177. Research Sponsor: Erasca; Lilly and Pfizer.

**Preliminary results from ERAS-007 plus palbociclib (palbo) in patients (pts) with KRAS/NRAS mutant (m) colorectal cancer (CRC) or KRASm pancreatic ductal adenocarcinoma (PDAC) in HERKULES-3 study: A phase 1b/2 study of agents targeting the mitogen-activated protein kinase (MAPK) pathway in pts with advanced gastrointestinal malignancies (GI cancers).**

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**Background:** The RAS/MAPK pathway is dysregulated in a broad range of cancers including CRC and PDAC, resulting in downstream activation of ERK1/2. ERAS-007 is a novel, orally bioavailable inhibitor of ERK. Palbo is an oral CDK4/6 inhibitor that inhibits cellular proliferation, an essential feature of tumor growth. Both in vitro & in vivo data exploring the combination of ERAS-007 and palbo in a panel of CRC and pancreatic CDX and/or PDX models have shown promising activity to support the potential combinatorial clinical benefit in RASm CRC and PDAC pts. **Methods:** HERKULES-3 is a Phase 1b/2 study to assess safety, tolerability, PK, and preliminary clinical activity of ERAS-007 combinations targeting the MAPK pathway in pts with advanced GI cancers. Within this study, we are currently evaluating the safety, tolerability, and PK of escalating doses of ERAS-007 twice daily-once a week (BID-QW) (75, 100 mg) in combination with palbo once daily (QD) (75, 100, 125 mg) in pts with KRASm/NRASm CRC or KRASm PDAC. **Results:** As of 30 November 2022, 30 pts were dosed with the combination of palbo and ERAS-007. Treatment emergent AEs (TEAEs) occurring in  $\geq 20\%$  pts were diarrhea (40%), nausea (40%), anemia (33%), vision blurred (27%), fatigue (23%), and neutrophil count decreased (20%). ERAS-007 treatment related AEs (TRAEs) were reported in 19 pts (63%), with the most frequently reported as diarrhea (40%), nausea (33%), and vision blurred (27%). Grade (Gr)  $\geq 3$  TEAEs were reported in 12 pts (40%), including 3 related to ERAS-007 (neutrophil count decreased, diarrhea and dermatitis acneiform). Neutrophil count decreased and anemia were the only Gr 3 events reported in  $\geq 2$  pts. No Gr 4 events were reported. Two Gr 5 events unrelated to any drugs (hemorrhage intracranial and malignant pleural effusion) and one Gr 5 event (anemia) related to palbo were reported. Two pts discontinued ERAS-007 due to TEAEs (Gr 5 malignant pleural effusion and Gr 2 neutrophil count decreased). Three pts reported 4 DLTs: 1 pt at 75mg ERAS-007 & 125mg palbo (Gr 3 maculopapular rash & Gr 4 sepsis), 1 pt at 100mg ERAS-007 & 100mg palbo (Gr 3 dermatitis acneiform), and 1 pt at 100mg ERAS-007 & 125mg palbo (Gr 3 thrombocytopenia). Based on preliminary PK, no clinically relevant PK interactions were identified between ERAS-007 and palbo. The evaluation of clinical activity is ongoing. **Conclusions:** ERAS-007 in combination with palbo in pts with KRASm/NRASm CRC or KRASm PDAC shows expected preliminary safety with reversible and manageable AEs. The highest dose evaluated and cleared by the safety review committee to date is ERAS-007 100 mg BID-QW in combination with the approved monotherapy dose of palbo 125 mg QD. Clinical trial information: NCT05039177. Research Sponsor: Erasca (Sponsor); Pfizer (collaborator).

**Novel GUCY2C targeting CAR-T therapy: Efficacy in advanced colorectal cancer.**

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**Background:** Chimeric antigen receptor (CAR) T cells have shown remarkable success in treating patients with hematologic malignancies. However, the majority of CAR-T cell clinical trials in solid tumors have limited success which is partially attributed to the detrimental tumor microenvironment and the lack of ideal target. Recently, GUCY2C emerges as an ideal therapeutic target which is highly expressed in colorectal cancer (CRC). Here, a novel nanobody-based GUCY2C targeting CAR-T cell developed by us is applied to treat advanced colorectal cancer patients who failed third-line therapy.

**Methods:** In this open-label, single-arm, investigator-initiated exploratory trial (ChiCTR2100044831), we primarily explore the safety and the low-dose efficacy of the autologous anti-GUCY2C CAR-T cells. All patients screened by IHC met our criteria for GUCY2C positivity. All enrolled patients had received at least 3 prior lines of therapy and had multiple metastases at baseline. Totally, 13 advanced CRC patients aged 18 to 65 years were infused with the anti-GUCY2C CAR-T cells at a dose range of 0.5-3 x10<sup>6</sup>/kg after standard Cy-Flu lymphodepletion. Adverse events (AE) were graded according to CTCAE 5.0, and objective tumor responses were assessed per RECIST 1.1 criteria. **Results:** Between November 2021 and December 2022, 13 subjects with refractory metastatic CRC (mCRC) were treated with the anti-GUCY2C-CAR-T cells infusions at a dose range of 0.5-3 x10<sup>6</sup>/kg. There were no dose-limiting toxicities, treatment-related death or any form of neurotoxicity occurred in this study. The most common TEAEs were diarrhea in 9/13 (69%) subjects (Grade 1: 2/13 (15.38%) Grade 2: 3/13 (23.07%) Grade 3: 4/13 (30.77%)). 8/13 (62%) mCRC subjects experienced grade 1 or grade 2 mouth ulcer generally appear as a single lesion lasting for a few days. All cytokine release syndromes (CRS) observed were grade 1. Totally 9 pts (69%) experienced grade 1 CRS and all resolved by supportive care without the need of tocilizumab. No grade 4 AEs except for decreased lymphocytes in 12/13 pts (92%), decreased white blood cells in 1/13 pts (7.7%) and decreased platelet in 1/13 pts (7.7%). Among 10 evaluable patients, 6 partial responses, 2 stable disease and 2 progressive disease were achieved. At all dose levels ORR and DCR were 60% and 80% respectively. Notably, all 3 patients infused at the dose level above 2x10<sup>6</sup>/kg reached partial responses. Among the 3 non-evaluable patients, 1 patient lost follow-up, 1 patient died of bone marrow failure and 1 patient died of COVID-19 related complications before the first efficacy evaluation. Additional data will be presented at the meeting. **Conclusions:** This clinical study indicated that the anti-GUCY2C CAR-T cell therapy was well tolerated and had promising efficacy in heavily pretreated mCRC patients. Clinical trial information: ChiCTR2100044831. Research Sponsor: None.

# Matching-adjusted indirect treatment comparisons (MAIC) of sotorasib vs trifluridine/tipiracil (T/T) and regorafenib as monotherapy in chemorefractory *KRAS* G12C-mutated metastatic colorectal cancer (mCRC).

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**Background:** In the single-arm phase 1/2 CodeBreak 100 study (NCT03600883) evaluating sotorasib monotherapy in *KRAS* G12C-mutated mCRC patients whose disease progressed on or after fluoropyrimidine, oxaliplatin, and irinotecan treatment, the overall response rate was 12%, and the median progression-free survival (PFS) and overall survival (OS) was 4.2 and 13.4 months, respectively (data cutoff March 2021, n = 91). To compare the efficacy and safety of sotorasib with standard of care (SOC) T/T and regorafenib as monotherapy treatments, MAICs were performed. **Methods:** Clinical outcomes and adverse events (AEs) were compared from representative studies in mCRC patients who received sotorasib 960 mg once daily in CodeBreak 100, T/T in the phase 3 RECURSE study (NCT01607957, n = 534), and regorafenib in the phase 3 CORRECT study (NCT01103323, n = 505). By weighting individual patient-level data from CodeBreak 100, differences in available baseline characteristics considered to be prognostic factors, including age, race, sex, type of cancer (colon vs. rectum), ECOG performance status, time from diagnosis of metastatic disease, number of metastatic sites, number of prior lines of therapy, and prior bevacizumab treatment, were adjusted with the MAIC. MAICs compared sotorasib with T/T and regorafenib separately. Odds ratios (ORs) were estimated for objective response rates, hazard ratios (HRs) were used for PFS and OS. AEs were compared descriptively. **Results:** The effective sample size of sotorasib with matched characteristics consisted of 29 and 56 patients in the T/T and regorafenib comparison, respectively. The MAIC results showed that treatment with sotorasib increases the likelihood of response to treatment, with an adjusted OR of 8.5 vs T/T and 16.1 vs regorafenib. Sotorasib decreased the risk of progression or death by 41% vs T/T and 45% vs regorafenib (Table). Grade 3+ treatment-emergent AEs occurred in 37%, 69%, and 78% of sotorasib-, T/T-, and regorafenib-treated patients, respectively. **Conclusions:** The analyses suggest that sotorasib monotherapy is associated with a statistically significant and clinically meaningful improvement in response rates, PFS and OS vs. T/T and regorafenib in heavily pretreated chemorefractory *KRAS* G12C-mutated mCRC patients. The analyses are limited by the decreased sample size for sotorasib, the lack of *KRAS* G12C-specific data in SOC studies, and the impact of differences in post-progression treatments on OS across studies. Research Sponsor: Amgen.

|                                  | Sotorasib vs T/T | Sotorasib vs regorafenib |
|----------------------------------|------------------|--------------------------|
| Objective response, OR (95% CI)* | 8.5 (3.2-22.1)   | 16.1 (5.5-46.6)          |
| PFS, HR (95% CI)                 | 0.59 (0.40-0.88) | 0.55 (0.39-0.78)         |
| OS, HR (95% CI)                  | 0.32 (0.18-0.57) | 0.42 (0.28-0.62)         |

\* Per central review for sotorasib, per investigator assessment for comparators CI: confidence interval.

### Determinants of permanent liver limited disease (pLLD) in metastatic colorectal cancer (mCRC).

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**Background:** CRC is a complex and heterogeneous disease, with the liver being the most frequent site of metastasis. Around 20% of patients will always progress exclusively in the liver. These patients may be candidates for more aggressive therapeutic procedures that will impact in their outcome. LIVERMET SURVEY Database (LMSD) is a prospective international database, focused on patients operated for CRC liver metastasis, whether resected or not, which purpose is to evaluate patient outcomes and prognostic factors for these resected patients. We propose to analyse all patients enrolled in the (LMSD) to better characterised those determinants that are associated with pLLD. **Methods:** We analyzed all patients included in the LMSD. Patients with relapse of their disease after the first liver surgery were selected. In order to test associations between hepatic only and extrahepatic metastasis, a univariate and multivariate logistic model was performed with the variables considered clinically more relevant. Imputation of missing data using the mice method (Multivariate Imputation via Chained Equations) was performed. All analyses were performed with R 4.1.1 software. **Results:** A total of 8715 patients out of 33581 (26%) included in the LMSD presented disease recurrence after a first liver surgery. During their oncological history, pLLD occurred in 1392 patients (16%), and extrahepatic relapse was presented in 7323 patients (84%). The characteristics of patients with pLLD were as follows: 58.4% patients presented synchronic disease, 20.2% had right sided primary tumor, 58.6% presented unilateral disease at time of first hepatic surgery and 20.4% of patients presented R1 surgery. In multivariant analysis, right sided and rectum (HR 0.82,  $p = 0.001$ ), unilateral liver involvement (HR 0.74,  $p < 0.001$ ) and  $> 3$ cm of diameter in greatest lesion with maximum of 3 lesions (HR 0.85,  $p = 0.02$ ) were predictive determinants of extrahepatic disease. Only synchronic metastases (HR 1.29,  $p < 0.001$ ) and male sex (HR 1.15,  $p = 0.028$ ) were associated with pLLD. Molecular information according to RAS, BRAF and MSI status was not evaluable in a majority of patients since this item has been recently implemented in the questionnaire. **Conclusions:** This study confirms that about 16% of patients with LLD mCRC will be pLLD mCRC. Despite clinical determinants like synchronic metastatic disease, which is associated with pLLD, further analyses including molecular determinants are needed. Identifying those determinants of pLLD in a scenario where more extreme surgeries and liver transplantations are being considered is a great challenge that needs to be addressed. Research Sponsor: None.

**A phase II study of regorafenib plus sintilimab as salvage-line treatments in non-MSI-H metastatic colorectal cancer (mCRC).**

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**Background:** PD-1 blockade is particularly ineffective in patients with microsatellite stable (MSS) or mismatch repair (MMR)-proficient colorectal cancer (CRC). Regorafenib (R) has been shown to modulate anti-tumor immunity by different mechanisms including reduction of tumor-associated macrophages (TAMs) and the immunosuppressive cells. Synergy between Regorafenib and anti-PD-1/PD-L1 antibodies has been shown in pre-clinical models, as well as in several clinical studies, compared with either treatment alone. The purpose of this study was to evaluate the activity of regorafenib in combination with immune checkpoint inhibitors. **Methods:** This is a single-arm, open-label, phase II trial assessing the efficacy and safety of Regorafenib (80mg QD 3weeks/4) + Sintilimab (S) (200mg every 3 weeks) combination in non MSI-H mCRC patients (pts). The primary endpoint was the overall survival (OS). Secondary endpoints included progression free survival (PFS), objective response rate (ORR), disease control rate (DCR) and Safety. **Results:** As of February 1, 2023, a total of 103 patients with mCRC were enrolled and received R+S. Median age was 57 (range, 28-75). Male 59.2%, ECOG PS 0/1/2 20.4%/66.0%/13.6%, left-sided colon/right-sided colon/rectum 39.8%/15.5%/44.7%, RAS mutant 42.7%. The ORR was 21.4% and DCR was 63.1%, with complete response (cCR) in 1 (1.0%), partial response (PR) in 21 (20.4%) and stable disease (SD) in 43 (41.7%) patients. With a median follow up of 19.9 months (range, 0.1-41.4), the median PFS was 4.0 months (95% CI, 3.1-4.4 months) and median OS was 13.0 months (95% CI, 12.0-16.5 months). The patients who achieved ORR have significantly longer PFS (20.8 vs. 3.2 months,  $p < 0.001$ ) and OS (not reach vs. 12.0 months,  $p < 0.001$ ) than the non-ORR patients. The median PFS were similar regardless the KRAS mutation status, primary sidedness, or liver metastases. Multivariate analysis revealed that male, ECOG PS2, the number of metastatic sites  $\geq 2$ , and RAS mutant were significantly associated with worse OS. The most common grade 3/4 adverse events were hand-foot syndrome (1.9%), stomatitis (1.9%), rash (1.0%), hypertension (1.0%), allergic reaction (1.0%), fatigue (1.0%) and fever (1.0%). 8(7.8%) patients experienced at least 1 dose modification or treatment interruption. No death was related to the treatment. **Conclusions:** In this study the R+S combination as salvage-line treatments in non-MSI-H mCRC achieved a promising clinical benefit, with a manageable safety profile. Female, ECOG PS0/1, single metastases, RAS wild type mCRC were associated with better OS. Clinical trial information: NCT04745130. Research Sponsor: None.

### Safety and efficacy of D-1553 in KRAS G12C-mutated colorectal cancer: Results from a phase I/II study.

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**Background:** KRAS G12C mutation is an oncogenic driver that occurs in 3-4% of colorectal cancer (CRC). D-1553 is a novel oral and potent KRAS<sup>G12C</sup> inhibitor. This phase I/II open-label study (NCT04585035) is an international multicohort study evaluating the safety, tolerability, pharmacokinetics (PK) and efficacy of D-1553 in patients (pts) with KRAS G12C mutated locally advanced or metastatic solid tumors. The Phase I part was conducted to determine the recommended phase 2 dose (RP2D) of D-1553. The Phase II part enrolled multiple expansion cohorts of different cancer types. The endpoints of the study include clinical activity, safety and PK. Here we report preliminary data from pts with locally advanced unresectable or metastatic CRC receiving  $\geq$  RP2D of D-1553 monotherapy.

**Methods:** Pts with locally advanced unresectable or metastatic CRC with progression after standard treatment were enrolled in the Phase I and Phase II parts of the study. Pts were required to have KRAS G12C mutations in tumor or ctDNA samples and no prior KRAS G12C directed therapy. The current analysis includes CRC patients who were treated with D-1553 at RP2D (600 mg BID in Phase I and II) and above (800 mg BID in Phase I) as monotherapy. **Results:** As of 30 December 2022, 24 pts with previously heavily treated locally advanced or metastatic CRC (54.2% male; median age, 61.5 years [range 44, 74]; ECOG PS 0/1: 45.8%/54.2%) were enrolled and received D-1553 600 mg (n = 23) or 800 mg (n = 1) BID monotherapy. 95.8% of pts had stage IV disease. 66.7% had  $\geq$  2 prior lines of therapy (median: 2 [range, 1, 6]). Median treatment duration was 5.75 (range 1.51, 11.83) months (mo) with a median follow-up of 6.64 (range 2.46, 13.11) mo. Confirmed ORR was 20.8% (5/24) (95% CI: 7.1%-42.2%), and DCR was 95.8% (23/24). Median PFS was 7.62 mo (95% CI, 2.89 to 9.53 mo). At the data cutoff date, 37.5% (9/24) of pts remain on study treatment. Treatment-related adverse events (TRAEs) of any grade occurred in 50% (12/24), most were grade 1 or 2 in severity. Two pts had grade 3/4 TRAEs (alanine aminotransferase increased, diarrhea, hypertension and hypokalaemia). No TRAEs were fatal or resulted in D-1553 discontinuation. The most common ( $\geq$  5%) TRAEs (any grade) were increased alanine aminotransferase or aspartate aminotransferase, increased total bilirubin or conjugated bilirubin, diarrhea, hypothyroidism and nausea. **Conclusions:** D-1553 demonstrated a tolerable safety profile and promising monotherapy activity in pts with heavily pretreated locally advanced or metastatic CRC and KRAS G12C mutations. This study is ongoing to further evaluate the safety and efficacy of D-1553 as monotherapy and in combination with cetuximab or chemotherapy in pts with locally advanced or metastatic CRC. Clinical trial information: NCT04585035. Research Sponsor: InventisBio Co., Ltd.

**Impact of sex on the efficacy of first-line mFOLFOX6 (plus or minus bevacizumab) versus mFOLFOX6 (plus or minus bevacizumab) plus Y90-radioembolization in patients with metastatic colorectal cancer: An exploratory, retrospective analysis of the phase III SIRFLOX trial.**

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**Background:** Clinical trials in metastatic colorectal cancer (mCRC) investigating systemic and local treatment options are usually conducted irrespective of sex. However, sex-associated differences relating to safety and efficacy in the treatment of mCRC are of gaining interest. **Methods:** The SIRFLOX trial investigated the efficacy and safety of adding radioembolization using yttrium-90 resin microspheres (SIRT) to standard fluorouracil, leucovorin, and oxaliplatin (FOLFOX)-based chemotherapy in patients with previously untreated mCRC comparing it to standard treatment with FOLFOX-based chemotherapy. In this post-hoc analysis, the study population was stratified for sex (male versus female) using Propensity Score Matching (PSM) with baseline variables with difference ( $p$ -value  $< 0.20$ ) in a gender specific comparison serving as matching covariates (age, BSA, sidedness, bilirubin). Groups were matched in a 1:1 ratio, with the nearest calculated propensity logit, with a caliper width of  $\leq 0.20$  of the SD of the propensity score logit. Evaluated efficacy endpoints were progression-free survival (PFS, primary endpoint of the trial) and overall survival (OS). **Results:** 356 (67.2%) male and 173 (32.6%) female patients were randomized and treated in the SIRFLOX trial. In the overall study population, there was no difference regarding PFS or OS between male and female patients. No difference in PFS and OS between male ( $n = 101$ ) and female ( $n = 101$ ) patients could be observed independently of treatment arm. After PSM, female patients showed significant benefit regarding PFS from SIRT + chemotherapy compared to chemotherapy alone (12.2 [9.9-17] vs. 9.9 [7.5-12.7],  $p = 0.047$ ) that translated into a trend to longer OS (27.3 [25.6-36.5] vs. 23.7 [17.8-28.8] months,  $p = 0.094$ , HR 0.68 [0.43-1.10]) while male patients did not show a difference regarding PFS and OS between treatment arms ( $p = 0.170$ , HR 1.41 [0.87-2.10]). In the experimental arm, female patients had significantly longer OS compared to male patients (27.3 [25.6-36.5] vs. 21.0 [17.9-26.7] months,  $p = 0.031$ , HR 0.64 [0.42-0.96]). Sex did not impact on OS in the chemotherapy arm ( $p = 0.410$ , HR 1.22 [0.76-2.02]). **Conclusions:** In the SIRFLOX trial, female patients seem to benefit from the addition of SIRT to chemotherapy regarding PFS and OS. This was not observed in male patients. To our knowledge, this is the first analysis to show sex-differences in the application of SIRT in patients with mCRC. Our results support the development of specific protocols according to sex. Clinical trial information: NCT00724503. Research Sponsor: None.

**A multicenter randomized phase II trial comparing CAPOXIRI + bevacizumab with FOLFOXIRI + bevacizumab as first-line treatment in patients with metastatic colorectal cancer: Primary results of the QUATTRO-II study.**

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**Background:** FOLFOXIRI plus bevacizumab (Bev) is highly effective for treating patients (pts) with metastatic colorectal cancer (mCRC); however, high incidences of hematologic adverse events (AEs) and pump infusion of 5-FU q2wk can complicate treatment continuation. According to the safety lead-in (Step 1), CAPOXIRI plus Bev with 1600-mg/m<sup>2</sup> capecitabine (Cap), 130-mg/m<sup>2</sup> oxaliplatin (Ox), 200-mg/m<sup>2</sup> irinotecan (Iri), and 7.5-mg/kg Bev q3wk was the recommended phase 2 dose (Kotani D, et al., Invest New Drugs, 2021). Here, we report the results of the randomized phase II part (Step 2) of the QUATTRO-II study, which examined the efficacy and safety of CAPOXIRI + Bev versus FOLFOXIRI + Bev. **Methods:** Enrolled pts were ECOG PS 0 or 1, and had chemotherapy-naïve mCRC with wild-type or single heterozygous UGT1A1 \*6/\*28 genetic polymorphism. Pts were randomly allocated to FOLFOXIRI + Bev (Arm A) or CAPOXIRI + Bev (Arm B) in a 1:1 ratio. The induction treatment in Arm A/B was continued for 8/6 cycles (12/8 cycles at maximum if feasible), and the maintenance treatment was either 5-FU + leucovorin + Bev or Cap + Bev at the discretion of the investigators. The primary endpoint was progression-free survival (PFS), with the two groups deemed equivalent if the hazard ratio (HR) of the point estimate was  $0.8 < HR < 1.25$ . Secondary endpoints were overall survival (OS), overall response rate (ORR), early tumor shrinkage (ETS), depth of response (DpR), and safety. **Results:** From June 2020 to June 2021, 103 pts (Arm A/B, 51/52 pts) were randomly assigned. Baseline characteristics, including age (median 60 years in both arms) and ECOG PS 0 (90%/94%), were well balanced between arms. At a median follow-up of 23.7 months, the median PFS (Arm A/B) was 10.6 months (95% CI 7.7–13.3)/10.9 months (95% CI 9.3–14.3; HR 1.119, P = 0.639), and the primary endpoint was met ( $HR: 0.8 < 1.119 < 1.25$ ). The 2-year OS rate (Arm A/B) was 65.5% (95% CI 49.5–77.6)/74.3% (95% CI 59.8–84.2), with the median OS not reached. Moreover, the ORR was 76.5% (95% CI 62.5–87.2)/84.6% (95% CI 71.9–93.1), ETS was achieved in 71.4%/82.0% of pts, and the median DpR was 43.0%/54.2%. Incidences of major grade  $\geq 3$  AEs (Arm A/B) were as follows: neutropenia (68.6%/40.4%), febrile neutropenia (9.8%/11.5%), diarrhea (7.8%/17.3%), and appetite loss (7.8%/17.3%). No treatment-related deaths occurred. **Conclusions:** The efficacy of CAPOXIRI + Bev was comparable to that of FOLFOXIRI + Bev. Although CAPOXIRI + Bev was associated with increased incidences of certain nonhematologic AEs, it was well tolerated, with a decreased incidence of neutropenia and no unexpected safety signal. Good survival and response results suggest that CAPOXIRI + Bev could become a new first-line treatment option in pts with mCRC. Clinical trial information: NCT04097444. Research Sponsor: Chugai Pharmaceutical.

# Associations between early tumor shrinkage (ETS)/depth of response (DpR) and overall and post-progression survivals (OS/PPS) from the Analysis and Research in Cancers of the Digestive System (ARCAD) database.

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**Background:** According to the various types of clinical trials for metastatic colorectal cancer (mCRC), ETS and DpR are suggested as the surrogates of OS. Whereas associations between ETS/DpR and OS/PPS in the era of chemotherapy (chemo) +anti-EGFR antibody (ab)/bevacizumab (bev) in 1st line therapy have not been elucidated. **Methods:** From 40,889 Individual patient data (IPD) from 59 studies in ARCAD mCRC database, 2,138 treatment-naïve pts with *RAS* wild-type (wt) mCRC were selected from 7 randomized studies (PRIME, CAIRO2, CRYSTAL, OPUS, CALGB80495, WJOG4407G, ATOM) of chemo with/without anti-EGFR ab or bev. The ETS was defined as 20% $\geq$  tumor shrinkage at 8  $\pm$  2wks (ETS+:  $\geq$ 20%, ETS-:  $<$ 20%). Multivariate Cox regression models for OS/PPS were performed to investigate associations between ETS+ and ETS- by primary tumor location (overall/left-sided/right-sided), adjusting for potential confounders. DpR was defined as tumor shrinkage at nadir. OS/PPS were evaluated across quartile limits of DpR by multivariate Cox regression models. **Results:** In pts with overall or left-sided *RAS* wt mCRC, adjusted hazard ratios (HRs) of OS/PPS between ETS- and ETS+ for chemo +anti-EGFR ab, chemo +bev, and chemo alone showed great improvement, respectively (Table). In pts with overall or left-sided *RAS* wt mCRC, HRs of ETS for OS/PPS are higher in pts treated with chemo +anti-EGFR ab compared to those treated with chemo +bev or chemo alone. Compared to the pts with highest quartile (defined as  $\geq$  75th percentile (Q3)) of DpR, HRs of OS/PPS for lower quartile groups showed upward trends in pts with *RAS* wt & overall (Table). **Conclusions:** ETS/DpR in pts with *RAS* wt mCRC might be a potential prognostic marker of OS/PPS regardless of targeted therapy and primary tumor location. Superior prognostic values of ETS+ are expected in pts treated with anti-EGFR ab. Research Sponsor: ARCAD foundation and ARCAD-Asia.

|     |                      | OS, HR (95%CI) *1              |                          |                           | PPS, HR (95%CI) *1             |                          |                           |
|-----|----------------------|--------------------------------|--------------------------|---------------------------|--------------------------------|--------------------------|---------------------------|
|     |                      | Chemo<br>+Anti-EGFR<br>(N=912) | Chemo<br>+BEV<br>(N=512) | Chemo<br>alone<br>(N=711) | Chemo<br>+Anti-EGFR<br>(N=581) | Chemo<br>+BEV<br>(N=426) | Chemo<br>alone<br>(N=452) |
| ETS | RAS wt & overall     | 2.03<br>(1.73-2.39)            | 1.64<br>(1.34-2.01)      | 1.46<br>(1.23-1.72)       | 1.82<br>(1.50-2.20)            | 1.45<br>(1.18-1.79)      | 1.32<br>(1.07-1.62)       |
|     | RAS wt & left-sided  | 2.04<br>(1.66-2.51)            | 1.48<br>(1.13-1.95)      | 1.44<br>(1.18-1.76)       | 1.86<br>(1.46-2.37)            | 1.26<br>(0.95-1.67)      | 1.37<br>(1.06-1.76)       |
|     | RAS wt & right-sided | 1.99<br>(1.43-2.77)            | 2.05<br>(1.27-3.31)      | 1.26<br>(0.86-1.84)       | 1.56<br>(1.07-2.29)            | 1.58<br>(0.94-2.66)      | 1.00<br>(0.63-1.60)       |
| DpR | RAS wt & overall     | <Q1<br>3.33<br>(2.75-4.03)     | 2.34<br>(1.75-3.13)      | 4.01<br>(3.16-5.08)       | 2.61<br>(2.07-3.30)            | 2.01<br>(1.49-2.72)      | 2.43<br>(1.80-3.26)       |
|     | $\geq$ Q1, <median   | 2.95<br>(2.40-3.63)            | 2.08<br>(1.54-2.81)      | 2.18<br>(1.71-2.78)       | 2.51<br>(1.96-3.21)            | 1.68<br>(1.24-2.27)      | 1.83<br>(1.35-2.48)       |
|     | $\geq$ Median, <Q3   | 1.84<br>(1.51-2.24)            | 1.43<br>(1.05-1.95)      | 1.94<br>(1.51-2.49)       | 1.66<br>(1.30-2.13)            | 1.42<br>(1.04-1.94)      | 1.87<br>(1.38-2.54)       |
|     | $\geq$ Q3            |                                |                          |                           |                                |                          |                           |

\*1 Adjusted for age, gender and ECOG-PS.

**Reversion of *RAS* mutations in metastatic colorectal cancer in the CCTG CO.26 clinical trial.**

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**Background:** *RAS* mutations in metastatic colorectal cancer (mCRC) drive resistance to anti-EGFR antibodies. It is unclear if *RAS* mutations are ever clonally lost, potentially uncovering a new therapeutic option for patients over time. **Methods:** We examined the temporal evolution of *RAS* mutation status among patients enrolled in CO.26 [NCT02870920] – a phase II clinical trial that assessed durvalumab + tremelimumab in patients with heavily pretreated mCRC – using whole exome sequencing (WES) of archival primary tumor tissue and circulating tumor DNA (ctDNA) sequencing of serial plasma samples that were taken at baseline, week 8 and on progression. **Results:** Six out of 95 (6.3%) patients diagnosed with *KRAS/NRAS*-mutated mCRC showed 'neo-*RAS*-wildtype' reversions at the time of baseline or week-8 ctDNA collection for the CO.26 trial. The majority (4/6) had falling tumor mutation burden (TMB) but stable or rising maximum mutation allele frequency (MAF) when reversions occurred. These were unlikely false negatives from non-secreting cancers as there were continued strong presence of other somatic clonal mutations (e.g., *TP53*, *ATM*). The majority (4/6) of reversions were transient, with mutations reappearing with progressive disease. 'Neo-*RAS*-wildtype' revertors had numerically longer median overall survival (OS) from date of cancer diagnosis to death compared those with persistent *RAS* mutations (7.7 vs. 4.2 yrs, HR = 0.65, 95% CI 0.31 to 1.36, log-rank  $P=0.26$ ). However, 'neo-*RAS*-wildtype' revertors were earlier-stage at diagnosis compared to those with persistent *RAS* mutations (33% vs. 63% had synchronous metastases,  $\chi^2 P=0.15$ ), and had lower disease burden upon enrollment (50% vs. 68.5% had liver metastases,  $P=0.17$ ; 33% vs. 75% had > 4 lesions,  $P=0.03^*$ ). Survival from stage IV diagnosis to death did not differ between those with reverted vs. persistent *RAS* mutations (median 3.8 vs. 3.2 yrs, HR = 0.77, 95% CI 0.35 to 1.72,  $P=0.52$ ). 'Neo-*RAS*-wildtype' reversions were not associated with side of primary tumors ( $P=0.41$ ), archival *BRAF/MEK/ERK*-mutant status ( $P=0.16$ , 1.00, 0.09), baseline *HER2* amplifications ( $P=1.00$ ), or baseline TMB ( $P=0.21$ ). **Conclusions:** We identified a 6.3% prevalence of 'neo-*RAS*-wildtype' reversions in the CO.26 trial, however only 2.1% of patients had persistent loss when serial time points were considered. Further research is needed to understand if 'neo-*RAS*-wildtype' revertors may benefit from anti-EGFR therapy. Clinical trial information: NCT02870920. Research Sponsor: The Canadian Clinical Trials Group is funded by the Canadian Cancer Society.

# Prognostic impact of depth of response (DpR) and early tumour shrinkage (ETS) in patients (pts) with *BRAF V600E* mutated (mut) metastatic colorectal cancer (mCRC) receiving targeted therapy (TT) as second-line treatment.

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**Background:** Encorafenib and cetuximab (EC) is the standard of care for pre-treated patients (pts) with *BRAF V600E* mut mCRC. DpR and ETS previously showed a strong correlation with survival outcomes in mCRC pts receiving 1<sup>st</sup>-line therapy. We assessed these tumour dynamic response parameters and their association with clinical outcome in pts with *BRAF V600E* mut mCRC treated with TT in second-line. **Methods:** Patients treated in a real-life setting with EC or EC plus binimetinib (ECB) in 20 Italian centres were included. Pts with measurable disease and at least one available disease reassessment by CT scan were eligible. Pts experiencing disease progression as best response (i.e. primary resistant) were not evaluable for DpR and ETS. Associations between DpR and ETS and progression free survival (PFS) and overall survival (OS) were tested by univariate and multivariate models. **Results:** 105 pts were included: 89 (85%) and 16 (15%) pts were treated with EC and ECB, respectively. Median PFS and OS were 5.2 and 10.3 mos, respectively. Twenty-nine pts (28%) were primary resistant, while 76 (72%) pts achieved disease control (51 [48%] and 25 [24%] pts had SD or CR/PR, respectively). Among baseline characteristics, the presence of peritoneal metastases was a predictor of primary resistance ( $p = 0.04$ ). Among pts evaluable for response parameters ( $n = 76$ ), median DpR was 15% and ETS occurred in 28 pts (37%). Mucinous histology was associated with a significantly lower magnitude of DpR ( $p = 0.005$ ) and a lower rate of ETS ( $p = 0.002$ ). A significant association between DpR and PFS was observed, both as a dichotomous (i.e.  $\geq$  or  $<$  median value) and continuous variable in univariate and multivariate analyses. Also RECIST response correlated with PFS in the two models (table). DpR was associated with OS in the univariate analyses, but this was not confirmed in the multivariate models (table). No correlation between ETS and survival, either as a dichotomous (i.e.  $\geq$  or  $<$  20%) or a continuous variable, was observed. **Conclusions:** Having a DpR of at least 15% predicts longer PFS and OS in patients with *BRAF V600E* mut mCRC receiving TT as second-line treatment. An independent cohort of pts treated with second-line chemotherapy +/- antiangiogenic is under investigation as control group. Research Sponsor: None.

|                 | DpR Continuous    | DpR $\geq$ 15% | DpR $<$ 15%      | RECIST CR+PR | RECIST SD        |
|-----------------|-------------------|----------------|------------------|--------------|------------------|
| PFS             |                   |                |                  |              |                  |
| HR (95% CI)     | 0.87* (0.77-0.96) |                | 0.56 (0.34-0.93) |              | 0.54 (0.33-0.88) |
| P               | .006              |                | .017             |              | .017             |
| Adj HR (95% CI) | 0.88* (0.78-0.98) |                | 0.55 (0.33-0.92) |              | 0.52 (0.30-0.90) |
| P               | .021              |                | .024             |              | .020             |
| OS              |                   |                |                  |              |                  |
| HR (95% CI)     | 0.88* (0.76-0.99) |                | 0.61 (0.34-1.07) |              | 0.58 (0.33-1.04) |
| P               | .038              |                | .075             |              | .079             |
| Adj HR (95% CI) | 0.93* (0.80-1.08) |                | 0.78 (0.43-1.42) |              | 0.70 (0.36-1.38) |
| P               | .293              |                | .417             |              | .307             |

\*for 10% increase.

**Predictive impact of *RNF43* mutation (mut) in patients (pts) with pMMR/MSS *BRAF V600E* mutated metastatic colorectal cancer (mCRC) treated with target therapy (TT) or chemotherapy (CT).**

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**Background:** Encorafenib plus cetuximab is a standard option in the treatment of *BRAF V600E* mut mCRC pts pre-treated with at least one systemic therapy. *RNF43* is a negative regulator of WNT pathway. A recent study showed that *RNF43* mutation is associated with better outcome among pMMR/MSS *BRAF V600E* mut mCRC patients treated with TT but not in an independent cohort of pts not treated with TT (Elez et al. Nat Med 2022). However, no comparison is available between TT vs CT as second-line (2L) treatment for pMMR/MSS *BRAF V600E* mut mCRC according to *RNF43* mutational status. **Methods:** The predictive impact of *RNF43* mut was evaluated in a real-life dataset of 126 pMMR/MSS *BRAF V600E* mut mCRC pts treated with TT (consisting of BRAF inhibitor + anti-EGFR antibody  $\pm$  MEK inhibitor) vs CT  $\pm$  target agent as 2L treatment. A cohort of 36 pts receiving TT after 2L was also analyzed. **Results:** Thirty-one (25%) and 95 (75%) out of 126 pMMR/MSS *BRAF V600E* mut tumours were *RNF43* mut and *RNF43* wt, respectively. In the *RNF43* mut group 14 (45%) received CT and 17 (55%) TT; in the *RNF43* wt group, 56 (59%) and 39 (42%) received CT and TT, respectively. Among *RNF43* mut pts, those treated with TT reported longer PFS (7.1 vs 3.0 months, HR: 0.35 95%CI: 0.16-0.76,  $p = 0.006$ ) and higher ORR (42% vs 0%,  $p = 0.009$ ) than those receiving CT. On the other hand, no significant difference was observed among *RNF43* wt patients in terms of PFS (4.3 vs 3.7 months, HR: 0.69 95% CI: 0.45-1.05,  $p = 0.080$ ) and ORR (28% vs 16%,  $p = 0.24$ ). However, no significant interaction between treatment effect and *RNF43* mutational status was reported in terms of PFS ( $p_{\text{interaction}} = 0.17$ ) and ORR ( $p_{\text{interaction}} = 0.96$ ). After excluding 36 pts in the CT group that received TT after 2L, no interaction effect was observed also in terms of OS ( $p_{\text{interaction}} = 0.53$ ). However, among *RNF43* mut pts, those treated with TT reported longer OS (16.5 vs 10.1 months; HR: 0.34 95% CI: 0.11-1.00,  $p = 0.049$ ), while no significant difference was observed among *RNF43* wt pts (10.6 vs 6.6 months; HR: 0.66 95% CI: 0.39-1.11;  $p = 0.12$ ). In the group receiving TT after 2L, 9 (25%) out of 36 cases were *RNF43* mut and achieved higher ORR (78% vs 26%,  $p = 0.014$ ) and longer PFS (10.1 vs 4 months; HR: 0.35 95%CI: 0.14-0.88;  $p = 0.020$ ) and OS (11.7 vs 7 months; HR: 0.35 95%CI: 0.15-0.82;  $p = 0.012$ ) than *RNF43* wt ( $N = 27$ ). **Conclusions:** pMMR/MSS *BRAF V600E* mut mCRC patients achieve benefit from TT vs CT independently of *RNF43* mutational status, but a higher magnitude of benefit from TT is observed among those with *RNF43* mut tumors. These findings deserve confirmation in past and current randomized trials (i.e. BEACON and BREAKWATER). Research Sponsor: None.

## Transcriptomic signatures of MSI-high metastatic colorectal cancer to predict efficacy of immune checkpoint inhibitors.

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**Background:** Microsatellite instability (MSI) is currently the only predictive biomarker of efficacy of immune checkpoint inhibitors (ICI) in metastatic colorectal cancers (mCRC). However, 10-40% of patients with MSI mCRC will experience a primary resistance to ICI. **Methods:** In a cohort of 103 patients with MSI mCRC treated with ICI, 3'RNAseq was performed from primary tumors resected before the beginning of ICI. Previously described single-cell transcriptomic signatures of tumor microenvironment (TME) were analysed. **Results:** The unsupervised clustering of this cohort allowed the identification of three clusters of tumors with distinct transcriptional profiles: cluster A ("stromal<sup>HIGH</sup>-proliferation<sup>LOW</sup>"), cluster B ("stromal<sup>HIGH</sup>-proliferation<sup>MED</sup>"), and cluster C ("stromal<sup>LOW</sup>-proliferation<sup>HIGH</sup>"), with an enrichment of patients progressing at first disease assessment under ICI in the cluster A (30% vs 12% in cluster B and 8.1% in cluster C,  $p = 0.074$ ). Progression-free survival (PFS) was also significantly shorter in patients belonging to cluster A, compared to clusters B or C ( $p < 0.001$ ) with 2-year PFS rates of 33.5%, 80.5% and 78.3%, respectively. Similar results were observed for overall survival (OS). In multivariate analysis, PFS was still significantly longer in patients belonging to cluster B (HR: 0.26 95%CI 0.11-0.58,  $p = 0.001$ ) and cluster C (HR: 0.35 95%CI 0.16-0.78,  $p = 0.01$ ), compared to patients belonging to cluster A (Table). No association of identified clusters with PFS during non-ICI-based regimens was identified. **Conclusions:** This unsupervised transcriptomic classification identified three groups of MSI mCRCs with different compositions of TME cells and proliferative capacities of TME/tumor cells. The "stromal<sup>HIGH</sup>-proliferation<sup>LOW</sup>" cluster is associated with a lower efficacy of ICI. Research Sponsor: None.

| Multivariate analysis for progression-free survival according to the three unsupervised clusters. |                 |                     |         |
|---------------------------------------------------------------------------------------------------|-----------------|---------------------|---------|
| Characteristics                                                                                   | HR <sup>1</sup> | 95% CI <sup>1</sup> | p-value |
| Unsupervised clustering                                                                           |                 |                     |         |
| Cluster A (stromal <sup>HIGH</sup> -proliferation <sup>LOW</sup> )                                | —               | —                   |         |
| Cluster B (stromal <sup>HIGH</sup> -proliferation <sup>MED</sup> )                                | 0.26            | 0.11, 0.58          | 0.001   |
| Cluster C (stromal <sup>LOW</sup> -proliferation <sup>HIGH</sup> )                                | 0.35            | 0.16, 0.78          | 0.010   |
| ICI-based treatment line                                                                          |                 |                     |         |
| 1                                                                                                 | —               | —                   |         |
| ≥2                                                                                                | 2.26            | 1.0, 5.12           | 0.051   |
| Type of ICI regimen                                                                               |                 |                     |         |
| monotherapy                                                                                       | —               | —                   |         |
| combination                                                                                       | 0.62            | 0.30, 1.26          | 0.2     |
| Age                                                                                               | 1.02            | 1.00, 1.05          | 0.10    |
| Gender                                                                                            |                 |                     |         |
| female                                                                                            | —               | —                   |         |
| male                                                                                              | 1.31            | 0.64, 2.68          | 0.5     |
| Number of metastatic sites at ICI start                                                           |                 |                     |         |
| 1 or 2 sites                                                                                      | —               | —                   |         |
| more than 2 sites                                                                                 | 1.59            | 0.78, 3.25          | 0.2     |
| Neutrophil-to-Lymphocyte Ratio                                                                    |                 |                     |         |
| < 3                                                                                               | —               | —                   |         |
| ≥3                                                                                                | 0.60            | 0.29, 1.26          | 0.2     |

<sup>1</sup>HR = Hazard Ratio, CI = Confidence Interval.

**Intermittent or continuous panitumumab plus FOLFIRI (FOLFIRI/PANI) for first-line treatment of patients (pts) with RAS/BRAF wild-type (wt) metastatic colorectal cancer (mCRC): An update of survival/toxicity and preliminary results of genomic alterations from IMPROVE study.**

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**Background:** IMPROVE is a randomized, non-comparative multicenter, phase 2 study which evaluated continuous, until progressive disease (PD), or intermittent FOLFIRI/PANI, in pts with unresectable and previously untreated RAS/BRAF wt mCRC. In the intermittent schedule 8 cycles were followed by a treatment free interval lasting until PD when another 8 cycles were restarted, continuing this strategy until PD occurred on treatment. Primary endpoints was progression-free survival on treatment (PFSOT) at 1 year. In a previous analysis the primary endpoint of the study was met and a reduced skin toxicity was observed in the intermittent arm. Here we report an updated analysis. **Methods:** Updated analysis includes PFSOT and toxicity with 9 months (mo) of additional follow-up. Moreover we report overall survival (OS), skin toxicity burden (STB) score and preliminary data on genomic alterations (GAs) in 46 pts with PD. STB score, incorporating both the frequency and the severity of skin toxicity, was obtained by summing all grades that the pts experienced across all treatment cycles. GAs were performed by NGS on circulating tumor DNA (ctDNA) from paired plasma samples (baseline and progression), focusing specifically on RAS, BRAF and PI3K mutations. **Results:** Pts received FOLFIRI/PANI continuously (arm A, 69 pts) or intermittently (arm B, 67 pts). Median number of FOLFIRI/PANI cycles administered per pt were (arm A/B) 13/16. In arm B, after induction treatment, 24/53 pts (45%) without PD had  $\geq 2$  rounds of 8 FOLFIRI/PANI cycles. ORR (arm A/B) was 63/57%. At a median follow-up of 28 mo (IQR: 21-37), median PFSOT (77% of events) was 11.4 mo (95% CI: 9.1-13.7) in arm A, and 18.1 mo (95% CI: 6.8-29.3) in arm B. Median PFSOT in left sided tumors was 11.7 mo (95% CI: 9.1-14.3) in arm A and 23.9 mo (95% CI: 15.0-32.9) in arm B, compared with 10.7 mo (95% CI: 7.3-14.1) and 7.9 mo (95% CI: 5.7-10.1) in right sided pts, respectively. OS (46% of events) was 31.0 mo (95% CI: 24.7-37.2) in arm A and 32.2 mo (95% CI: 23.6-40.8) in arm B. Main grade  $\geq 3$  toxicities were (arm A/B): skin 30/18%, neutropenia 25/24%; diarrhea 13/15%. Median STB score was 0.77/cycle (IQR: 0.20-1.06) in arm A and 0.36/cycle (IQR: 0-0.77) in arm B. GAs in baseline ctDNA were evidenced in 12/46 (26%) pts, persisting to PD in all but one pt. Among the 34 pts without baseline GAs, only 8 (23%) developed  $\geq 1$  acquired GAs (Acq-GAs) to PD. **Conclusions:** Updated analyses confirmed that intermittent FOLFIRI/PANI strategy produces a long PFSOT and a reduced skin severe and skin burden toxicity without any detrimental effect on OS. Preliminary data on Acq-GAs suggest that classical mutations associated with anti-EGFR resistance are infrequent with up-front use of anti-EGFR/chemotherapy. Clinical trial information: NCT04425239. Research Sponsor: partially funded by Amgen.

# Impact of WNT/B-catenin alterations and metastasis location among patients with metastatic colorectal cancer (mCRC) treated with immune checkpoint inhibitors (ICI) combinations.

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**Background:** Emerging evidence suggests that WNT mutations and liver/peritoneal metastases in mCRC may have an immunosuppressive role that could affect ICI activity. **Aim:** To evaluate the impact of WNT alterations and liver/peritoneal metastasis among patients with mCRC treated with ICI. **Methods:** Patients from Vall d'Hebron Hospital, with mCRC treated with ICI from 2017-2022 were included. WNT alterations (APC, AXIN1/2, CTNNB1, FBXW7, EPHB2, RNF43 and SOX9) were evaluated using NGS (tissue). Clinical outcomes were calculated using survival Kaplan-Meier curves. Patients' characteristics were collected retrospectively. **Results:** Overall, 104 patients were included (66 MSI patients and 38 MSS patients). Among MSI patients, median age was 63 years (22-95), with 53% female, and 64% of patients received immunotherapy in 1st or 2nd line. Regarding tumor characteristics, 72% were right-sided and 82% harbored WNT alterations. 75% of patients presented with liver/peritoneal metastases. Patients with WNT mutations and peritoneal/liver metastases exhibit lower ORR (46% vs 57% p 0.5 and 45% vs 75%, p 0.03 respectively). Peritoneal/liver metastases were associated with lower PFS (HR 3.6 CI95% 1.27-10.24 p 0.02 respectively). Overall, tumors with WNT pathway alterations tend to have shorter PFS and OS. Liver metastases were associated with lower OS (NR vs 34 months HR 2.49 CI95% 1.01-6.17 p 0.05). Table summarizes outcomes. Among MSS patients, median age was 57 years (41-75), with 25% female and 92% of patients receiving immunotherapy  $\geq$  3rd line. Regarding tumor characteristics, 78% were left-sided and 90% harboured WNT alterations. 79%, of patients presented with liver or peritoneal metastases. WNT mutations were not associated with ORR, patients without liver/peritoneal metastases tend to have higher ORR (12.5% vs 3.3% p 0.335). Patients with WNT alterations had worse PFS (9.23 vs 1.87 months, p 0.12), and liver metastases were associated with lower PFS (6.98 vs 1.79 months HR 2.87 CI95% 1.17-7.09 p:0.02). Regarding OS, tumors harboring WNT alterations have shorter OS (8.4 vs 12 months p 0.63). The presence of liver or peritoneal metastases was associated with lower OS (NR vs 7.85 months HR 3.69 CI95% 1.09-12.55 p: 0.04). **Conclusions:** In our cohort, WNT pathway mutations, and liver metastases were associated with worse ORR, PFS, and OS regardless of MSI status. These findings need further validation in a prospective cohort. Research Sponsor: This research has been partially funded by CaixaResearch Advanced Oncology Research Program supported by Fundació La Caixa (LCF/PR/CE07/50610001).

|              | MSS n:38    |                  |                           |                    | MSI n:66    |                  |                           |                    |
|--------------|-------------|------------------|---------------------------|--------------------|-------------|------------------|---------------------------|--------------------|
|              | Liver (Y/N) | Peritoneal (Y/N) | Liver or Peritoneal (Y/N) | WNT mutation (Y/N) | Liver (Y/N) | Peritoneal (Y/N) | Liver or Peritoneal (Y/N) | WNT mutation (Y/N) |
| ORR (%)      | 3.3 vs 12.5 | 12.5 vs 3.3      | 3 vs 12.5                 | 3.7 vs 0           | 47 vs 57    | 44 vs 65         | 45 vs 75                  | 46 vs 57           |
| PFS (months) | 1.79 vs 6.9 | 2 vs 1.86        | 1.79 vs 6.9               | 1.87 vs 9.23       | 7.26 vs 16  | 6.5 vs NR        | 7.75 vs NR                | 10.25 vs NR        |
| OS (months)  | 7.8 vs NR   | 9.13 vs 8.28     | 7.8 vs NR                 | 8.44 vs 12         | 34 vs NR    | 45 vs NR         | 45 vs NR                  | 39 vs NR           |

**Real-world comparison of triplet- versus doublet-chemotherapy with bevacizumab in patients with newly diagnosed metastatic colorectal cancer (mCRC).**

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**Background:** Meta-analysis of five randomized intensification trials in mCRC demonstrated an overall survival (OS) benefit of triplet chemotherapy (5FU/leucovorin, oxaliplatin, irinotecan) plus bevacizumab (Triplet/Bev) compared to doublet chemotherapy plus bevacizumab (Doublet/Bev) (HR 0.81). Guidelines recommend first-line Triplet/Bev for patients with excellent performance status and normal organ function who can withstand increased toxicity, but such patients are less represented in the real world. In this retrospective cohort study, we examine the real-world effectiveness of Triplet/Bev compared to Doublet/Bev in the U.S. **Methods:** We used the nationwide Flatiron Health electronic health record-derived database, comprising de-identified patient-level structured and unstructured data curated via technology-enabled abstraction from ~ 280 cancer clinics. Included were mCRC patients treated with first-line Triplet/Bev or Doublet/Bev between 10/23/14 and 10/31/22. OS by treatment was assessed using the Kaplan Meier method and adjustment for confounding variables was performed using Cox proportional hazards modeling with stabilized inverse probability of treatment weighting (IPTW). Pre-specified confounding variables included age, gender, race/ethnicity, year of metastatic diagnosis, health insurance, practice setting, *KRAS/NRAS/BRAF* mutation status, MMR/MSI status, synchronous/metachronous metastases, primary tumor sidedness, CEA, renal dysfunction, hepatic dysfunction, and ECOG performance status. Missing data were imputed using multiple imputation with chained equations. **Results:** Among 10,016 patients, 391 (3.9%) received Triplet/Bev and 9,625 (96.1%) received Doublet/Bev. Patients who received Triplet/Bev were younger (median age 52 vs 63 years) and were more likely to be treated at academic practices (27% vs 8%), to have synchronous metastatic disease (83% vs 65%), and to have *KRAS/NRAS/BRAF* wild-type disease (30% vs 17%). In the univariate analysis, median OS was 30.0 months (95% CI 24.7 – 34.7) in the Triplet/Bev cohort versus 23.4 months (95% CI 22.8 – 24.1) in the Doublet/Bev cohort (HR 0.73; 95% CI 0.62 – 0.85;  $p < 0.001$ ). In Cox proportional hazards modeling with IPTW, there was no significant difference in hazard of death between Triplet/Bev and Doublet/Bev (HR 0.92; 95% CI 0.75 – 1.12;  $p = 0.4$ ). **Conclusions:** In this large, national, real-world population of patients with mCRC, first-line treatment with Triplet/Bev was not associated with improved survival compared to Doublet/Bev after accounting for differences in baseline patient characteristics. The benefit to Triplet/Bev observed in randomized trials does not translate well to a more heterogeneous, real-world population. Sub-group analyses including by age, tumor sidedness, *KRAS/NRAS/BRAF* status, and baseline CEA level will be performed. Research Sponsor: None.

# Modified FOLFOXIRI plus cetuximab and avelumab as initial therapy in RAS wild-type unresectable metastatic colorectal cancer: Results of the phase II AVETRIC trial by GONO.

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**Background:** The modified schedule of FOLFOXIRI (mFOLFOXIRI) in combination with an anti-EGFR agent showed a manageable safety profile and remarkable activity in RAS wild-type (wt) metastatic colorectal cancer (mCRC). The association of an active cytotoxic regimen with cetuximab (cet) may increase the exposure of tumour-associated neoantigens and induce immunogenic cell death and antibody-dependent cell-mediated cytotoxicity thus enabling the effect of immune checkpoint inhibitors. The AVETRIC study aimed at exploring the efficacy and safety of first-line mFOLFOXIRI plus cet and avelumab (ave) in RAS wt mCRC patients (pts). **Methods:** AVETRIC is a prospective, open label, multicenter, phase II, single arm trial in which initially unresectable and previously untreated RAS wt mCRC pts received mFOLFOXIRI (irinotecan 150 mg/sqm, oxaliplatin 85 mg/sqm, folinate 200 mg/sqm leucovorin [LV], and 5-fluorouracil [5FU] 2400 mg/sqm) plus cet (500 mg/sqm) and ave (800 mg) every 2 weeks up to 12 cycles followed by maintenance with 5FU/LV plus cet and ave until disease progression. A safety run-in phase including the first 6 enrolled pts was planned. Due to the occurrence of grade 3-4 diarrhoea in 2 (33%) pts, the protocol study was amended to reduce the irinotecan dose to 130 mg/sqm. Primary endpoint was Progression Free Survival (PFS). Fifty-eight pts were needed to detect an increase in median PFS (mPFS) from 10.0 to 19.4 months (mos), setting one-sided  $\alpha$  and  $\beta$  errors at 0.05 and 0.10, respectively. The trial is registered at Clinicaltrial.gov, NCT04513951. **Results:** Between Jun 2020 and Dec 2021, 62 pts were enrolled in 16 Italian centres. Main pts' characteristics were: median age 56 yrs, ECOG PS 0 87%, synchronous metastases 94%, liver-only disease 42%, left-sided primary tumour 89%; all pts had BRAF wt and proficient MMR (pMMR) tumours. The primary endpoint was met. At a median follow-up of 16.0 months, 39 (63%) events were recorded and mPFS was 14.1 months (90% CI 12.0-16.7, Brookmeyer-Crowley test  $p < 0.001$ ). Response rate and disease control rate were 82% and 98%, respectively, and R0 resection rate was 21% (27% in liver-only subgroup). Early tumour shrinkage was achieved in 74% pts and median deepness of response was 56%. In pts treated after study amendment ( $n = 56$ ), main grade 3-4 adverse events were neutropenia (27%), diarrhoea (27%), skin rash (14%), asthenia (14%), nausea (11%), stomatitis (7%), febrile neutropenia (2%). Grade 3-4 immune-related adverse events occurred in 6% of pts. Overall survival results are still immature. **Conclusions:** AVETRIC study met its primary endpoint, showing that combining mFOLFOXIRI plus cet and ave achieves promising results in terms of PFS as well as response rate, in pts with pMMR RAS and BRAF wt mCRC. Translational analyses to evaluate the impact of immune-related biomarkers are ongoing. Clinical trial information: NCT04513951. Research Sponsor: GONO Foundation; This study was financially supported by Merck Serono S.p.A., Rome, Italy, an affiliate of Merck KGaA, Darmstadt, Germany (CrossRef Funder ID: 10.13039/100009945).

### Exploring the prognostic and predictive impact of genomic loss of heterozygosity (LOH) in patients with metastatic colorectal cancer (mCRC).

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**Background:** Genomic LOH consists in the loss of chromosomal regions and is associated with the homologous recombination repair (HRR) system deficiency (dHRR). In ovarian cancer, LOH-high predicts benefit from platinum-based chemotherapy and PARP inhibitors. In mCRC, the role of LOH has been poorly investigated. **Methods:** An NGS-based assay (FoundationOne CDx; Foundation Medicine, Inc. Cambridge, MA) was used to determine the percentage of genomic LOH and the presence of pathogenetic mutations in HRR-related genes in archival chemo-naïve tumor tissues of mCRC patients included in a real-world registry and in the AtezoTRIBE (NCT03721653) and AVETRIC (NCT04513951) studies. Both these trials assessed the combination of an anti-PDL1 (atezolizumab or avelumab) with the triplet FOLFOXIRI plus bevacizumab or cetuximab. The prespecified cut-off of  $\geq 14.08$  for LOH-high described for mCRC (Sokol et al., JCO Precis Oncol 2020) was adopted. Tumors with at least one biallelic alteration in any of the 27 genes involved in the HRR pathway (Riaz et al., Nat Commun 2017) included in the FoundationOne CDx panel (BARD1, BRCA1, BRCA2, BRIP1, MRE11A, NBN, PALB2, RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54L, RBBP8, XRCC2, ABL1, ATM, ATR, BAP1, CDK12, DNMT3A, ERCC4, FANCA, FANCC, FANCG, FANCL, PARP1) were defined dHRR. **Results:** Overall, 196 samples were analysed. None of 7 MSI-H tumors were classified as LOH-high. Fourteen (7%) and 6 (3%) of 189 MSS tumors were classified as LOH-high and dHRR, respectively. In particular, in LOH-high subgroup, 3 (21%) tumors were defined dHRR, while 3 (50%) tumors were classified LOH-high among dHRR tumors. LOH-high tumors were more frequently *BRAF* mutated ( $p = 0.019$ ) and dHRR ( $p = 0.006$ ) compared to LOH-low. Among patients receiving triplet chemotherapy +/- biologic agent alone ( $N = 55$ ) or with an anti-PDL1 ( $N = 58$ ), an interaction effect was shown between the effect of the addition of the checkpoint inhibitor and LOH with higher benefit in the LOH-high subgroup ( $N = 10$ ) in terms of both PFS ( $p_{\text{interaction}} = 0.002$ ) and OS ( $p_{\text{interaction}} < 0.001$ ). In the cohort of patients not receiving the anti-PDL1, longer PFS was observed in patients with LOH-low ( $N = 125$ ) respect to LOH-high ( $N = 6$ ) tumors (12.1 vs 5.1 months, HR: 0.11, 95%CI: 0.04-0.26,  $p < 0.001$ ). No differences in PFS or OS were reported between patients treated with first-line oxaliplatin- ( $N = 40$ ) vs irinotecan-based doublets ( $N = 25$ ) or with the triplet FOLFOXIRI ( $N = 55$ ) vs doublets ( $N = 65$ ) according to LOH status. No prognostic or predictive impact of HRR deficiency was shown. **Conclusions:** In MSS mCRC, LOH-high was associated with biallelic alterations in the HRR system, worse prognosis and higher benefit from the addition of anti-PDL1 agents to chemotherapy. Considering the low number of LOH-high tumors in our study, these results deserve confirmation in larger cohorts. Research Sponsor: None.

### Analysis of primary and acquired resistance to cetuximab from multiomic data in the New EPOC trial.

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**Background:** The use of cetuximab (cetux) with chemotherapy (chemo) in patients with operable liver metastases from colorectal cancer (CRC) conferred a survival disadvantage in New EPOC. Whilst genetic alterations such as emergent sub-clonal mutations in *RAS/BRAF*, *ERBB2* and *MET* are recognized as mechanisms of resistance to EGFR inhibition (EGFRi), divergent mechanisms such as transcriptomic changes may also be responsible. Of the CRC intrinsic subtypes (CRIS), CRIS-C has elevated EGFR signalling and is thought to have greater sensitivity to EGFRi. **Methods:** Resected primary tumors (n = 205; pre neoadjuvant treatment) and/or liver metastases (n = 144; post neoadjuvant treatment) from 223 patients, wild type for *KRAS* (codons 12/13/61) at trial entry, randomized to chemo +/- cetux underwent targeted NGS and/or transcriptome profiling. Primary clinical endpoints were progression free (PFS) and overall survival (OS). **Results:** Repeat NGS identified additional *RAS/BRAF* mutations in primary tumors from 49 patients (25 *KRAS*, 15 *NRAS*, 9 *BRAF V600E*). Of the 148 patients with confirmed *RAS/BRAF* wild type primary tumors, 46% were classified as CRIS-C with the remainder being CRIS-A (9.5%), CRIS-B (11%), CRIS-D (8.8%) or CRIS-E (8.1%). CRIS-C patients had a similar PFS with ChemoCetux vs Chemo (HR 0.84 95% CI 0.47-1.5 p = 0.55) whereas non-CRIS-C had a shorter PFS (HR 2.12 95%CI 1.24-3.63 p = 0.006; p interaction 0.021). OS results were similar (p interaction 0.067). In liver metastases, genetic changes were comparable (Chemo vs ChemoCetux): *KRAS* (4.1% v 2.1%), *NRAS* (4.1% v 0%), *BRAF V600E* (0% v 0%), *ERBB2* gain/amplification (20% v 21%) and *MET* gain/amplification (25% v 18%). However, gain/amplification of *MET* resulted in shorter OS with ChemoCetux vs Chemo (HR 5.13 95%CI 1.38-19.15 p = 0.015) whereas those without *MET* gain/amplification had a similar OS (HR 1.32 95%CI 0.76-2.28 p = 0.32; p interaction 0.029). The same association was found after excluding liver metastases with *RAS/BRAF* mutations (n = 101, p interaction 0.030). Differential expression/mutation analyses did not identify other stratifiers after false discovery correction. **Conclusions:** The shorter survival with ChemoCetux was restricted to patients with a non CRIS-C subtype before treatment supporting the use of this transcriptomic classifier to stratify for EGFRi. After neoadjuvant treatment, gain/amplification of *MET* was associated with a shorter survival with ChemoCetux. By contrast MAPK pathway mutations were largely unchanged supporting earlier findings that alternative resistance mechanisms predominate when EGFRi is combined with chemotherapy. In summary these results highlight the heterogeneity and differential responses to EGFRi that exist within *RAS/RAF* wild type CRC and provide insights into the substantial survival detriment observed in the New EPOC trial. Clinical trial information: ISRCTN22944367. Research Sponsor: Cancer Research UK (C317/A7275), Medical Research Council UK.

### Single nucleotide polymorphisms (SNPs) in MHC class I and II genes to predict outcome in patients (pts) with metastatic colorectal cancer (mCRC): Data from FIRE-3, MAVERICC, and TRIBE trials.

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**Background:** The immune system is alerted for virally infected cells in the body by the antigen presentation pathway, which is in turn mediated by the major histocompatibility complex (MHC) class I and II molecules. Cancer cells overcome immune evasion as a major hallmark by downregulation of antigen presentation pathway molecules. Therefore, the present study aimed to explore the effect of genetic variants in MHC class I and II pathways on first-line treatment outcome in mCRC pts. **Methods:** Genomic DNA from blood samples of 775 pts enrolled in three independent, randomized, first-line trials: TRIBE (FOLFIRI-bevacizumab [bev], N = 215), FIRE-3 (FOLFIRI-bev, N = 107; FOLFIRI-cetuximab [cet], N = 129) and MAVERICC (FOLFIRI-bev, N = 163; FOLFOX-bev, N = 161) was genotyped through OncoArray, a custom array manufactured by Illumina including approximately 530K SNP markers. The impact on outcome of 40 selected SNPs in 22 genes of MHC class I and II pathways (*ERAP1*, *ERAP2*, *TAP1*, *TAP2*, *TAPBP*, *B2M*, *HLA-A*, *HLA-B*, *HLA-C*, *HLA-E*, *HLA-F*, *HLA-G*, *CIITA*, *HLA-DMA*, *HLA-DMB*, *HLA-DOA*, *HLA-DOB*, *HLA-DPA1*, *HLA-DPB1*, *HLA-DQA1*, *HLA-DQB1* and *HLA-DRA*) was analyzed. **Results:** We identified several SNPs in multiple genes associated with targeted treatment benefit across different treatment arms in our study population ( $P < .05$ ). Germline variants in *ERAP1* rs2287987 were associated with worse PFS in pts receiving FOLFIRI-bev in TRIBE (10.4 vs 8.8 months, HR 1.48, 95%CI 1.04-2.12,  $P = .035$ ) and FIRE-3 trials (11.1 vs 9.9 months, HR 3.46, 95%CI 1.18-10.12,  $P = .049$ ), while rs26653 SNP in the same gene was associated with better PFS in MAVERICC (10.1 vs 14.5 months, HR 0.54, 95%CI 0.35-0.84,  $P = .0062$ ). *TAP1* rs1135216, *TAP2* rs1800454 and rs1044043, *HLA-B* rs2770, *HLA-G* rs1610696, *CIITA* rs4774, *HLA-DRA* rs7192 were associated with longer OS and/or PFS in cet-treated pts in FIRE-3; whereas *TAP2* rs241447, *TAPBP* rs3106191, *HLA-DMB* rs10751, *HLA-DOB* rs11244, *HLA-DPB1* rs3097671 showed worse PFS and/or OS. SNPs in *TAP2* (rs1800454), *HLA-C* (rs1049281), *HLA-G* (rs1063320), *CIITA* (rs1139564), *HLA-DMB* (rs1042337), *HLA-DOA* (rs375256, rs3129303), *HLA-DPA1* (rs1042190) and *HLA-DRA* (rs7192) were specifically associated with clinical outcomes in the FOLFOX-bev arm of MAVERICC but not in control cohorts of pts treated with FOLFIRI-bev. Treatment-SNP interaction analyses with targeted agents (bev vs cet) and chemotherapy backbone (FOLFIRI vs FOLFOX) confirmed a significant treatment interaction for *HLA-G*, *TAP2*, *CIITA*, and *HLA-DMB* SNPs ( $P < .05$ ). **Conclusions:** Our results highlight an important role for MHC SNPs as prognostic and predictive biomarkers for first-line treatment in mCRC, with differential effects based on biologic agent and chemotherapy backbone. These biomarkers, when further validated, may contribute to personalized treatment strategies for mCRC patients. Research Sponsor: National Cancer Institute, Gloria Borges WunderGlo, Dhont Family, San Pedro Peninsula Cancer Guild, Daniel Butler Research Fund, Victoria and Philip Wilson Research Fund, Fong research project, Ming Hsieh research fund.

**Nipavect: Phase II study of niraparib and panitumumab in advanced RAS WT colorectal cancer.**

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**Background:** Panitumumab (Pmab) is a recombinant monoclonal antibody that binds specifically to the human epidermal growth factor receptor (EGFR) and it is indicated for the treatment of metastatic colorectal carcinoma (mCRC). However, the objective response rates (ORR) for the single agent are historically about 8-11%. EGFR inhibition induces synthetic lethality with poly ADP ribose polymerase inhibitors (PARPi) such as Niraparib, by attenuating DNA repair pathways. Combining PARP and EGFR inhibition has the potential to confer synergistic benefit, while also ameliorating resistance mechanism to EGFRi. We conducted this study to define the safety and efficacy of the combination of Niraparib and Pmab in RAS wildtype (WT) mCRC. **Methods:** Patients (pts) with RAS WT mCRC who have progressed on at least one line of systemic chemotherapy were eligible for the trial. Other selected inclusion criteria were  $\geq 18$  years of age, ECOG PS 0-1 and measurable disease per RECIST 1.1. Pmab was administered at 6 mg/kg IV on days 1 & 15 of each 28-day cycle, while Niraparib was taken orally at 200mg or 300mg (based on body weight and platelet count) daily throughout the cycle. The primary endpoint was clinical benefit rate (CBR) defined as complete (CR) + partial (PR) response + stable disease (SD) per RECIST v1.1. Multiple secondary endpoints included safety/tolerability, ORR, progression-free survival (PFS), overall survival (OS), and duration of response (DoR). **Results:** A safety run-in cohort of 6 pts was initially enrolled. Following a preplanned safety analysis showing an acceptable toxicity profile, an additional 19 pts were enrolled. Of the total 25 enrolled pts, 24 were evaluable for response. Male - 50%; Whites/African Americans/Asians - 65.2%/21.7%/13%; median age - 58.5yrs. The majority had left sided tumor (92%) and the median line of prior therapy was 2 (range 1-4). CBR was 83.3% (0 CR, 6 PR, 14 SD) and ORR was 25%. mPFS - 5.6mos (95% CI: 3.7, 6.9); mOS - 20.9mos (95% CI: 9.2, NR). Six-month PFS and OS rates were 48.4% and 100% respectively. At a median follow up time of 7.5mos (95% CI: 4.2, 10.4), 3 patients remained on treatment and DoR was yet to be determined. Mucocutaneous adverse events (AEs) of any grade included rash (58.4%), oral mucositis (20.8%), dry skin (16.7%) and paronychia (4.2%). Most common (grade  $> 2$ ) treatment related AEs (TRAEs) were anemia (G3; 12.5%), dermatitis, oral mucositis, hypertension and neutropenia (G3; 4.2% each). There were no grade 4-5 TRAEs. **Conclusions:** The combination of Pmab and Niraparib had an acceptable safety profile, and showed considerable antitumor activity in pts with advanced RAS WT mCRC compared to historical rates. Additional biomarker analyses such as survival correlation with immune cell infiltration in paired skin biopsies and HER2/BRAF mutational status are ongoing. Funding and product (Niraparib) for the study were provided by GSK (NCT03983993). Clinical trial information: NCT03983993. Research Sponsor: GSK.

**OniLon: Phase II trial of trifluridine/tipiracil (TAS-102) and nanoliposomal irinotecan (nal-IRI) in advanced colorectal cancer.**

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**Background:** TAS-102 (FTD/TPI) is a combination of a nucleoside analogue and a thymidine phosphorylase inhibitor, and has showed activity in 5FU-resistant CRC. Nal-IRI achieves higher intra-tumor concentrations than irinotecan (Iri; 142-fold) and its major metabolite, SN-38 (9-fold). This resulted in superior anti-tumor activity compared to free Iri in multiple tumor xenografts. Furthermore, multiple clinical trials have established the activity of nal-IRI in combination with 5FU in pancreaticobiliary cancers. The combination of nal-IRI with the more potent nucleoside analogue TAS-102 may thus result in a more effective systemic therapy. We conducted this study to define the safety and efficacy of the combination of TAS-102 and nal-IRI in advanced CRC. **Methods:** Eligible patients (pts) on this investigator-initiated phase II study had advanced CRC and must have had disease progression on at least one prior therapy. Additional inclusion criteria - measurable disease, ECOG PS 0-1 & adequate organ function. Based on previously published dose escalation phase I data, the pts were treated at the recommended phase II dose of TAS-102 (given orally at 35mg/m<sup>2</sup> bid on days 1-5) and nal-IRI (60mg/m<sup>2</sup> IV on day 1) in 14-day cycles. The primary endpoint was objective response rate (ORR) = complete (CR) + partial (PR) response per RECIST v1.1. Multiple secondary endpoints included safety/tolerability, progression-free survival (PFS), overall survival (OS), disease control rate (DCR) and duration of response (DoR). **Results:** Twenty-two pts were enrolled, of whom 20 were evaluable for response. Male - 50%; ECOG PS 1 - 75%; African Americans - 38%; median age - 58yrs (interquartile range [IQR] 49-67). Median line of prior therapy was 1 (IQR 1-2) and 13.6% (3 pts enrolled during the dose escalation phase) had received prior Iri. 47.6% received concurrent Bevacizumab. ORR was 15% (3 PR, 0 CR). 60% had stable disease (SD) as best response; DCR of 75%. mPFS - 9.7 mos (95% CI: 5.6, 14.2); mOS - 10.1 mos (95% CI: 7.3, 15.9). 12-month PFS and OS rates were 34.6% and 39.5% respectively. At a median follow up of 11.9 mos, DoR was yet to be determined and 50% of the pts had maintained response for 12 mos. Median duration of progression free among pts with SD was 7.6 mos (95% CI: 4.6, 13.2), with a 12-month survival rate of 47.7% (95% CI: 15.5%, 74.5%). Median time to response (TTR) was 4.8mos. Most common treatment related toxicities included fatigue and neutropenia (G3; 27.3% each); anemia and leucopenia (G3; 18.2% each). Other G3 AEs included febrile neutropenia, diarrhea, hypokalemia, lymphopenia and nausea (4.5% each). **Conclusions:** We demonstrated that the combination of TAS-102 and nal-IRI had an acceptable safety profile, and showed antitumor activity in patients with advanced CRC. Additional biomarker analyses such as survival correlation with UGT1A1 phenotype and RAS mutational status are ongoing. (NCT03368963). Clinical trial information: NCT03368963. Research Sponsor: Taiho; Ipsen.

### Deep learning-derived spatial organization features on histopathology images to predict prognosis in patients with colorectal liver metastasis, after hepatectomy.

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**Background:** Histopathological images of colorectal liver metastasis (CRLM) contain rich morphometric information that may predict patient outcomes, but current indicators depend on labor-intensive and subjective visual estimation. Herein, we aimed to develop an automated framework for tissue classification of routine H&E stained whole-slide images and establish a risk-scoring model for better prognosis prediction in patients with CRLM. **Methods:** Using 161,371 hand-delineated image patches, we trained a robust deep convolutional neural network-CRLM-SPA for accurate classification of CRLM into seven tissue types. With the tissue classification results on two independent in-house cohorts (SYSUCC:  $n = 433$ , BJCH:  $n = 404$ ), we systematically quantified spatial organization features (SOFs), involving whole-slide, tumor-infiltrating and tumor-distal SOFs for different tissue types as well as the interactions between tumor and non-tumor tissues. Subsequently, univariate Cox proportional hazards regression analysis was performed to investigate the association between various SOFs and patient outcomes. Nonredundant SOFs that are clinically relevant were selected to build a risk-scoring model for prognosis using multivariate Cox regression analysis in the SYSUCC cohort, followed by validation in the BJCH cohort. **Results:** CRLM-SPA achieved an overall classification accuracy of  $> 93\%$  in an independent set of 17,653 image patches. With the classification result, we calculated various SOFs and built a four-SOF risk-scoring model that significantly predicted overall survival (OS) in the discovery cohort, SYSUCC ( $P = 1.40 \times 10^{-5}$ ; HR = 2.26; 95%CI: 1.55 - 3.29) and the independent validation cohort, BJCH ( $P = 4.59 \times 10^{-4}$ ; HR = 2.00; 95%CI: 1.35 - 2.97). The prognostic performance of our SOF risk-scoring model is independent of the clinical risk score (CRS) system. Further stratification analyses in patients without preoperative chemotherapy (CTx) indicated that adjuvant CTx consistently improved OS in the SOF high-risk subgroups in both cohorts ( $P = 0.018$  and  $0.049$ ), but not in the SOF low-risk subgroups. Furthermore, a combined scoring system incorporating SOF and CRS considerably improved the prognostic performance in both cohorts over the individual SOF and CRS systems. **Conclusions:** CRLM-SPA showed a high accuracy in tissue classification and robustness in extracting prognostic information from H&E images. The SOF risk-scoring system demonstrated a strong and robust prognostic value that is independent of CRS, and could therefore provide a time- and cost-efficient tool to assist clinical decision making for patients with CRLM. Research Sponsor: Science Technology and Innovation Commission of Shenzhen Municipality; Research Grants Council of the Hong Kong Special Administrative Region.

### Efficacy and safety of fruquintinib plus investigator's choice of chemotherapy as second-line therapy in metastatic colorectal cancer: A multicenter, single-arm phase 2 trial.

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**Background:** Emerging evidence suggested that chemotherapy in combination with anti-angiogenic targeted agents can achieve higher response in multiple solid tumors. Fruquintinib is a highly selective small-molecule VEGFR inhibitor that has been approved for the third-line treatment in metastatic colorectal cancer (mCRC) patients in China. Here, we assessed the efficacy and safety of fruquintinib plus investigator's choice of chemotherapy as second-line therapy in pretreated advanced mCRC patients. **Methods:** In this prospective, open-label, multicenter, single-arm phase 2 trial (ChiCTR2200059280), patients with mCRC progressed after first-line prior systemic treatment were administered fruquintinib (5~3mg, D1-21, Q4w) and investigator's choice of chemotherapy (fluorouracil ± irinotecan ± oxaliplatin, Q3w) for up to 8 cycles. Patients without disease progression (PD) were followed by fruquintinib maintenance until PD or intolerable toxicity. The primary endpoint was progression-free survival (PFS). Secondary endpoints included objective response rate (ORR), disease control rate (DCR), duration of response (DOR), overall survival (OS), and safety. **Results:** As the data cutoff on February 10, 2023, 37 patients have been enrolled and treated, with 31 evaluable for efficacy. Median age was 63 years (range, 44-76) and 22 (59.5%) were male. The left colon cancer involved in 28 patients (75.7%). All patients were microsatellite-stable phenotype and 11 (29.7%) had mutations in KRAS gene. 16 (43.2%) patients received prior anti-VEGF therapy. 20 (54.1%) and 11 (29.7%) patients had liver and lung metastases respectively. At a median follow-up of 8.4 months, 28 patients are still on treatment. The ORR is 48.4%, with 15 partial response. The DCR is 90.3%. At data cutoff, median PFS has not yet reached. 23 patients (74.2%) remained progression free at 6 months. In terms of safety, the regimen was well tolerated, mainly grade 1/2 adverse events (AEs). Grade 3/4 AEs were neutropenia (21.6%), leukopenia (10.8%), thrombocytopenia (5.4%), proteinuria, diarrhea, hand-foot syndrome and hypertension accounted for 2.7% respectively. 5 pts received reduced doses of fruquintinib. No treatment-related deaths occurred. **Conclusions:** Fruquintinib combined with chemotherapy followed by fruquintinib maintenance shows promising efficacy and manageable safety profile for mCRC patients in second-line setting. Updated follow up data will be presented in the future. Clinical trial information: ChiCTR2200059280. Research Sponsor: National Natural Science Foundation of China (grant no.82102954).

## The impact of sodium-glucose cotransporter-2 inhibitors on the outcome of patients with colorectal adenocarcinoma.

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**Background:** The mortality of colorectal cancer remains high despite the development of novel anti-neoplastic agents. Preclinical studies have shown that colorectal cancer upregulates the expression of sodium-glucose cotransporter 2 (SGLT2) channels and inhibition of these channels by SGLT2 inhibitors (SGLT2i) reduces tumor proliferation. We aimed to investigate the impact of SGLT2i on the outcome of patients with colorectal cancer. **Methods:** We conducted a retrospective cohort study by including all adult patients with colorectal adenocarcinoma and type 2 diabetes mellitus in two tertiary centers in Taiwan. SGLT2i and non-SGLT2i patients were matched 1:1 based on age, sex, and cancer stage. The primary outcome was overall survival (OS) and progression-free survival (PFS), and the secondary outcomes were previously reported serious adverse events associated with the use of SGLT2i. **Results:** We identified 1347 patients with colorectal cancer and type 2 diabetes mellitus, from which 92 patients in the SGLT2i cohort were matched to the non-SGLT2i cohort. Compared to non-SGLT2i recipients, SGLT2i recipients had a higher rate of OS (5-year OS: 86.2% [95% CI: 72.0-93.5] vs. 62.3% [95% CI: 50.9-71.8],  $p = 0.013$ ) and PFS (5-year PFS: 76.6% [95% CI: 60.7-86.7] vs. 57.0% [95% CI: 46.2-66.4],  $p = 0.021$ ). In Cox proportional hazard analyses, the use of SGLT2i was associated with a 50-70% reduction in the risk of all-cause mortality and disease progression. The rate of cancer-associated mortality was lower in the SGLT2i group (7% vs. 21%,  $p = 0.005$ ). SGLT2i were not associated with an increased risk of sepsis, hypoglycemia, or acute kidney injury. We did not detect any cases of urosepsis or diabetic ketoacidosis in the SGLT2i group. **Conclusions:** The use of SGLT2i was associated with a higher rate of survival in colorectal cancer patients with diabetes mellitus. Research Sponsor: None.

Cox Regression hazard analysis comparing SGLT2i and non-SGLT2i.

| Outcomes              | SGLT2i<br>No. of<br>cases | Non-<br>SGLT2i<br>No. of<br>cases | Univariate<br>hazard ratio<br>(95% CI) | P-<br>value | Multivariate<br>hazard ratio <sup>a</sup><br>(95% CI) | P-<br>value |
|-----------------------|---------------------------|-----------------------------------|----------------------------------------|-------------|-------------------------------------------------------|-------------|
| <b>Primary</b>        |                           |                                   |                                        |             |                                                       |             |
| All-cause mortality   | 7 (8%)                    | 26 (28%)                          | 0.36 (0.15-0.84)                       | 0.018       | 0.28 (0.10-0.81)                                      | 0.018       |
| Disease progression   | 13 (14%)                  | 32 (35%)                          | 0.47 (0.24-0.90)                       | 0.024       | 0.40 (0.18-0.86)                                      | 0.019       |
| <b>Secondary</b>      |                           |                                   |                                        |             |                                                       |             |
| Acute kidney injury   | 5 (5%)                    | 3 (3%)                            | 3.59 (0.63-20.3)                       | 0.149       | 2.94 (0.38-22.5)                                      | 0.30        |
| Hypoglycemia          | 3 (3%)                    | 4 (4%)                            | 0.91 (0.20-4.09)                       | 0.90        | 2.68 (0.32-22.4)                                      | 0.36        |
| Sepsis                | 7 (8%)                    | 12 (13%)                          | 0.89 (0.33-2.40)                       | 0.82        | 0.64 (0.18-2.30)                                      | 0.50        |
| Urosepsis             | 0 (0%)                    | 6 (7%)                            | -                                      | -           | -                                                     | -           |
| Diabetic ketoacidosis | 0 (0%)                    | 0 (0%)                            | -                                      | -           | -                                                     | -           |

<sup>a</sup>Adjusted for age, sex, cancer stage, Eastern Cooperative Oncology Group (ECOG) Performance Status, chemotherapy regimen, radiotherapy, hypertension, hyperlipidemia, chronic kidney disease, and chronic obstructive pulmonary disease.

**A phase II study of nivolumab and ipilimumab with radiation therapy in patients with metastatic, microsatellite stable colorectal cancer.**

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**Background:** Immune checkpoint inhibitors (ICIs) have limited efficacy in patients with microsatellite stable (MSS) colorectal cancer (CRC). Radiation therapy (RT) may increase the response rate to ICIs through multiple mechanisms. In our phase 2 trial (NCT03104439), 40 patients with metastatic MSS CRC were enrolled to receive ipilimumab and nivolumab with RT (24 Gy/3 fractions) starting on C2D1. Among the 27 patients who received RT (33% dropout rate), the disease control rate (DCR) was 37% and objective response rate (ORR) was 15%. To confirm this signal and address dropout prior to RT, we conducted a phase 2 study of nivolumab and ipilimumab with RT moved to C1D1. **Methods:** In this open-label, single-arm, phase 2 study (NCT04361162), eligible patients had histologically confirmed metastatic MSS CRC, ECOG PS 0-1, and progressed on at least one line of chemotherapy. Treatment consisted of ipilimumab 1 mg/kg every 6 weeks for the first 4 cycles, nivolumab 240 mg every 2 weeks on a 6-week cycle, and RT with 24 Gy/3 fractions to one site starting on C1D1. Treatment continued until disease progression, discontinuation, or withdrawal. The primary endpoint was ORR outside of the RT field by RECIST 1.1 with radiological evaluations every 3 months. The treatment regimen was considered to have promising activity if at least 3 of 30 patients achieved an objective response in unirradiated lesions. Secondary endpoints included DCR, PFS, OS, and safety. A single-stage design was used to enroll 30 patients for intention-to-treat analysis of patients receiving at least one dose of study treatment. The per protocol analysis included patients who completed C1D1. The treatment regimen was considered to have promising activity if at least 3 of 30 patients achieved an objective response in unirradiated lesions, providing 85% power to reject 4% ORR in favor of 15% ORR at a significance level of 15%. **Results:** We enrolled and treated 30 patients (median age 56 years [range 28-85], 60% male, 83% white) from 10/2020 to 05/2022. Patients received a median of 2 (range, 1-7) prior lines of chemotherapy. All patients in the intention-to-treat population also met criteria for inclusion in the per protocol analysis. The ORR was 13% (4/30; 95% CI, 4-31%), DCR was 33% (10/30; 95% CI, 17-53%), median PFS was 2.4 months (95% CI, 1.8-2.9 months), and median OS was 10.6 months (95% CI, 6.8-17.8 months). 16 patients had grade 3+ treatment-related serious adverse events, including lymphopenia (3 patients with grade 4), anemia, diarrhea, colitis, vomiting, alkaline phosphatase increase, hypothyroidism, fatigue, and myositis. **Conclusions:** Treatment with ipilimumab, nivolumab, and RT starting on C1D1 showed promising activity in patients with traditionally immunoresistant metastatic MSS CRC. Further analyses are ongoing to evaluate optimal patient selection and radiation strategies. Clinical trial information: NCT04361162. Research Sponsor: Bristol Myers Squibb.

**Efficacy and safety of irinotecan-eluting HepaSphere transarterial chemoembolization combined with hepatic arterial infusion chemotherapy for unresectable colorectal liver metastases.**

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**Background:** Both drug-eluting bead transarterial chemoembolization (DEB-TACE) and hepatic arterial infusion chemotherapy (HAIC) are recommended for unresectable colorectal liver metastases (CRLM) treatment. However, the combined application of DEB-TACE and HAIC is not widely accepted. The aim of this single-center retrospective study was to evaluate the efficacy and safety of Irinotecan-eluting HepaSphere chemoembolization combined with HAIC for unresectable CRLM. **Methods:** Patients with age older than 18 years, histologically confirmed CRLM and treated with Irinotecan-eluting HepaSphere chemoembolization plus HAIC from Oct 2020 to Jan 2022 were enrolled. Patients who had synchronously received other local treatments were excluded. Hepatic progression-free survival (hPFS) and PFS were calculated using Kaplan-Meier method. Adverse events (AE) were evaluated with CTCAE 5.0. **Results:** The eligible population was 101, composed of 66 males and 35 females. Among them, 54% patients had one of KRAS or NRAS or BRAF gene mutation and ECOG of 62% patients was 1. In addition, 59% patients were refractory to second standard line or above systemic therapy and mean interventional treatment cycles were 3.3. As the follow-up cutoff date was Dec 31, 2022, median duration of follow-up was 17.9 months (95% CI, 16.185-19.615). Median hPFS was 8.7 months (95% CI, 6.744-10.658) while median PFS was 6.2 months (95% CI, 5.048-7.352). For the patients who were refractory to second line or above systemic therapy, hPFS and PFS was 6.2 months (95% CI, 4.899-7.501) and 5.2 months (95% CI, 3.682-6.718) respectively. Overall survival has not been reached yet. There were 7 patients achieved clinical complete response. Overall response rate was 41.6% and disease control rate was 82.2%. There was no treatment-related death. 28 patients (27.7%) experienced grade 3 or higher toxicities. The most common treatment related AE were aspartate transaminase/alanine transaminase elevation (41.6%) and bilirubin elevation (40.6%). The hematologic AE included anemia (27.7%), leukopenia (27.7%), neutropenia (14.9%) and thrombocytopenia (28.7%). **Conclusions:** The combination of Irinotecan-eluting HepaSphere chemoembolization and HAIC is effective and safe for unresectable CRCLM, even for patients who are refractory to second or above systemic therapy, indicating it is a promising regional treatment with improved outcome. Research Sponsor: None.

# **Efficacy and safety of IBI351 (GFH925) monotherapy in metastatic colorectal cancer harboring KRAS<sup>G12C</sup> mutation: Preliminary results from a pooled analysis of two phase I studies.**

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**Background:** IBI351 (GFH925) is an irreversibly covalent inhibitor of KRAS<sup>G12C</sup> and has demonstrated promising anti-tumor activity with acceptable safety in advanced solid tumors. Here, we report a pooled analysis of two phase I studies (NCT05005234, NCT05497336) evaluating the efficacy and safety of IBI351 (GFH925) monotherapy for metastatic colorectal cancer (CRC) harboring KRAS<sup>G12C</sup> mutation. **Methods:** Eligible metastatic CRC patients (pts) with KRAS<sup>G12C</sup> mutation were included. Pts received IBI351 (GFH925) orally at dose levels of 700mg once daily (QD), or 450/600/750mg twice daily (BID). The primary endpoint was objective response rate (ORR) assessed by investigator per RECIST v1.1. Data cutoff for the analyses was November 30, 2022 unless otherwise specified. **Results:** A total of 45 pts were enrolled (median age: 58.0 years; male: 57.8%; ECOG PS 1: 71.1%; pts with ≥2 prior lines of treatment: 66.7%), including 3, 4, 37, and 1 pts in 700mg QD, 450mg BID, 600mg BID, and 750mg BID cohorts, respectively. The median exposure to therapy was 84 days (range, 7-286), and 36 pts (80.0%) were still on treatment including one patient at 450mg BID with treatment exposure for 286 days. As of December 15, 2022, ORR was 47.5% (19/40, 95% CI: 31.5%-63.9%) and disease control rate (DCR) was 85.0% (95% CI: 70.2%-94.3%) for the efficacy-evaluable pts across all dose levels. The median duration of response (DOR) was not reached. For 32 efficacy-evaluable patients at 600mg BID, ORR and DCR were 43.8% (14/32) and 87.5%, respectively. Treatment-related adverse events (TRAEs) occurred in 40 (88.9%) pts, with the most common being anaemia, white blood cell count decreased, alanine aminotransferase increased, and pruritus. Grade ≥ 3 TRAEs occurred in 9 (20.0%) pts. No drug-related adverse events leading to treatment discontinuation or death occurred. **Conclusions:** IBI351 (GFH925) monotherapy demonstrated promising anti-tumor activity with manageable safety profile in metastatic CRC patients harboring KRAS<sup>G12C</sup> mutation. These two studies are still ongoing and longer follow-up will provide more solid evidence. Updated data will be presented at the meeting. Clinical trial information: NCT05005234, NCT05497336. Research Sponsor: Innovent Biologics (Suzhou) Co., Ltd.

### Understanding resistance in V600E BRAF advanced colon cancer treated with BRAF inhibitors plus anti-EGFR antibodies +/- MEK inhibitors: The URBAN study.

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**Background:** The combination of BRAF inhibitors (BRAFi) plus anti-EGFR antibodies is a new standard of care in V600E BRAF mutated (mut) metastatic colorectal cancer (mCRC). Nevertheless, resistance develops during the target therapy (TT). We designed the URBAN study, a translational prospective project, in order to identify possible primary and acquired resistance mechanisms. **Methods:** Patients (pts) with V600E BRAF mut mCRC treated with BRAFi + anti-EGFR +/- MEK inhibitors at Veneto Institute of Oncology were enrolled. Clinical data and liquid biopsy at baseline and progression were collected. The ctDNA derived from plasma was analyzed by the AVENIO expanded kit, a hybridization capture sequencing-based 77 genes pan-cancer assay contained in NCCN Guidelines. Survival outcomes were calculated using Kaplan–Meier curves, log-rank tests and univariate Cox regression models were also performed. The study is exploratory and no formal hypothesis has been postulated. **Results:** Forty consecutive V600E BRAF mut mCRC pts were enrolled. Median age was 63 years (42–77), 47.5% of pts were males. Right CRC were 65% and 20% were MSI-H. Only 5% of pts received TT after second line; doublet regimen was administered in 60% of pts while triplet in 40%. According to the mPFS of doublet arm in the BEACON trial (4.2 months, mo), our population was divided in responder (R), 24 (60%), and non-responder (NR), 16 (40%). In R vs NR group, mPFS was 9 vs 3.2 mo while mOS was 21.6 vs 10.7 mo, respectively. The V600E BRAF mut was detected in 85% of the pre-treatment plasma samples without statistically significant differences in the genomic alterations between R and NR groups, but there was a higher frequency of *MET* and *EGFR* amplification in NR group. At progression, the mutation of *BRAF* was lost in 2 cases in R group. After receiving TT, the most common acquired mutations involved *RAS* genes: 16 pts (40%) acquired at least one activating mutation in *KRAS* and/or *NRAS*. Among these, 9 pts showed multiple mutations of the same *RAS* gene probably due to both intra- and inter-lesional heterogeneities; none of these pts had MSI-H mCRC. We found a higher number of *RAS* and *MAP2K1* acquired mutations in NR and a trend to acquire *EGFR* amplification in R group. Inactivating mutations in *RNF43* gene was observed in 2 cases in R group. These data did not reach statistical significance, probably due to the low number of cases. Interestingly, 37% of NR pts acquired three or more molecular alterations vs 13% in R group. Furthermore, a higher number of genetic alterations was acquired in pts treated with doublet vs triplet regimen. **Conclusions:** This prospective, observational molecular profiling study provided further evidences to support the use of ctDNA in capturing the dynamic somatic mutational spectrum in V600E BRAF mut mCRC and to identify potential mechanisms of resistance to TT. An expansion of study population is ongoing. Research Sponsor: Partially funded by the Italian Ministry of Health grant “Ricerca Finalizzata Giovani Ricercatori” [grant number GR2019-12368903].

### Optimal molecular-targeted therapies as first-line treatment for *RAS* wild-type, right-sided metastatic colorectal cancer from the Analysis and Research in Cancers of the Digestive System (ARCAD) database.

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**Background:** Although anti-EGFR antibody plus doublet chemotherapy has become the standard of first-line treatment for *RAS* wild-type (wt), left sided metastatic colorectal cancer (mCRC), the optimal molecular-targeted therapy for *RAS* wt, right-sided mCRC is not clear. **Methods:** To evaluate the efficacy between anti-EGFR antibody vs. bevacizumab combination therapy, from 40,889 Individual patient (pt) data from 59 studies in ARCAD mCRC database, 723 pts with *RAS* wt, right-sided mCRC who received a first-line molecular-targeted therapy with backbone chemotherapy (FOLFOX/FOLFIRI when anti-EGFR antibody combination, and FOLFOX/FOLFIRI/CAPOX/FOLFOXIRI when bevacizumab combination) were selected from 10 randomized studies (FIRE-3, CALGB80495, CRYSTAL, PRIME, CAIRO2, OPUS, TRIBE, TRIBE2, ATOM, and CCOG1201). Primary objective was overall survival (OS), and secondary objectives were progression-free survival (PFS) and overall response rate (ORR); Hazard ratio (HR) and Odds ratio (OR) were adjusted for ECOG-PS, age, and gender. Propensity score matching (PSM) and inverse probability treatment weighting (IPTW) as sensitivity analysis were also performed. Secondary analyses were limited to *BRAF* wt pts. **Results:** Anti-EGFR antibody and bevacizumab were administrated in 329 and 394 pts, respectively, of whom 162 and 151 pts were *BRAF* wt, respectively. Baseline characteristics were as follows (anti-EGFR antibody/bevacizumab): median age, 63.0 years both; female, 47.1/46.4%; and ECOG-PS 0, 53.2/65.2%. The OS and PFS were significantly prolonged in pts receiving bevacizumab combination therapy compared to those receiving anti-EGFR antibody combination therapy (Table). These trends were more pronounced when limited to *BRAF* wt pts (Table). There were no significant differences in ORR for both overall and *BRAF* wt pts. These results were similar in the sensitivity analysis (Table). **Conclusions:** For patients with *RAS* wt or *RAS/BRAF* wt, right-sided mCRC, bevacizumab combination therapy is preferred first-line treatment. Research Sponsor: ARCAD, ARCAD Asia.

|                    |                           | Median OS, months | Median PFS, months | ORR, %           |
|--------------------|---------------------------|-------------------|--------------------|------------------|
| <i>RAS</i> wt      | Anti-EGFR vs. bevacizumab | 18.4 vs. 21.5     | 8.0 vs. 9.4        | 57.5 vs. 56.6    |
|                    | HR/OR (95%CI)             | 1.19 (1.01-1.40)  | 1.21 (1.03-1.42)   | 1.11 (0.81-1.52) |
|                    | PSM; HR/OR (95%CI)        | 1.27 (1.07-1.50)  | 1.27 (1.08-1.49)   | 1.01 (0.86-1.19) |
|                    | IPTW; HR/OR (95%CI)       | 1.28 (1.09-1.51)  | 1.26 (1.08-1.48)   | 1.03 (0.88-1.22) |
| <i>RAS/BRAF</i> wt | Anti-EGFR vs. bevacizumab | 18.7 vs. 25.8     | 8.3 vs. 11.1       | 63.5 vs. 66.0    |
|                    | HR/OR (95%CI)             | 1.52 (1.18-1.96)  | 1.68 (1.31-2.17)   | 0.98 (0.59-1.61) |
|                    | PSM; HR/OR (95%CI)        | 1.49 (1.15-1.92)  | 1.68 (1.30-2.16)   | 0.98 (0.76-1.27) |
|                    | IPTW; HR/OR (95%CI)       | 1.57 (1.21-2.03)  | 1.67 (1.30-2.14)   | 1.11 (0.84-1.47) |

**Overall survival of patients with BRAF-mutant metastatic colorectal cancer treated with encorafenib-cetuximab in a real-world nationwide study in the Netherlands.**

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**Background:** Approximately 10-15% of patients with metastatic colorectal cancer (mCRC) have BRAF<sup>V600E</sup> mutated tumors. Encorafenib plus cetuximab (EC) combination treatment was approved following the results of the randomized phase III BEACON trial and is currently recommended for all pretreated patients with BRAF<sup>V600E</sup>-mutant mCRC. Selection of patients in randomized controlled trials is based on restrictive eligibility criteria and often not representative of the patient population in daily clinical practice. Complementary real-world effectiveness studies can improve knowledge on the generalizability of trial results, and support decision making in clinical practice. **Methods:** This population-based study included all mCRC patients in the Netherlands treated with EC since approval in October 2020 until June 2022. Individual patient data and original pathology reports were collected. Primary endpoint was overall survival (OS), defined as time from EC initiation until death by any cause, analyzed by Kaplan-Meier curves. WebPlotDigitizer was used to reconstruct the Kaplan-Meier survival curve of the BEACON trial. Uni- and multivariable analyses were performed. Subgroup analyses were conducted for patients who would have been eligible for the BEACON trial based on key eligibility criteria: BRAF<sup>V600E</sup> mutation, 1-2 prior regimens, world health organization performance status (WHO PS) of 0-1, normal neutrophil count, absence of brain metastases, no prior RAS/RAF/MEK treatment, and no concurrent malignancies. **Results:** 155 patients were included with a median follow-up time of 11.1 months. Patient characteristics differed from BEACON in mean age (64 *versus* 60 years), primary tumor sidedness (69% *versus* 50% right-sided) and WHO PS (23% *versus* 51% WHO 0). Median OS of the total real-world cohort was 6.6 months (95% CI: 6.0-8.3) and differed significantly from BEACON (9.3 months; 95% CI: 8.0-11.3;  $p = 0.003$ ). 35% of real-world patients treated with EC would not have been eligible for the BEACON trial, showing an inferior median OS of 6.0 months (95% CI: 4.6-9.2) compared to 7.0 months (95% CI: 6.4-9.8) in trial eligible real-world patients. WHO PS was independently associated with poor survival in multivariable analysis. Patients with WHO PS  $\geq 2$  had a median OS of 3.9 months (95% CI: 2.4-NA) with a hazard rate of 2.5 (95% CI: 1.1-5.5;  $p = 0.003$ ). **Conclusions:** This largest to date - unselected - population-based cohort of mCRC patients treated with EC showed an efficacy-effectiveness gap for OS. These outcomes should be considered in clinical practice for treatment decision making. Omitting EC for subgroups demonstrating very short OS, such as patients with WHO PS  $\geq 2$ , needs to be considered. Further research is warranted to adapt treatment guidelines towards a more personalized approach for patients with BRAF<sup>V600E</sup>-mutated mCRC. Research Sponsor: Zorginstituut.

### Phase II study of biweekly TAS-102, irinotecan and bevacizumab in pre-treated metastatic colorectal cancer (TABAsCO).

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**Background:** The efficacy of 2<sup>nd</sup> line FOLFIRI plus VEGF inhibition (VEGFi) is limited for metastatic colorectal cancer (mCRC). TAS-102 is a novel oral anti-metabolite approved for pts with mCRC following standard treatment with FP, oxaliplatin and irinotecan. TAS-102 has a distinct mechanism of action from 5-FU and overcomes 5-FU resistance in preclinical models, and in the clinic. We hypothesized that the combination of TAS-102 with irinotecan (TAS-IRI) and bevacizumab (BEV) would prove superior to FOLFIRI/VEGFi. **Methods:** Phase II, single-arm study of TAS-IRI and BEV in 2<sup>nd</sup>-line mCRC. Eligible patients had received prior treatment with a fluoropyrimidine and oxaliplatin in the advanced setting or experienced recurrence within 12 mos of this regimen in the adjuvant setting. Adequate organ function, ECOG PS 0-1, measurable disease (RECIST 1.1), no prior irinotecan or TAS-102 exposure and candidacy for BEV were required. Pts were treated with BEV 5 mg/kg IV, irinotecan 180 mg/m<sup>2</sup> IV (day 1 and 15), and TAS-102 25 mg/m<sup>2</sup> orally bid (days 2-6 and 16-20) in 4-week cycles (PMID: 31924737). Treatment continued until disease progression or unacceptable toxicity. The primary endpoint was median progression-free survival (PFS). Assuming a median PFS for historic control (FOLFIRI/VEGFi) of 6 mos vs. 9 mos for TAS-IRI plus BEV (HR: 0.67), treatment of 36 evaluable pts would achieve 80.5% power (at  $\alpha = 0.1$ ) to detect such an effect. **Results:** Forty-nine patients were enrolled; 42 were eligible and 38 had at least one disease assessment (median age 59 yr, 47% male, 74% colon primary, tumor KRAS/NRAS mutated in 55%/21%). The median PFS was 8.7 mos (range: 6.7-11.8 mos,  $p = 0.009$ ), meeting the primary endpoint. The most common reason for study treatment discontinuation was progressive disease (53%). The objective response rate was 16% (90% CI: 8.0-27.3%); the median OS was 16.5 mo (range: 11.8-25.3). Twenty-eight pts (67%) had a treatment-related adverse event (TRAE) G3 or higher (Table). The most common TRAEs were gastrointestinal and hematologic. Grade 3/4 neutropenia occurred in 34%, with only 1 episode of febrile neutropenia, though G-CSF use was frequently needed to maintain dose density/intensity. **Conclusions:** TAS-IRI plus BEV is an effective 2<sup>nd</sup> line therapy for patients with mCRC. The median PFS appears higher compared to historical controls (FOLFIRI/VEGFi). Neutropenia is common and can affect dose density/intensity mandating use of G-CSF. A randomized study versus standard of care therapy is warranted. (Clinical trial registration ClinicalTrials.gov NCT04109924). Clinical trial information: NCT04109924. Research Sponsor: NCCN Oncology Research Program.

| TRAE                      | Total (%) | Grade 3/4(%) |
|---------------------------|-----------|--------------|
| Neutropenia               | 27 (71)   | 13 (34)      |
| Diarrhea                  | 25 (66)   | 6 (16)       |
| Nausea                    | 25 (66)   | -            |
| Fatigue                   | 22 (58)   | 1 (3)        |
| Alopecia                  | 14 (37)   | -            |
| Anemia                    | 12 (32)   | 5 (13)       |
| Anorexia                  | 12 (32)   | 1 (3)        |
| Vomiting                  | 11 (29)   | -            |
| Thrombocytopenia          | 9 (24)    | 2 (5)        |
| Constipation              | 7 (18)    | -            |
| Epistaxis                 | 7 (18)    | -            |
| Infusion related reaction | 6 (16)    | -            |
| Abdominal pain            | 5 (13)    | 3 (8)        |

**Real-world treatment trends and outcomes in elderly patients with metastatic colorectal cancer in the United States.**

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**Background:** Prospective data to guide management of elderly patients with metastatic colorectal cancer (mCRC) is limited. Whether elderly patients derive the same degree of benefit from doublet chemotherapy as younger patients is not well understood. In this study we utilized real-world data from patients in the United States (US) to evaluate treatment trends and compare overall survival (OS) in younger versus older patients with mCRC who receive first-line (1L) chemotherapy. **Methods:** The nationwide Flatiron Health electronic health record-derived de-identified database was used to select patients  $\geq 50$  years old (yo) with mCRC treated with 1L fluoropyrimidine-based chemotherapy with or without the addition of targeted therapy. Overall survival from the initiation of 1L therapy, stratified by receipt of a doublet or single agent cytotoxic chemotherapy regimen was assessed independently within three age cohorts (50-69; 70-74,  $\geq 75$ ) using both a cox proportional hazard model and a propensity score weighted analysis to control for potential confounding variables. A subgroup analysis was performed in patients receiving bevacizumab as part of 1L therapy. **Results:** Of 14,440 patients who received 1L chemotherapy, 5,874 (40.7%) were  $\geq 70$  yo, including 3,656 (25.4%) who were 75 or older. Doublet chemotherapy was received by 7,505 (87.6%) patients age 50-64 yo, 1,892 (85.3%) patients age 70-74 yo, and 2,297 (62.2%) patients age 75 and above. In all three groups, multivariate analysis of single agent chemotherapy vs doublet chemotherapy showed a benefit to doublet chemotherapy with a hazard ratio of 1.14, (95% CI 1.04-1.24,  $p = 0.006$ ), 1.22 (95% CI 1.05-1.42,  $p = 0.011$ ) and 1.19 (95% CI 1.10-1.29,  $p < 0.001$ ), respectively. Similar results were noted when comparing doublet to single agent chemotherapy in the age 70-74 and  $\geq 75$  groups using a propensity score weighted analysis. In the subgroup of patients age  $\geq 70$  who received 1L bevacizumab, 2,604 received doublet chemotherapy and 441 received single agent chemotherapy. Median OS was 19 mos (95% CI: 18-20) vs 13 (95% CI: 11-15) vs with a propensity score weighted HR of 1.35 (95% CI 1.12-1.63,  $p = 0.001$ ). **Conclusions:** A smaller percentage of patients over age 75 receive 1L doublet chemotherapy for mCRC in the US compared to patients age 50-69 or 70-74. Regardless of age, patients who receive doublet fluoropyrimidine-based chemotherapy appear to have improved overall survival compared to single agent chemotherapy. This retrospective US data is in contrast with recently presented data from a prospective phase III trial in Japan (JCOG1018). Elderly patients in the US should not be excluded from receiving doublet chemotherapy for mCRC without further prospective data. Research Sponsor: None.

**MicroOrganoSpheres as a clinically applicable precision oncology platform for the discovery of novel therapies in colorectal cancer.**

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**Background:** Patient-derived models of cancer, such as cell lines, patient-derived organoids, and patient-derived xenografts, are useful models of patient response in the clinic. However, these models are often not clinically applicable within the time periods necessary to inform clinical decision making, as they can take weeks to months to develop. An ideal platform using patient-derived models would be generated from a core biopsy with a subsequent drug screen within 10-14 days to minimize delay in therapy. We recently reported the development of MicroOrganoSpheres (MOS) that can be used in drug screens within 14 days of obtaining a biopsy. In the current study, we use this MOS system as precision oncology platform in colorectal cancer (CRC) to identify new therapies and predict response to therapy.

**Methods:** CRC patient tissue samples were collected under a Duke Institutional Review Board approved protocol (Pro00089222). Resections or biopsies were mechanically and enzymatically digested to obtain a single cell suspension. Cells were then plated in Matrigel at a ratio of 20,000 cells:5  $\mu$ L Matrigel to establish “mini-bulk” organoid cultures. After establishment for 5-7 days, cultures were harvested with subsequent generation of MOS at a ratio of 50 cells per MOS. After growing for 3-4 days, MOS were used for dose-response curves using oxaliplatin, SN38, and 5-Fluorouracil (5-FU) as well as high-throughput drug screens with the NCI Approved Oncology Drugs Set VI library. **Results:** We developed and optimized a MOS pipeline on over 50 CRC specimens, including 9 primary rectal, 35 primary CRC, 12 CRC liver metastasis, and 1 CRC lung metastasis lines with a success rate of 80% and an average of 10-21 days from biopsy to MOS generation. The high success of generating CRC MOS in a clinically applicable time frame led to the next phase of the project where a total of 10 CRC MOS were tested against standard of care chemotherapy agents used in CRC (oxaliplatin, irinotecan and 5-FU) as well as the NCI Approved Oncology Drugs Set VI within 14-21 days of establishment. We noted a range of sensitivity of approximately 100-fold for standard of care agents. The most sensitive drugs found in the high-throughput screen were Bortezomib, Carfilzomib, and Panobinostat and the most resistant were Gefitinib, Chlorambucil, and Procarbazine hydrochloride. **Conclusions:** These results demonstrate that our MOS pipeline can be used as a precision oncology platform within a clinically applicable time frame to potentially guide therapy. We are now in the process of correlating drug response in MOS to patient outcome data and these findings will be presented at the annual meeting. Research Sponsor: Discretionary funds.

### Management of Early-onset Metastatic Colorectal Cancer (ERMIONE): A single institution analysis.

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**Background:** Despite rising incidence and mortality reported worldwide for CRC diagnosed in pts aged < 50 yrs, currently early onset metastatic CRC (EOMCRC) are treated as their older counterparts. We aimed to investigate how this specific subgroup is treated in real world. **Methods:** This an observational, retrospective, monocentric study aiming to describe features, management and prognosis of EOMCRC. Pts with EOMCRC treated at our Institution between Apr2002 and Dec2022 were included. Applying a descriptive method, counts and percentages were reported for categorical variables, while median and range for continuous variables. PFS and OS were estimated with the Kaplan-Meier method. A multivariate Cox regression analysis was performed. **Results:** 172 pts were included, of those 60.5% were female and 66% had an ECOG PS of 0. Median age at diagnosis was 43 yrs (range 12-49 yrs). Metastatic disease was mainly synchronous (72.1%), while only 12.2% and 15.7% were stage II and III at diagnosis and developed metachronous metastases. Primary tumor was left-sided in 70.1%. Metastatic site was most frequently liver (67.4%), followed by peritoneum (41.3%), lungs (33.7%), ovary (23.2%) and bones (9.9%). Disease was mostly widespread, while only 30.2% had a single metastatic site. MMR status was available for 87.2% of pts, being proficient in 90% and deficient in 10%. RAS/BRAF status was available for 95.3% of cases, of those 47.5% was RAS/BRAF wt, 48.2% was RAS mt and 4.3% was BRAF mt. 42.4% of cases had a family history positive for cancer. Germline pathogenic or likely pathogenic variants were identified in 6.4% of cases, of those 63.6% involved MMR genes and 18.2% involved HRD genes. Median number of lines of treatment received was 2 (range 1-6). Most frequent first line regimen was a doublet CT (69.8%), followed by a triplet CT (23.8%) and immunotherapy (4.1%), CT regimens were associated to bevacizumab in 45.3% of cases and to antiEGFRs in 29.1% of cases. Throughout the whole continuum of care 8.7% of pts received immunotherapy and 21.5% received treatment within a clinical trial. 70.3% of pts received surgery and/or local ablative treatments (LATs) with radical intent (52.9% surgery, 12.2 both, 4.6% LATs). At a median FU of 38.6 m, mPFS for first line was 13.5 m (95%CI 12.1-15.0 m) and mOS was 41.5 m (95%CI 33.9 - 44.1 m). Median OS was significantly longer for pts who received surgery and/or LATs compared to those who did not (43.4 vs 23.6 m,  $p < .0001$ ). At multivariate analysis, besides surgery and/or LATs ( $p = .0007$ ), BRAF status ( $p = .0165$ ) and ECOG PS ( $p = .0209$ ) independently correlated with OS. **Conclusions:** We confirmed that EOMCRC is more frequently diagnosed as synchronous disease, due to delayed diagnosis. Despite the small population and the retrospective nature, we showed that combining surgery and/or LATs to systemic therapy is associated with increased OS in EOMCRC. These evidence warrant further validation in prospective setting. Research Sponsor: None.

### Effect of trifluridine/tipiracil in combination with bevacizumab on ECOG-PS in refractory metastatic colorectal cancer: An analysis of the phase 3 SUNLIGHT trial.

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**Background:** SUNLIGHT, an international, open-label, randomized, phase 3 study comparing trifluridine/tipiracil (FTD/TPI) in combination with bevacizumab versus FTD/TPI monotherapy in patients with metastatic colorectal cancer (mCRC) met its primary endpoint (overall survival [OS]) and key secondary endpoint (progression free survival [PFS]). Primary and key secondary outcomes in patients with maintained ECOG-PS are reported. **Methods:** SUNLIGHT enrolled patients with ECOG-PS 0/1. ECOG-PS was evaluated at baseline, at each treatment cycle, and at withdrawal visit. Worst ECOG values and time to ECOG worsening of more than 2 were reported. Here we report a post-hoc analysis assessing OS and PFS in patients who remained at ECOG-PS 0 or 1. **Results:** Of the 492 randomized patients, 491 had ECOG-PS 0 or 1 at baseline. The median OS was improved by 3.3 months with FTD/TPI plus bevacizumab (10.8 months with FTD/TPI plus bevacizumab vs 7.5 months with FTD/TPI), hazard ratio of 0.61 (95% CI, 0.49 to 0.77;  $P < 0.001$ ), and median PFS was of 5.6 months vs 2.4 months favoring the FTD/TPI plus bevacizumab group (hazard ratio, 0.44; 95% CI, 0.36 to 0.54;  $P < 0.001$ ). The results showed that FTD/TPI plus bevacizumab significantly improved time to ECOG-PS worsening from 0 or 1 to  $\geq 2$  when compared with FTD/TPI monotherapy (9.3 months with FTD/TPI plus bevacizumab vs 6.3 months with FTD/TPI (hazard ratio, 0.54; 95% CI, 0.43 to 0.67). Highest ECOG-PS distribution showed comparable proportions over cycles with no clinically meaningful difference ( $> 10\%$ ) in both treatment arms. Considering patients who had discontinued study treatment as of the clinical cut-off date of July 5, 2022, 189/208 (91%) patients in the FTD/TPI plus bevacizumab and 200/235 (85%) patients in the FTD/TPI remained at ECOG-PS 0 or 1 at treatment discontinuation. In patients with maintained ECOG-PS, median OS demonstrated a prolonged survival in the FTD/TPI plus bevacizumab group vs the FTD/TPI group and a decreased death risk (10.58 months [range, 9.03 to 11.24] vs 8.71 months [range, 7.39 to 10.18]; hazard ratio, 0.78; 95% CI, 0.61 to 0.99). PFS consistently showed benefit in patients with maintained ECOG 0 or 1 favoring FTD/TPI plus bevacizumab (5.22 months [range, 4.17 to 5.75] vs 2.55 months [range, 2.1 to 3.58]; hazard ratio, 0.49; 95% CI, 0.4 to 0.61). **Conclusions:** Consistent with the results of the overall study population, FTD/TPI in combination with bevacizumab prolonged overall survival and PFS in patients with maintained ECOG-PS. Maintenance of performance status may allow patients to receive further therapeutic options during the continuum of care. Clinical trial information: NCT04737187. Research Sponsor: Institut de Recherches Internationales Servier; Taiho Oncology.

**PolyPEPI1018 vaccine in combination with TAS-102 in participants with late-stage microsatellite-stable metastatic colorectal cancer (MSS mCRC): A phase Ib study to evaluate safety, tolerability, immunogenicity and efficacy (OBERTO-201).**

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**Background:** PolyPEPI1018 is an off-the-shelf, multi-peptide vaccine containing 12 immunogenic epitopes derived from 7 tumor-specific antigens frequently expressed in patients with mCRC. Following early evidence of clinical activity of PolyPEPI1018 in first-line MSS mCRC, here we report the results of a phase Ib study of PolyPEPI1018 vaccine plus trifluridine/tipiracil (TAS-102) in late-stage mCRC patients. **Methods:** Patients with MSS mCRC who have progressed on  $\leq 2$  lines of prior chemotherapy regimen for mCRC received PolyPEPI1018 subcutaneously on days 1 and 15 and TAS-102 orally twice daily on days 1-5 and 8-15 of a 28-day cycle. Treatment continued for up to 7 cycles, until disease progression or unacceptable toxicity. Immunomonitoring was performed at both blood and tumor levels prior to and on study treatment. The primary endpoint of the study was safety and tolerability. Data on objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), overall survival (OS) and correlation studies will be presented. **Results:** 15 patients started treatment. Median age was 55 years (range 31–71), 73% had liver metastases (mets), 73% had KRAS mutant tumors. The most common side effect related to PolyPEPI1018 was Grade (Gr) 1-2 local skin reactions in 93% of patients. Gr 3 events (n = 15) at least possibly related to treatment were fatigue, decreased white blood cell, lymphocyte and neutrophil counts, nausea, diarrhea, myalgia, and maculo-papular rash. There were no Gr 4 or 5 events. The ORR was 0%. The DCR was 53.3% in the overall population and 36% and 100% for the patients *with* liver mets and *without* liver mets, respectively. The mPFS was 4 months (95%CI 2.2-6.1) in the overall population and 2.3 months (95%CI 2.1-NE) and 7.6 months (95%CI 4.4-NE) for the patients *with* liver mets and *without* liver mets, respectively (HR = 5.73 (95%CI 1.21, 27.06); p = 0.015). At the data cut-off date (Feb 10, 2023), the mOS was 8.7 months (95%CI 6.9-NE; not mature) in the overall population and it was not reached in patients *without* liver mets. Patients with increased PFS ( $\geq 24$  weeks) had more robust vaccine-specific humoral and T cell responses induced by higher number of vaccine peptides than patients with PFS < 24 weeks. **Conclusions:** To our knowledge, this is the first study investigating combination of a cancer vaccine with TAS-102 chemotherapy in advanced MSS mCRC. Our results show that PolyPEPI1018 plus TAS-102 was well-tolerated with few grade 3 AEs beyond what is expected with TAS-102 monotherapy. Despite no objective tumor responses could be detected, correlative data suggest contribution of PolyPEPI1018-induced immunological responses to the improved clinical benefit and the combination warrants further testing. Clinical trial information: NCT05130060. Research Sponsor: US Department of Defense.

**VIC regimen (vemurafenib/irinotecan/cetuximab) versus bevacizumab plus chemotherapy as first-line treatment for *BRAF V600E*-mutated advanced colorectal cancer.**

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**Background:** The need for safe and effective therapies for untreated, *BRAF V600E*-mutated, unresectable locally advanced or metastatic colorectal cancer remains unmet. The purpose of this study was to compare the efficacy and safety of the VIC (Vemurafenib/Irinotecan/Cetuximab) regimen versus bevacizumab plus chemotherapy as first-line setting in Asian patients. **Methods:** In the single-center prospective cohort study, 78 untreated, *BRAF V600E*-mutant, locally advanced or metastatic CRC patients were enrolled. Every two weeks, the VIC regimen and bevacizumab plus doublet or triplet chemotherapy are administered. We evaluated the objective response rate (ORR), the disease control rate (DCR), and the conversion resection rate. In progression-free survival (PFS) and overall survival, the Kaplan-Meier method was utilized (OS). **Results:** In the evaluable population, 32 patients received VIC regimen and 35 patients received bevacizumab plus chemotherapy. The ORR and DCR in the VIC group were significantly higher than in the bevacizumab-therapy group (ORR: 68.8% versus 40.0%,  $P = 0.018$ ; DCR: 100.0% versus 80.0%,  $P = 0.023$ ). The VIC regimen was significantly superior to bevacizumab plus chemotherapy for PFS (median, 11.9 vs 7.9 months; hazard ratio [HR] = 0.48, 95% CI, 0.27-0.87;  $P = 0.012$ ) and OS (median, 24.5 vs 14.4 months; HR = 0.36, 95% CI, 0.16-0.78;  $P = 0.007$ ). In the VIC group, the conversion resection rate for liver metastases was 42.1% (8 of 19 patients), and for local CRC it was 66.7% (6 of 9 patients with initially unresectable local CRC). Rates of treatment-related adverse events of Grade 3 to 4 were 32.4% and 31.1% for the VIC regimen and bevacizumab plus chemotherapy, respectively. **Conclusions:** Among Asian patients with *BRAF V600E*-mutated advanced CRC, the VIC regimen was superior to bevacizumab plus chemotherapy in terms of tumor response and oncological survival, with a tolerable and manageable toxicity profile in the first-line setting. Clinical trial information: NCT05540951. Research Sponsor: None.

### Upfront vs deferred monoclonal antibodies in metastatic colorectal cancer: A target trial emulation using the GEMCAD 14-01 prospective cohort.

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**Background:** There are no randomized trials comparing the addition monoclonal antibodies (MAB: bevacizumab, cetuximab, panitumumab) to first line chemotherapy (upfront use) versus deferring their addition to the second-line chemotherapy (deferred use) in pts with metastatic colorectal cancer (mCRC). We emulated a target trial comparing upfront vs deferred use of MAB using the GEMCAD 14-01 observational registry. **Methods:** We first specified the (hypothetical) target trial to fully articulate the research question and then emulated it using real-world data. The eligibility criteria of the target trial were a diagnosis of mCRC, being treatment naïve, and a ECOG PS <2. The target trial would randomize pts to the following strategies: (1) initiation of MAB within 2 months of starting first line chemotherapy ("upfront MAB") and (2) initiation of MAB within 2 months of starting second line chemotherapy ("deferred MAB"). The primary outcome of the target trial would be overall survival and the causal contrast (or estimand) would be the effect under complete adherence. We emulated this target trial using data from the GEMCAD 1401 registry (ClinicalTrials.gov identifier: NCT02254941), which collected data prospectively from 47 Spanish centers from June 2014 to June 2018. The emulation used the same definitions of eligibility criteria and treatment strategies, and classified individuals according to their baseline data using clones. The effect under complete adherence was estimated by censoring pts when they deviated from the assigned treatment strategy and by using time-varying weights to adjust for baseline and post-baseline confounding. **Results:** A total of 627 pts were eligible in the "upfront MAB" and 397 pts in the "deferred MAB". Median age was in the "upfront vs deferred" 64.6 (interquartile range: 56-71) vs 67.8 (61-75) years, 96% vs 87% had an ECOG 0-1, 80% vs 79% had a Charlson score < 3, 46% vs 60% had a RAS mutation, 6% vs 4% had a BRAF mutation, 72% vs 69% had left side primary location, 74% in both strategies had liver metastasis and 42% vs 50% had LDH levels above the normal threshold. Pts in the "upfront MAB" group contributed a total 16,057 months of follow-up and 502 if them died. Pts in the "deferred MAB" contributed a total of 7,774 months of follow-up, and 222 of them died. The 48-month overall survival was 26.7% (95% CI 21.4-38.2%) in the "upfront MAB" group and 21.6% (14.2-41.7%) in the "deferred MAB" group, corresponding to a 48-month survival difference ("upfront MAB" is the reference) of -5.0% (95% CI -17.9 to 17.95) and a hazard ratio of 1.15 (0.88-1.40). **Conclusions:** Our study suggests little or no survival detrimental effect of deferring the use of MAB to the second line of treatment compared with the use of MAB as part of the first line of treatment among pts with mCRC. Clinical trial information: NCT02254941. Research Sponsor: Supported by Instituto Carlos III PI13-01728 and an unrestricted support grant from Grupo Español Multidisciplinar en Cancer Digestivo (GEMCAD).

### Preoperative assessment in lung-limited metastatic patients with colorectal cancer: The Meta-Lung Score.

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**Background:** Due to the inconsistent data on curative resection of isolated lung metastases (LM) from colorectal cancer (CRC) and the lack of specific recommendations, the therapeutic approaches for metastatic liver disease are mostly applied to LM treatment, despite differences in their biological behavior. Like Fong's criteria in CRC liver metastases, our study proposed a score associated with clinical outcomes in patients (pts) undergoing CRC lung metastasectomy, aiming to better pts' selection for surgery and the most appropriate therapeutic strategy. **Methods:** We retrospectively collected data from 260 pts (aged 18-85) who underwent CRC lung metastasectomy with curative intent from December 2002 to January 2022 at four Italian Centers: the Division of Thoracic Surgery at "A. Businco Cancer Center" in Cagliari, the Division of Thoracic Surgery at "Città della Salute e della Scienza" in Tourin, the Department of Thoracic Surgery at "IRCCS Azienda Ospedaliero-Universitaria" in Boulogne, and the Medical Oncology Unit of the University Hospital of Cagliari. Statistical analysis was performed with MedCalc (survival distribution: Kaplan-Meier; survival comparison: log-rank test; association between categorical variables: Fisher's exact test). **Results:** We analyzed the impact of different clinicopathological features on overall survival (OS). At the univariate analysis: higher baseline CEA levels ( $p = 0.0001$ ), disease-free survival less than or equal to 12 months (m) ( $p = 0.0043$ ), LM size larger than 2 cm ( $p = 0.0187$ ), multiple resectable nodules ( $p = 0.0083$ ), and positive nodal status of the primary tumor ( $p = 0.0011$ ) were associated with poorer prognosis. In a Cox regression model, these five characteristics retained their independent role for OS ( $p < 0.0001$ ) and were chosen as criteria to be assigned one point each for clinical risk score. The 5-year survival rate in pts with 0 points was 88%, while no pts with a 5-points score survived at 2 years. Based on the 0-2 versus 3-5 score range, we obtained a significant difference in median OS: 101.7 m (95%CI 64.6 - 101.7) vs. 39.5 m (95%CI 27.3 - 87.5) respectively ( $p < 0.0001$ ) stratifying pts into good and poor prognosis. **Conclusions:** The Meta-Lung Score appeared to be an exciting prognostic tool in selecting CRC pts' candidates for radical surgical treatment when LM were diagnosed. Whether early surgery should be considered in pts with a score 0-2, the same choice should be taken with caution in pts with a score 3-5, for whom early chemotherapy would allow better assessment of tumor biology and appropriate selection for surgery. Research Sponsor: None.

| Score | Survival (%) |      |      |      |      | Median OS (m)              |
|-------|--------------|------|------|------|------|----------------------------|
|       | 1-yr         | 2-yr | 3-yr | 4-yr | 5-yr |                            |
| 0     | 97%          | 91%  | 88%  | 88%  | 88%  | NR                         |
| 1     | 97%          | 90%  | 78%  | 71%  | 60%  | NR                         |
| 2     | 97%          | 80%  | 65%  | 54%  | 46%  | 45.9 (95%CI 36.1 to 75.5)  |
| 3     | 90%          | 74%  | 58%  | 45%  | 34%  | 41.7 (95%CI 29 to 87.5)    |
| 4     | 73%          | 72%  | 41%  | 27%  | 27%  | 29.3 (895%CI 8.8 to 62.2)  |
| 5     | 34%          | 0    | 0    | 0    | 0    | 12.3 (95%CI 4.6 to 19.5)   |
| Total | 95%          | 83%  | 70%  | 60%  | 55%  | 75.6 (95%CI 57.6 to 101.7) |

**Targeting EZH2 to overcome the resistance to immunotherapy in microsatellite stable colorectal cancer: Results from the CAIRE study.**

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**Background:** Profound epigenetic and transcriptomic changes induced by EZH2 in tumor cells and immune cells mobilize the elements of the TME, leading to immune-suppressive activity of solid tumors. Targeting EZH2 can enhance anti-tumor immunity by reshaping the tumor microenvironment (TME). The CAIRE study is first study investigating the impact of EZH2 inhibition in combination with immune checkpoint inhibitor in solid tumors. **Methods:** This is a single-arm open-label multicentric phase II trial assessing the efficacy and safety of tazemetostat (800 mg BID) combined with durvalumab (1120 mg every 3 weeks) in patients with advanced microsatellite stable colorectal cancer. The primary endpoint was the disease control rate within 24 weeks after treatment onset defined as the proportion of patients with at least a tumor assessment showing confirmed or unconfirmed complete response, confirmed or unconfirmed partial response or stable disease based on central review according to RECIST 1.1. Secondary endpoints included: 1-year progression free survival (PFS), 1-year overall survival (OS), and safety using NCI-CTCAE v5.0. All patients underwent sequential blood and tissue sampling for translational studies. **Results:** Between July 2021, and June 2022, 47 pts were enrolled at Institut Bergonié (Bordeaux, Centre). Median age was 67.7 (range 39.6 – 84.0). Median number of previous treatment lines was 4: (range 1 – 6). The most common treatment related adverse events were grade 1/2 asthenia, nausea. No death was related to the treatment. Among the 34 pts for whom blinded central review of imaging was available for at least one imaging tumor assessment, best tumor response was confirmed partial response, stable disease and progressive disease in 1 (2.9%), 11 (32.3%) and 22 (64.7%) patients respectively. The disease control rate was 35.3% (95%CI 19.7 – 53.5). **Conclusions:** The CAIRE study met its first endpoint for disease control rate in patients with advanced colorectal cancer. Efficacy data on the whole cohort of patients and full biomarkers analyses will be presented at the meeting. Clinical trial information: NCT04705818. Research Sponsor: Astrazeneca; Epizyme, Inc.

### Molecular landscape and survival outcomes on early phase clinical trials in sporadic young onset colorectal cancer.

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**Background:** Sporadic young onset colorectal cancer (YOCRC, ages 30-49) is a public health crisis with a persistent rise in incidence globally. While a proportion of patients (pts) develop YOCRC due to hereditary predisposition, the majority develop sporadic microsatellite stable (MSS) colorectal cancer with limited options beyond standard of care in advanced settings. We report the early phase clinical trial experience at a large academic cancer center to elucidate clues regarding molecular landscape and impact of clinical trials in refractory MSS YOCRC. **Methods:** Patient and clinical characteristics were summarized using descriptive statistics. Kaplan-Meier method was used to estimate the probabilities of overall survival (OS) and progression free survival (PFS). Log-rank tests were used to assess the differences in OS and PFS between subgroups. Cox proportional hazards regression models were fit to assess the association between OS or PFS and patient characteristics. Statistical analyses were performed using SAS and Splus. **Results:** 240 MSS YOCRC pts were analyzed. Median age 42.8 (20.5-49), > 80% were white and only 10% black. 21% received at least 3 lines of therapy. The top five mutations were *TP53*, *KRAS*, *APC*, *PI3CKA* and *BRAF*<sup>V600E</sup>. Median OS was 39.2 months (95% CI: 34.8 – 42.5 months). *CTNNB1* mutation was associated with a lower risk of death (HR = 0.28, 95% CI: 0.09 – 0.87, p-value = 0.03) while *BRAF*<sup>V600E</sup> mutation was associated with an increased risk (HR = 3.28, 95% CI: 2.00 – 5.38, p-value < 0.001). Multivariable Cox model for OS revealed both *CTNNB1* and *BRAF*<sup>V600E</sup> mutations are significant prognostic factors for OS, unlike *KRAS*. There was no difference in OS or PFS among pts who entered a mutation specific clinical trial vs. those enrolled on an unmatched clinical trial (p = 0.35, p = 0.68, respectively, log-rank test). Median PFS was 1.9 months (95% CI: 1.8 – 2.1 months). *CTNNB1* mutation was associated with a lower risk of progression/death (HR = 0.29, 95% CI: 0.09 – 0.92, p-value = 0.04) while *NRAS* mutation was associated with an increased risk (HR = 2.13, 95% CI: 1.19– 3.80, p-value = 0.01). Among 145 pts who have data on response, 9 had partial response, 41 had stable disease and 95 had progressive disease. *BRAF*<sup>V600E</sup> mutation is associated with a higher probability of response (OR = 6.89, 95% CI: 1.64 – 28.94, p-value = 0.01) and pts enrolled on matched clinical trials had a higher probability of response (OR = 6.23; 95% CI: 1.24 – 31.09, p-value = 0.03). However, increased response did not translate to improved OS. No survival differences were appreciated based on a pts race or BMI. **Conclusions:** *CTNNB1*, *BRAF*<sup>V600E</sup> & *NRAS* mutations may have prognostic implications in YOCRC. Pts enrolled on mutation specific clinical trials did not achieve improved outcomes compared to unselected trials, highlighting aggressive biology, current lack of therapeutic targets and need for novel drug development in sporadic YOCRC. Research Sponsor: None.

### Analysis of fruquintinib adverse events of special interest from phase 3 of the FRESCO-2 study.

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**Background:** Fruquintinib (F) is a highly selective and potent inhibitor of VEGFRs-1, -2 & -3 designed with improved kinase selectivity to minimize potential off-target toxicities. In the phase 3 FRESCO-2 study (NCT04322539), F demonstrated a statistically significant improvement vs placebo (P) in overall survival (hazard ratio, HR = 0.66) and progression-free survival (HR = 0.32), with a manageable toxicity profile and without deterioration in quality of life in heavily pre-treated patients (pts) with metastatic colorectal cancer (mCRC). Here we report the treatment-emergent adverse events of special interest (AESI). **Methods:** FRESCO-2 was a double-blind, P-controlled multiregional study with 2:1 randomization to F + best supportive care (BSC) or P + BSC. F or P was given 5 mg PO, QD, 3 weeks (w) on, 1 w off, in a 28-day (d) cycle. AEs were coded using MedDRA v25.0 and graded (G) by NCI-CTCAE v5.0. Clinically relevant class toxicities were grouped into respective AESI categories. **Results:** The safety population had 686 pts (F: 456 vs P: 230); 47% were  $\geq 65$  yrs, 56.9%/43.1% with ECOG 1/0, 71.6% with hepatic metastases, and 50.5% with medical history of hypertension (HTN) at baseline. The median number of prior lines of anti-cancer treatment for metastatic disease F vs P was 4 (2, 16) vs 4 (2, 12); 96.4% with prior anti-VEGF agent; 91.6% with prior TAS-102; and 47.8% with prior regorafenib. Median duration of exposure was longer with F 3.06 months (m) (range, 1.84-5.5) vs P 1.84 m (range, 0.95-2.27). 368 pts (80.7%) on F and 122 pts (53.0%) on P had an AESI. The most frequent AESIs (all G) in  $\geq 25\%$  of pts (F vs P) were HTN (38.4% vs 8.7%), dermatological toxicity (34.4% vs 11.7%), and thyroid dysfunction (27.0% vs 1.7%).  $G \geq 3$  AESIs in  $\geq 5\%$  of pts in the F vs P, were HTN (14.0% vs 0.9%), hepatic function abnormal (8.3% vs 9.1%), dermatological toxicity (6.8% vs 0.4%), and infections (6.6% vs 5.7%). AESIs (F vs P) that led to dose reductions (DR) occurred in 13.6% vs 0.9% of pts, drug discontinuation (DD) in 8.3% vs 6.1% of pts. AESIs leading to death were 1.8% vs 1.3% of pts, with infections (1.1% vs 0.4%) being most common. The median time to first occurrence of HTN AESI was in cycle (C) 1, with hypertension (96%) being the most common AE reported. In pts with HTN AESI, 9.7% vs 0.6% had dose reduction (DR) and 1.7% vs 0% had DD. The median time to first occurrence of dermatological toxicity was C1, with palmar plantar erythrodysesthesia (PPE) being most common AE reported (56.1%); 27.3% vs 0% had DR and 3.4% vs 0% had DD. The median time to first occurrence of thyroid dysfunction was C2, with hypothyroidism (76.4%) being most common and none with DR or DD. **Conclusions:** F was well tolerated in heavily pretreated pts and despite underlying medical conditions. AESIs including HTN, PPE and thyroid dysfunction were manageable with low rates of dose reductions and dose discontinuation, which further supports F as a potential safe and new treatment option for refractory mCRC. Clinical trial information: NCT04322539. Research Sponsor: HUTCHMED LTD.

**Real-world rates of FDA-approved targeted therapy and immunotherapy prescriptions for patients with metastatic colorectal cancer in the VA's National Precision Oncology Program (NPOP).**

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**Background:** Colorectal cancer is the fourth most common cancer among Veterans and the third leading cause of cancer-related death in the USA. Use of comprehensive genomic profiling (CGP) to guide administration of FDA-approved biomarker directed therapies can improve outcomes among metastatic CRC (mCRC) patients. We sought to compute the rates of actionable biomarkers and prescriptions of associated FDA-approved therapies among Veterans in NPOP. **Methods:** The NPOP database was queried to identify mCRC patients who had undergone CGP via tissue or liquid biopsy between February 2019 and July 2022 and had one of the following 5 actionable biomarker profiles: *NRAS/KRAS/BRAF* wildtype, *BRAF* V600E, MSI-H, TMB-H, or *NTRK* fusion or rearrangement. The VA's Corporate Data Warehouse (CDW) was queried to extract prescription data for seven FDA-approved biomarker-directed therapies (targeted agents and immune checkpoint inhibitors (ICIs)). Rates of CGP-directed therapy prescriptions were assessed based upon biomarker and patient characteristics (sex, race, ethnicity, and rurality). **Results:** A total of 908 mCRC patients underwent CGP, with 81.4% bearing colon adenocarcinoma (COAD) and 18.6% rectal adenocarcinoma (READ). Rates of actionable biomarkers associated with FDA-approved therapies were as follows: *NRAS/KRAS/BRAF* wildtype (34.4%), TMB-H (9.6%), *BRAF* V600E (7.7%), MSI-H (5.6%), TMB-H and MSI-H (5.6%), and *NTRK* Fusion or rearrangement (0.3%). The combined rates of any actionable variant were 47.4% for COAD and 44.4% for READ patients. Relative to patients without actionable biomarkers, patients with *BRAF* V600E mutations were more likely to be older and white; patients with *NRAS/KRAS/BRAF* wildtype were more likely to be younger (all  $p < 0.001$ ). Among the 424 eligible patients, the frequencies of FDA-approved CGP-directed therapy prescriptions were as follows: MSI-H (70.7%), TMB-H (47.4%), *NRAS/KRAS/BRAF* wildtype (38.5%), and *BRAF* V600E (17.1%). Across all included biomarkers, African Americans (53.4%) were more likely to receive these therapies than whites (36.8%); and patients with prescriptions were more likely to be younger than those without (all  $p < 0.01$ ). **Conclusions:** Nearly 30% of patients with MSI-H mCRC did not receive efficacious ICIs, and though disease laterality data was not readily available, a substantial number of eligible patients also did not receive EGFR inhibitors. This underuse of EGFR inhibitors has been reported previously [1]. There were racial and age differences in prescription rates. Further studies should evaluate the barriers to prescribing CGP-directed therapies in the care of mCRC patients. **Keywords:** molecular testing, metastatic colorectal cancer, comprehensive genomic profiling, actionable biomarkers, FDA approved therapies, veterans. [1] Becker et al. 2021 PMID 34250412. Research Sponsor: VA National Precision Oncology Program.

**Early tumor shrinkage (ETS) as clinical factor to select maintenance with cetuximab (cet) monotherapy in patients (pts) with *RAS/BRAF* wild-type (wt) metastatic colorectal cancer (mCRC): A secondary endpoint analysis of the ERMES study.**

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**Background:** ERMES study aimed to demonstrate non-inferior PFS of maintenance with cetuximab (cet) monotherapy (arm B) compared to FOLFIRI+cet until progression (arm A) in *RAS* and *BRAF* wt mCRC pts. Although study did not meet its primary endpoint it showed better toxicity profile and OS for cet alone and comparable ORR between arms. Here we present results of ETS, a secondary endpoint. **Methods:** ETS was defined as  $\geq 20\%$  reduction of the sum of target lesions at 8 weeks from the start of treatment, and was assessed by blinded independent central review. PFS and OS were compared between ETS+ and ETS- pts according to treatment arm in the modified Per-Protocol (mPP) population. **Results:** 327 of 337 (97%) pts were evaluable for ETS. In arm A, no statistically significant differences were observed between ETS+ and ETS- cohort for PFS (11.9 m, 95%CI 11.28-13.19, vs 12.2 m, 95%CI 10.82-14.93; HR 1.05, 95%CI 0.73-1.50;  $p$  0.77) and OS (30.75 m, 95%CI 25.09-37.73, vs 25.46 m, 95%CI 19.96-34.63; HR: 0.79, 95%CI 0.50-1.24;  $p$  0.29). In arm B, a statistically significant difference was observed between ETS+ and ETS- cohort for PFS (10.62 m, 95%CI 9.86-13.09, vs 8.52 m, 95%CI 7.89-9.37; HR 0.70, 95%CI 0.49-1.00;  $p$  0.036) and for OS (38.88 m, 95%CI 34.53-43.25, vs 27.43 m, 95%CI 20.59-36.64; HR 0.60, 95%CI 0.38-0.93;  $p$  0.019). ETS+ pts reported no statistically significant difference between arm B and arm A in terms of PFS (HR 1.12, 95%CI 0.83-1.49;  $p$  0.43) while experiencing a better OS for arm B compared to arm A (HR 0.72, 95%CI 0.50-1.05;  $p$  0.08). **Conclusions:** In pts achieving ETS, induction treatment with FOLFIRI+cet may be de-escalated to cet alone without detrimental effect on PFS, resulting in less toxicity and better OS. These data support the hypothesis that ETS might be a clinical dynamic predictor of efficacy of a less intensive maintenance strategy, allowing to plan de-escalation to cet alone after 4 months of FOLFIRI+cet induction. Clinical trial information: NCT02484833. Research Sponsor: This research was financially supported by Merck Serono S.p.A., Italy, an affiliate of Merck KGaA, Darmstadt, Germany (CrossRef Funder ID: 10.13039/100009945).

### Subgroup analyses of safety and efficacy by number and types of prior lines of treatment in FRESCO-2, a global phase III study of fruquintinib in patients with refractory metastatic colorectal cancer.

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**Background:** Effective treatment options are limited for patients (pts) with refractory metastatic colorectal cancer (mCRC). Fruquintinib (F), a highly selective, potent, oral tyrosine kinase inhibitor of VEGFR-1, -2, and -3, was evaluated in the global phase III FRESCO-2 study (NCT04322539) and demonstrated a clinically meaningful and statistically significant improvement in OS and PFS with a favorable safety profile. Here we report subgroup analyses of efficacy and safety by prior lines (PL) and types of anti-cancer treatment (Tx) for metastatic disease (MD). **Methods:** FRESCO-2 was conducted in the US, Europe, Japan & Australia, comparing F + best supportive care (BSC) with Placebo (P) + BSC. Pts were given 5 mg PO, QD, 3 wks on, 1 wk off, in a 28-day cycle. Eligible pts received prior chemotherapy, anti-VEGF, and if *RAS* wild type, anti-EGFR therapies; if *BRAF*V600E mutant or MSI-H, and appropriate targeted regimen; and had progressed on, or were intolerant to, trifluridine/tipiracil (TAS-102) and/or regorafenib (R). Subgroup (sbgrps) analyses for efficacy and safety were performed according to number of prior lines of tx (LOT) and by types of therapy. **Results:** A total of 691 pts were randomized; F:461 vs P:230. The median number of prior lines of anti-cancer tx for metastatic disease F vs P was 4 (2, 16) vs 4 (2, 12). F improved OS and PFS compared to P for all sbgrps and prior tx for MD, consistent with those of the ITT population (pop). (Table: OS reported by sbgrps and prior tx for MD). Occurrence of adverse events (AEs) and serious adverse events were generally balanced between F vs P and consistent across all subgroups. The most common  $\geq$  G3 AEs in  $\geq$ 5% of pts on F within majority of sbgrps were hypertension, asthenia and palmar plantar erythrodysesthesia and were consistent with the overall safety population. Additional analyses based on duration of prior anti-VEGF and last therapy prior to F will be presented at the conference. **Conclusions:** F demonstrated clinically meaningful improvement in OS, PFS with an acceptable safety profile across all sbgrps. These results were consistent with the effect observed in the overall population; irrespective of previous tx with anti-VEGF, anti-EGFR, TAS-102 and R further supporting F as a potential new tx option for pts with refractory mCRC. Clinical trial information: NCT04322539. Research Sponsor: HUTCHMED LTD.

| Subgroups<br>(n = F, P)          | F<br>(n) | P<br>(n) | HR;<br>95% CI         | Subgroups<br>(n = F, P)  | F<br>(n) | P<br>(n) | HR;<br>95% CI         |
|----------------------------------|----------|----------|-----------------------|--------------------------|----------|----------|-----------------------|
| ITT population<br>(461, 230)     | 7.4      | 4.8      | 0.662;<br>0.549-0.880 | Anti-VEGF<br>(445, 221)  | 7.4      | 4.9      | 0.683;<br>0.565-0.827 |
| $\leq$ 3 prior LOT,<br>(125, 64) | 7.6      | 5.2      | 0.714;<br>0.488-1.043 | Anti-EGFR<br>(180, 88)   | 7.4      | 4.4      | 0.689;<br>0.507-0.936 |
| 4 prior LOT,<br>(122, 59)        | 7.4      | 4.7      | 0.661;<br>0.454-0.963 | TAS-102<br>(240, 121)    | 7.7      | 5.1      | 0.723;<br>0.557-0.938 |
| 5 prior LOT,<br>(93, 47)         | 8.0      | 3.4      | 0.310;<br>0.199-0.483 | R<br>(40, 18)            | 10.2     | 8.2      | 0.772;<br>0.379-1.573 |
| $\geq$ 6 prior LOT,<br>(121, 60) | 6.7      | 5.2      | 0.818;<br>0.566-1.183 | TAS-102 & R<br>(181, 91) | 6.8      | 4.4      | 0.600;<br>0.447-0.805 |

### Cost-effectiveness analysis of later-line therapies for metastatic colorectal cancer (mCRC) based on a novel methodology of network meta-analysis (NMA) of survival curves.

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**Background:** With advances in the management of mCRC, survival outcomes of patients have improved. While some patients still progress after first and second lines of treatment, there remain options for third or later-line treatment. This study aimed to determine the cost-effectiveness of later-line (>3) treatments for patients with mCRC in the US, based on a NMA of survival curves. Unlike conventional NMA using constant hazard ratios, this method is not constrained by the proportional hazard assumption and uses parametric fitting that incorporates the shape and scale parameters to provide time-varying treatment effects. **Methods:** The NMA compared the efficacy of 6 treatments, atezolizumab+/-cobimetinib (ATE+/-COB), fruquintinib (FRU), regorafenib (REG), TAS-102+/-bevacizumab (TAS+/-BEV), including biosimilar, to placebo (PBO). The study used a 3-state partitioned survival model over a 5-year time horizon incorporating drug acquisition, administration, adverse events, monitoring, and end of life costs, and utilities sourced from literature. Total costs, life-years (LY) and quality-adjusted LYs (QALY) for each treatment were determined. Incremental cost-effectiveness (ICER) and incremental cost-utility ratios (ICUR) were estimated using PBO as a common comparator. **Results:** Over the 5-year period and across the 6 therapies, survival ranged from 0.62 LYs for PBO to 1.15 LYs for TAS+BEV, translating to QALYs ranging from 0.44 for PBO to 0.85 for TAS+BEV. Total costs of treatment ranged from 278,877 for PBO to 417,495 for TAS+BEV (405,002 with biosimilar). TAS+BEV yielded the lowest ICER of an additional 261,421 (237,860 with biosimilar)/LY gained (g) and ICUR of an additional 343,458 (312,504 with biosimilar)/QALYg; while ATE presented the least favorable ICER and ICUR of an additional \$522,602/LYg and \$701,381/QALYg, respectively (Table). **Conclusions:** Among the 6 treatments evaluated, TAS+BEV emerged as the most cost-effective later-line treatment for mCRC while atezolizumab was the least cost-effective. Additionally, replacing BEV with a biosimilar reduces costs and enhances cost-effectiveness, improving access for patients. Research Sponsor: None.

Base case cost-effectiveness and cost-utility analyses.

| Regimen            | LY   | QALY | Cost (\$) | ICER      | ICUR      |
|--------------------|------|------|-----------|-----------|-----------|
| PBO                | 0.62 | 0.44 | 278,877   | Reference | Reference |
| ATE                | 0.69 | 0.50 | 316,170   | 522,602   | 701,381   |
| ATE+COB            | 0.79 | 0.57 | 342,976   | 369,374   | 507,025   |
| FRU                | 0.84 | 0.62 | 355,796   | 352,422   | 437,455   |
| REG                | 0.85 | 0.61 | 353,604   | 325,797   | 439,791   |
| TAS                | 0.81 | 0.58 | 334,000   | 290,850   | 387,582   |
| TAS+BEV            | 1.15 | 0.85 | 417,495   | 261,421   | 343,458   |
| TAS+BEV_biosimilar | 1.15 | 0.85 | 405,002   | 237,860   | 312,504   |

**Cancer of unknown primary with gastrointestinal profiles: A distinct CUP subset.**

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**Background:** Cancer of unknown primary (CUP) with a “gastrointestinal (GI) profile” appears to be a clinically distinct subset, based on immunophenotyping, specifically staining with cytokeratins 20 (CK20) and 7 (CK7), a type I and II keratin, respectively and caudal type homeobox 2 (CDX2) protein, a transcription factor expressed in nuclei of intestinal epithelial cells. However, only a limited clinicomolecular account of this entity exists. Comprehensive profiling is needed to substantially impact personalized therapeutics and improve prognosis. **Methods:** We identified 401 pts evaluated at MD Anderson Cancer Center. Pts were classified into 3 cohorts based on immunohistochemistry: lower GI profile CUP (LGI-CUP), other-GI profile CUP (OGI-CUP), or non-GI CUP (NGI-CUP). A control group of known lower GI primary cancers was derived from MSK-IMPACT data (cBioPortal, 1075 patients, LGI-KP). Clinical and pathological data including molecular profiling, therapy and survival were logged. Fisher-exact test was used. Kaplan-Meier method was used to estimate overall survival (OS) and compared with log-rank test. **Results:** Among 748 pts, 401 (53.6%) had adequate immunostaining for analysis. Of these, 72 (18.0%), 226 (56.4%) and 103 (25.7%) were classified as LGI-CUP, OGI-CUP, and NGI-CUP, respectively. LGI-CUP were enriched for adenocarcinoma (88% v 86% v 35%,  $P < 0.0001$ ) and good risk Culine score (59% v 51% v 37%,  $P = 0.02$ ), compared to OGI-CUP and NGI-CUP, respectively. While NGI-CUP had greater proportions of poorly differentiated histology (91% v 55% vs 66%,  $P < 0.0001$ ) and bone metastases (30% vs 7% v 24%,  $P = 0.0001$ ) compared to LGI-CUP and OGI-CUP, respectively. Comparison of key mutations is shown in table. Median OS of LGI-CUP (14.6 months [95%CI: 12.0 – 17.2]) was similar to OGI-CUP (14.3 months [95%CI: 10.7 – 18.0]) and NGI-CUP (16.2 months [95%CI: 12.4 – 20.0]),  $P = 0.75$ , but significantly lower than LGI-KP cohort (28.6 months, [95%CI 24.4 – 32.7],  $p < 0.001$ ). LGI-CUP was treated preferentially with fluoropyrimidine-based GI regimen [5-FU/capecitabine +/- oxaliplatin or irinotecan] (77% vs 43% v 5%) compared to OGI-CUP and NGI-CUP, respectively. However, median OS for LGI-CUP treated with 5-FU-based regimens was higher (16.0, 95%CI 13.0 – 19.0) than those treated with non-5-FU-based treatments (10.9, 95%CI 5.6 – 16.2),  $P = 0.002$ . **Conclusions:** LGI-CUP appears to be a molecularly distinct entity from and NGI-CUP, as well as lower GI CUP from known entities. LGI-CUP patients treated with 5-FU-based regimens appear to have improved survival. Research Sponsor: None.

**Key mutations.**

|      | LGI-CUP % (reference) | OGI-CUP | P    | NGI-CUP | P    | LGI-KP | P        |
|------|-----------------------|---------|------|---------|------|--------|----------|
| TP53 | 39                    | 39      | 1    | 44      | 0.78 | 70     | 0.003    |
| APC  | 27                    | 7       | 0.02 | 0       | 0.02 | 70     | < 0.0001 |
| KRAS | 31                    | 24      | 0.42 | 15      | 0.11 | 47     | .05      |

**Impact of vitamin d on the colon tumor immune microenvironment: Results of a randomized clinical trial of preoperative vitamin D supplementation in patients with stage I-III colon cancer.**

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**Background:** Although the anti-tumor properties of vitamin D in colorectal cancer (CRC) have been broadly reported, its potential role as a tumor microenvironment (TME) immunomodulator has not been well established. Vitamin D receptor (VDR) and CYP27B1 (1- $\alpha$ -hydroxylase; required to convert vitamin D to its active form) are critical factors in the vitamin D signaling pathway and harbor prognostic significance for CRC. Pre-clinical studies suggest that higher stromal VDR expression enhances chemotherapy efficacy. To characterize the effects of vitamin D on the TME in humans, we conducted a randomized placebo-controlled trial of preoperative high-dose vitamin D supplementation in stage I-III colon cancer patients. **Methods:** Forty-two patients were randomized to receive vitamin D3 50,000 IU/day x 7 days followed by 10,000 IU/day versus placebo prior to resection. Paired pre-treatment biopsy specimens and post-treatment surgical resection specimens from 19 patients who completed the assigned treatment and had sufficient tissue for analysis were collected for spatially-resolved immune cell profiling and evaluation of VDR and CYP27B1 expression using custom multiplex immunofluorescence. Blood samples were collected pre- and post-treatment to assess plasma 25-hydroxyvitamin D [25(OH)D] levels. Linear mixed effects models were used to compare tissue parameters; plasma 25(OH)D level changes were assessed using the Wilcoxon rank-sum test. **Results:** Patients randomized to high-dose vitamin D had increased post-treatment plasma 25(OH)D (median 18.0 to 63.6 (ng/ml)) whereas placebo-randomized patients did not (median 19.1 to 18.6 (ng/ml)) ( $p < 0.001$ ). Analysis of both biopsy and resection tissue revealed highly heterogeneous VDR and CYP27B1 expression. VDR expression was highest in tumor cells, followed by immune and then stromal cells. Although resection tissue from vitamin D-treated patients showed lower VDR expression than specimens from placebo-treated patients in all cells ( $p = 0.03$ ), VDR was preferentially expressed by tumor cells at the tumor invasive front compared to tumor center in the vitamin D arm, while the opposite was observed in the placebo arm. Vitamin D supplementation did not alter CYP27B1 expression. While treatment did not change total immune cell density, resection tissue from vitamin D-treated patients had higher overall CD3<sup>+</sup>CD8<sup>+</sup> T cell density ( $p = 0.04$ ), particularly CD3<sup>+</sup>CD8<sup>+</sup> memory T cell density ( $p = 0.02$ ). Additionally, vitamin D supplementation led to greater co-localization between CD3<sup>+</sup>CD8<sup>+</sup> T cells and tumor cells ( $p < 0.001$ ) compared to placebo. **Conclusions:** In patients with stage I-III CRC, vitamin D supplementation led to changes in vitamin D pathway signaling and evidence of an anti-tumor effect in the TME. Further investigation into the underlying mechanism of action is warranted. Clinical trial information: NCT02172651. Research Sponsor: U.S. National Institutes of Health.

### Predictive value of tumor-infiltrating lymphocyte (TIL) dynamics in the tumor microenvironment (TME) during preoperative chemoradiotherapy (CRT) on pathologic complete response (pCR) in microsatellite-stable (MSS) locally advanced rectal cancer (LARC).

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**Background:** Preoperative CRT followed by consolidation nivolumab before surgery showed a promising pCR rate in MSS LARC; both high PD-L1 expression and an elevated CD8<sup>+</sup> T cell/effector regulatory T cell ratio analyzed by pre-CRT samples were independently related to high pCR rate (Bando H, Clin Cancer Res. 2022). Here, we applied AI-powered spatial TIL analysis to the VOLTAGE study to investigate whether dynamic change of TIL in TME may predict pCR in MSS LARC. **Methods:** The VOLTAGE study is a multicenter phase I/II study to evaluate the efficacy of CRT followed by 5 cycles of nivolumab and surgery in patients (pts) with LARC. In this study, tumor samples were obtained at multiple time points; pre-CRT (B1), post-CRT/pre-nivolumab (B2), post-3 cycles of nivolumab (B3), and surgery. In this analysis, a total of 86 H&E whole-slide images (WSI) harvested at B1 and B2 time points from all the pts enrolled in the VOLTAGE study were included in the analysis. Lunit SCOPE IO (Lunit, Republic of Korea), AI-powered H&E-WSI analyzer developed based on 16,443 WSI with pathologists' annotations, was applied to quantify TIL density in TME. **Results:** In a total of 43 pts, pCR rate of MSS and microsatellite instability-high (MSI-H) were 28.9% (11/38) and 60% (3/5), respectively. In MSS subgroup (n = 38), which has a higher unmet need for pCR prediction, tumor samples with pCR (n = 11) had increased TIL density in TME (tTIL) in post-CRT (B2, median [IQR] 726 [249-1607]/mm<sup>2</sup>) compared to pre-CRT (B1, 598 [366-905]/mm<sup>2</sup>), whereas those without pCR (n = 27) had decreased tTIL in post-CRT (405 [148-748]/mm<sup>2</sup>), compared to pre-CRT (B1, 748 [460-1153]/mm<sup>2</sup>). Intratumoral TIL density changes were more prominent than stromal TIL density changes in MSS tumor samples with pCR (mean fold change x17.7 vs. x1.5). MSS Pts with tTIL change from B1 to B2 (during CRT) more than x1.8 times achieved 75% (6/8) pCR rate, whereas those with tTIL change less than x1.8 had 16.7% (5/30) pCR rate (p = 0.0035). **Conclusions:** Change of TIL density in TME during CRT was significantly correlated with favorable clinical outcome of preoperative CRT and consolidation nivolumab, resulting in high pCR rate in MSS LARC. Research Sponsor: Lunit Inc.

# Combined associations of a healthy lifestyle and body mass index on colorectal cancer recurrence and survival: A cohort study.

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**Background:** Colorectal cancer (CRC) risk is associated with modifiable lifestyle factors including smoking, physical inactivity, Western diet, and excess body weight. The impact of lifestyle factors on survival is less known. A cohort study was conducted to investigate the combined effects of a pre-diagnostic healthy lifestyle and body mass index (BMI) on prognosis following CRC diagnosis. **Methods:** Treatment and follow-up data were collected from the patient files of 1098 participants from the Swedish Colorectal cancer low-risk study cohort including stage I-III CRC patients. A healthy lifestyle and BMI (HL) score was computed using self-reported data on smoking status, physical activity, adherence to a Mediterranean diet pattern, and BMI five years before diagnosis, and divided into four categories ranging from least to most healthy. Survival analyses were performed to assess recurrence-free survival and overall survival across categories of exposure, using the Kaplan-Meier method and Cox proportional hazards model, the latter adjusted for age, sex, and educational level. **Results:** Among 1098 participants with stage I-III CRC, 233 (21.2%) had an HL score of 0-1 (least healthy), 354 (32.2%) HL score of 2, 357 (32.5%) HL score of 3 and 154 (14.0) HL score of 4 (most healthy). Participants missing follow-up data (n = 29) or relapsing  $\leq 6$  months (n = 29) were excluded from the recurrence-free survival analysis. 221 events of cancer recurrence were observed among 1040 participants during a median follow-up time of 4.3 years, and 542 deaths among 1098 participants during a median follow-up time of 6.3 years. Patients with the healthiest lifestyle had an improved recurrence-free survival (HL 4 vs HL0-1, HRadj 0.51 (95% CI 0.31-0.83) and overall survival (HL 4 vs HL 0-1, HRadj 0.52 (95% CI 0.38-0.70)). Stratifying these results for cancer stage (I-III) and tumor site (colon/rectum) did not alter estimated HR:s or 95% CI:s. Avoidance of smoking and being physically active were independently associated with improved recurrence-free survival and overall survival: non-smoking vs smoking HRadj 0.62 (95% CI 0.43-0.90), high vs low levels of physical activity HRadj 0.71 (95% CI 0.54-0.94)). **Conclusions:** Our study indicates that pre-diagnostic adherence to a healthy lifestyle may increase the recurrence-free and overall survival of patients with stage I-III CRC. Avoidance of smoking and being physically active were the strongest risk-reducing factors for these outcomes. Research Sponsor: Stockholm County Council (ALF project).

| HL-score | Recurrences, n | Deaths, n | Univariate     |           |                 |           | Multivariate   |           |                 |           |
|----------|----------------|-----------|----------------|-----------|-----------------|-----------|----------------|-----------|-----------------|-----------|
|          |                |           | CRC recurrence |           | All-cause death |           | CRC recurrence |           | All-cause death |           |
|          |                |           | HR             | 95% CI    | HR              | 95% CI    | HR             | 95% CI    | HR              | 95% CI    |
| 0-1 p    | 65             | 132       | 1 (ref)        |           | 1 (ref)         |           | 1 (ref)        |           | 1 (ref)         |           |
| 2 p      | 60             | 164       | 0.57           | 0.40-0.81 | 0.70            | 0.55-0.67 | 0.57           | 0.40-0.81 | 0.63            | 0.50-0.79 |
| 3 p      | 72             | 177       | 0.66           | 0.47-0.92 | 0.84            | 0.67-1.06 | 0.66           | 0.47-0.93 | 0.72            | 0.57-0.90 |
| 4 p      | 24             | 69        | 0.51           | 0.32-0.81 | 0.65            | 0.48-0.87 | 0.51           | 0.31-0.83 | 0.51            | 0.38-0.70 |

**Immunotherapy for localized MSI-H colorectal cancer: Characterizing endoscopic and imaging response.**

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**Background:** Early data suggests that immunotherapy (IO) for localized colorectal cancer (CRC) is associated with high rates of pathologic complete response (pCR) and durable remission. This study aims to characterize endoscopic, imaging, and pathological outcomes among patients who received IO for localized CRC. **Methods:** This was a single-institution retrospective analysis of patients with MSI-H CRC who received at least one cycle of anti-PD-1 therapy for localized (stage I-III) MSI-H CRC. Patient and tumor characteristics were obtained from the clinical record. Endoscopy reports were assessed using standardized criteria to determine response and imaging was assessed using RECIST v1.1 where applicable. Endoscopic complete response (endoCR) was characterized by flat, white scar and/or telangiectasia without residual ulcer or nodularity. Categorical variables were compared using Fisher's exact test. **Results:** 37 patients who received a median of 8 cycles of pembrolizumab (n = 36) or nivolumab (n = 1) for localized CRC were identified, of which 16 (43%) had a rectal primary. 10 patients (27%) had mucinous features on pathology. The best endoscopic response was complete response (CR) in 13 of 30 (43%) patients, which was achieved after a median of 6 cycles. The rate of endoCR after  $\leq 4$  cycles of IO was similar to the rate of endoCR after  $> 4$  cycles (29% vs 50%,  $p = 0.284$ ). The best imaging response was CR in 6 of 32 (19%) of patients. The rate of CR on imaging after  $\leq 4$  cycles and  $> 4$  cycles of IO was 6% and 36% respectively ( $p = 0.064$ ). In cases where imaging and endoscopy were available (n = 24), discrepancy in best response was noted in 14 (58%) cases. The most common discrepancy was partial response on imaging when endoCR was noted (n = 6 cases). 16 patients proceeded to surgical resection (after a median of 8 cycles) of which 11 (69%) had pCR despite non-CR on either endoscopy (n = 7) or imaging (n = 9). The endoscopic reports indicating residual disease prior to surgery included findings such as ulceration, scarring, strictures, and an obstructing mass. Of 15 patients who did not proceed to surgery and had follow-up more than 6 months after completion of IO, 13 (87%) had no evidence of progression at a median follow-up of 19.3 months from IO start. **Conclusions:** Discrepancies between endoscopic, imaging, and pathological outcomes were frequent, indicating a need for improved clinical response criteria. Nevertheless, immunotherapy was associated with deep and durable responses in most patients with localized MSI-H CRC. Research Sponsor: None.

### The role of image-guided volume-adapted high-dose-rate endorectal brachytherapy boost in total neoadjuvant treatment of distal locally advanced rectal cancer.

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**Background:** Endorectal brachytherapy has been used as palliative or preoperative treatment for advanced or locally advanced rectal cancer, with moderate radiation induced toxicity. This study aimed to explore the efficacy of high-dose-rate  $^{192}\text{Ir}$  brachytherapy combined with external beam radiotherapy (EBRT) in total neoadjuvant treatment (TNT) of distal rectal cancer. **Methods:** From January 2017 to December 2022, eligible rectal adenocarcinoma patients were assessed as clinical stage II-III, with primary tumor located in distal rectum ( $\leq 5$  cm from anal verge). The local staging was determined using endorectal ultrasound and magnetic resonance imaging (MRI). In addition, cases included in this study underwent preoperative semiweekly endorectal brachytherapy of 8-12Gy/2-3F/1-1.5w, followed by EBRT of 45-50.4Gy/25-28F, fluorouracil-based concurrent and consolidation chemotherapy. The brachytherapy was image-guided volume-adapted, and delivered by multi-channel  $^{192}\text{Ir}$  source applicators with different diameters. Radiation proctitis was graded from 0 to 4 using Radiation Therapy Oncology Group (RTOG) criteria. **Results:** A total of 40 cases were enrolled in this retrospective study, biopsy-proven and diagnosed as clinical stage II (n = 8), stage III (n = 32). The rates of T4 and positive circumferential resection margin (CRM) were 35% and 87.5% respectively. The median value of the interval from completion of radiotherapy to operation was 86 days (interquartile range: 75-98 days). After neoadjuvant treatment, 2 cases were undergoing the watch-and-wait strategy with sustained clinical complete response (cCR), 38 patients received operation and R0 resection rate was 97.4%. The pathological complete response (pCR) rate was 28.9%, and major pathological regression rate was 67.5%. The theoretical rate of anus preservation was 62.5%, including 6 of 21 cases underwent abdominoperineal resection but achieved pCR, 17 cases underwent low anterior resection or intersphincteric resection, and 2 cases with sustained cCR. After a median follow-up of 37 months (range 12-68 months), the 3-year disease-free survival, local-regional recurrence-free survival and overall survival was 81.1% (95%CI: 64.3%-91.2%), 94.9% (95%CI: 80.6%-98.5%) and 96.9% (95%CI: 82.1%-99.4%), respectively. No patients experienced Grade  $\geq 3$  radiation proctitis. The median Wexner fecal incontinence score of anus-preserving cases was 1 (range 0-8). Subgroup analysis showed that the complete response rate (pCR or sustained cCR) was lower in cases received 2 fractions brachytherapy than those received 3 fractions (27.8% vs 36.4%). **Conclusions:** The addition of image-guided volume-adapted high-dose-rate  $^{192}\text{Ir}$  endorectal brachytherapy boost to TNT could improve the pathological tumor response and anus preservation rate in distal locally advanced rectal cancer. Research Sponsor: Oncology Leading Discipline Construction Support Project, Zhongnan Hospital Of Wuhan University (XKJS202005); Discipline Cultivation Funding (Oncology), Zhongnan Hospital of Wuhan University (ZNXPY2020012).

**The number of lymph nodes examined as a poor prognosis factor in stage II and stage III colon cancer patients undergoing curative surgery.**

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**Background:** The number of lymph nodes (LN) examined is a key factor in determining the stage of colorectal cancer. Over the past 20 years, the examination of fewer than 12 LN has been regarded as a poor prognosis. However, surgical procedures and pathologic examination improvements have increased the number of LN examined. This raised the question of reassessing the best cutoff point for staging colon cancer patients undergoing curative surgery. **Methods:** We reviewed a total of 3,126 eligible patients with stage II-III colon cancer collected from the Yonsei Cancer Center Registry (YCC) database between 2005 and 2015. As a validation cohort, we used clinicodemographic data from 9,604 patients with stage II-III colon cancer identified by the Netherlands Cancer Registry (NCR) between 2006 and 2015. The hazard ratio (HR) obtained from Cox-proportional regression was used to investigate the effect of LNs' yield on 6-year overall survival (OS). The optimal LN yield was evaluated by analyzing every cutoff from 3 to 165. We used the Kaplan-Meier method to compare the effect of higher vs. lower optimal LN yield on a 6-year OS. Univariate and multivariate Cox regressions were conducted.

**Results:** The proportion of patients with fewer than 12 LN examined dramatically decreased between 2005 and 2015 (17.4% in 2005 vs. 0.6% in 2015,  $P < 0.001$ ). Based on the 12 LN examined as the conventional cutoff number, there was no significant association between 6-year OS and LN yield in all stages II-III patients (HR = 1.21,  $P = 0.116$ ;  $n = 3,126$ ), stage II (HR = 1.39,  $P = 0.068$ ;  $n = 1,570$ ), and stage III (HR = 1.18,  $P = 0.297$ ;  $n = 1,557$ ) colon cancer. Interestingly, a higher cutoff of 20 LN examined was associated with a significant increase in 6-year OS in all patients (HR = 1.51,  $P < 0.001$  in patients with LN examined less than 20). Multivariate Cox-proportional hazard regression showed a significant decrease in 6-year OS in stage II (HR = 1.39,  $P = 0.026$ ) and stage III (HR = 1.54,  $P < 0.001$ ) with less than 20 LN yield. Consistent with the result in the YCC database, less than 20 of LN yield was associated with poorer 6-year OS in stage II-III patients (HR = 1.25,  $P < 0.001$ ;  $n = 9,604$ ), stage II (HR = 1.43,  $P < 0.001$ ;  $n = 4,689$ ), and stage III (HR = 1.13,  $P = 0.007$ ;  $n = 4,915$ ) in the NCR, respectively. **Conclusions:** Less than 20 LN examined is associated with a poorer prognosis in patients with surgically treated colon cancer, compared to the current cutoff value of 12. Further validation of re-evaluating criteria on the inadequacy of LN retrieval in high-risk stage II colon cancer is warranted. Research Sponsor: A grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : HI22C0353); The National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2022R1A2C4001879).

**Efficacy and safety of mFOLFOX6 with apatinib as postoperative adjuvant chemotherapy in stage IIIB or IIIC colorectal cancer.**

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**Background:** Antiangiogenic therapy has shown benefit in metastatic colorectal cancer. However, the role of apatinib in adjuvant treatment of stage IIIB and IIIC colorectal cancer remains unclear. **Methods:** Patients received modified FOLFOX6 once every 2 weeks for a 6-month period (control group) or modified FOLFOX6 for 6 months plus apatinib (500mg) once a day for a 12-month period (experimental group). Progression free survival (PFS), overall survival (OS) and treatment-related adverse events were evaluated in two groups. **Results:** 63 patients have been enrolled in this study. This trial reported a 3-year PFS of 74.2% for modified FOLFOX6 plus apatinib vs. 61.5% for modified FOLFOX6 ( $P < 0.05$ ). 5-year OS was significantly improved in the experimental group compared with the control group (86.3% vs. 72.5%;  $P < 0.05$ ). The most common grade 3 adverse events were hypertension and hand-foot syndrome. No treatment-related grade 4 adverse events or treatment-related deaths were observed. **Conclusions:** Apatinib can prolong PFS and OS when added to adjuvant chemotherapy in resected stage IIIB and IIIC colorectal cancer. The toxicities associated with apatinib were generally acceptable. Clinical trial information: NCT03365765. Research Sponsor: Jiangsu Hengrui Medicine Co., Ltd.

## The significance of mucin on rectal MRI in patients with locally advanced rectal cancer being considered for watch-and-wait after neoadjuvant therapy.

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**Background:** Neoadjuvant therapy (NAT) leads to a clinical complete response (cCR) in a significant proportion of patients with locally advanced rectal cancer (LARC) allowing for possible non-operative management. Along with physical exam and proctoscopy, magnetic resonance imaging (MRI) is used to evaluate for residual tumor following NAT. The presence of mucin on MRI leads to uncertainty about residual disease and appropriateness of watch-and-wait strategy (WW) in patients with no evidence of disease on proctoscopy (endoscopic cCR). We set out to determine the impact of radiographic mucin in patients with LARC after NAT. **Methods:** MRI reports between July 2016 to January 2020 at Memorial Sloan Kettering Cancer Center were queried for the presence of mucin in the tumor bed on post-treatment rectal MRI in patients with LARC following NAT. The clinicodemographic, pathologic, and outcomes data from these patients were compiled and analyzed. **Results:** Seventy-one patients were included in the final analysis who fit the pre-specified inclusion criteria (Table). Of the 71 patients with mucin present on post-treatment MRI, 19 patients (27%) entered a watch-and-wait (WW) strategy and 52 patients (73%) were planned for surgery (non-WW). Comparing the WW and non-WW cohorts, there was no difference in age, sex, clinical nodal status, or neoadjuvant regimen. Patients entering a WW protocol had a higher proportion of cT1-T2 tumors (32%) compared to non-WW (4%) ( $P < 0.01$ ). All WW patients had endoscopic cCR, while only one non-WW patient achieved endoscopic cCR. Of 52 non-WW patients, 49 underwent resection and 3 did not have surgery due to disease progression. The pathologic complete response (pCR) rate in the non-WW patients who underwent surgery was 10%. Of 19 WW patients, 4 experienced regrowth (21%), while 15 (79%) have no local regrowth, with a median follow up of 4.2 years (range 2.4-6.3 yrs). Two patients (11%) experienced distant failure. **Conclusions:** The presence of mucin after NAT for LARC should not necessarily preclude a WW strategy in otherwise appropriate candidates who achieve an endoscopic cCR. Research Sponsor: U.S. National Institutes of Health.

Characteristics of patients with mucin on MRI after NAT for LARC who entered a watch-and-wait (WW) protocol or did not (Non-WW).

| Characteristic                                            | Patients (No.)     |                    | P-value |
|-----------------------------------------------------------|--------------------|--------------------|---------|
|                                                           | WW<br>(n = 19)     | Non-WW<br>(n = 52) |         |
| Age, median (range), yrs.                                 | 60.6 (30.3 – 75.5) | 58.5 (35.2 – 82.8) | 0.90    |
| Male                                                      | 13 (68)            | 31 (60)            | 0.58    |
| Clinical tumor (T) classification                         |                    |                    |         |
| cT1-T2                                                    | 6 (32)             | 2 (4)              | < 0.01  |
| cT3-T4                                                    | 13 (68)            | 50 (96)            |         |
| Clinical nodal (N) classification                         |                    |                    |         |
| cN1-2                                                     | 18 (95)            | 43 (83)            | 0.27    |
| Neoadjuvant Regimen                                       |                    |                    |         |
| Chemotherapy only                                         | 0 (0)              | 1 (2)              | 0.60    |
| Chemoradiotherapy only                                    | 1 (5)              | 6 (12)             |         |
| Total Neoadjuvant Therapy                                 | 18 (95)            | 45 (86)            |         |
| Time from NAT completion to surgery, median (range), yrs. | 1.1 (0.9-2.5)      | 0.2 (0.1-2.8)      | < 0.01  |
| Pathologic tumor (T) classification                       | (n = 3)            | (n = 49)           |         |
| pCR                                                       | 0 (0)              | 5 (10)             | < 0.01  |
| pT1-T2                                                    | 2 (67)             | 5 (10)             |         |
| pT3-T4                                                    | 1 (33)             | 39 (80)            |         |

### Mismatch Repair system protein deficiency as a factor of resistance to chemo-radiotherapy treatment in patients with locally advanced rectal adenocarcinoma and its influence on disease-free survival.

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**Background:** Available data on Mismatch Repair system deficit and microsatellite instability are conflicting and are generally derived from a small number of patients due to the rarity of this condition in rectal cancer. Our study aimed to evaluate the frequency and therapeutic implications of Mismatch Repair proteins (MMR) status in patients with locally advanced rectal cancer (LARC). **Methods:** We retrospectively collected data from 318 patients affected by LARC adenocarcinoma (cT3-4 +/- N1-2) treated at the Medical Oncology Unit of the University Hospital of Cagliari, Italy, the Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy and at the Medical Oncology Unit, AOU Ospedali Riuniti, Ancona, Italy. All patients included in the study underwent neoadjuvant concurrent capecitabine and long-course radiotherapy (RT) (total dose of Gy 50.4), afterwards a total mesorectal excision (TME) was performed. MMR expression was evaluated through immunohistochemistry. The primary objective was major TRG (0-1 Ryan's score) while secondary objectives were pathological complete response, disease-free survival (DFS) and overall survival (OS). **Results:** 160 patients (148 pMMR and 12 dMMR) were included in exploratory cohort and 158 (146 pMMR and 12 dMMR) were included in validation cohort. A major TRG has been shown in 64/148 (42,6%) and 63/146 (43,1%) patients with pMMR in exploratory cohort and validation cohort, respectively; while no major TRG have been shown in dMMR patients in exploratory cohort nor in validation cohort. Both exploratory and validation cohorts showed a statistically significant higher median DFS in pMMR patients compared to dMMR ones: NR vs 14 months in exploratory cohort ( $p = 0,003$ ) and NR vs 17 months in validation cohort ( $p = 0,02$ ). **Conclusions:** Our retrospective study indicated an association between dMMR and poor or no response to preoperative chemoradiotherapy. These results are consistent with the role of MMR as a predictor of poor-response to chemoradiotherapy and represent a hypothesis-generating study for the selection of these patients for immunotherapy in the neoadjuvant setting, in which the available data are immature. Research Sponsor: None.

### The Ave-Rec trial: Phase II trial of PD-L1/PD-1 blockade with avelumab plus chemoradiotherapy for locally advanced resectable T3b-4/N1-2 rectal cancer—Toxicity and interim efficacy data.

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**Background:** Neoadjuvant long course chemoradiotherapy (LCCRT) for locally advanced rectal cancer (LARC) results in a complete pathological response rate in 10-30% of patients (pts), but with 20-40% non-responders and 10-15% have local recurrence. Radiotherapy (RT) is immuno-stimulatory by enhancing local/distant tumour cell death, but also immunosuppressive as it stimulates PDL1 production and myeloid-derived suppressor cell activity. Hence PDL1 inhibition may be required to enhance the immuno-stimulatory effects of RT. **Hypothesis:** In pts with resectable LARC, the anti-PDL1 antibody Avelumab given post LCCRT may enhance the pathological/imaging response rates whilst reducing local/distant relapse rates. **Methods:** Phase II single arm trial. All pts had standard LCCRT (50.4Gy RT plus 5FU [225mg/m<sup>2</sup>/day/CI] or Capecitabine [825mg/m<sup>2</sup> BID on days of RT] over 5.5 weeks). Post LCCRT (prior to surgery), pts received 4 cycles Avelumab (AV) (10mg/kg, q2 weeks), then surgical resection at 10-12 weeks post LCCRT. Fresh tumour biopsy and ctDNA sampling taken pre LCCRT, pre Cycle 1 AV and at surgery. Response by FDG PET/CT and pelvic MRI at 8 weeks post LCCRT, pre surgery. **Inclusion Criteria:** pts with LARC (MRI stage T3b-4/N1-2/M0), planned for LCCRT followed by curative resection, tumoural lower border within 12cm from anal verge, measurable disease (RECIST1.1), ECOG 0-1, adequate organ function and no contraindications to AV therapy. **Endpoints:** (a) *Primary*; Complete pathological response rate post-LCCRT (Target ≥ 35%), documented by central pathologist, (b) *Secondary*; MRI and FDG PET/CT imaging responses by RECIST1.1 and PERCIST, respectively. Toxicity. (c) *Exploratory*; Tumoural immune cell subsets/checkpoint expression (by multiplex immunohistochemistry and in-vitro functional assays) and ctDNA analysis. Distant relapse-free survival and the sites of relapse. ANZCTR: NCT03299660. **Results:** 37 pts across 6 sites, with 33 patients (89%) received allocated interventions: LCCRT, AV and surgery (where possible). Age median 55 years (31-76). M:F 23:10. Baseline tumoural stage: T3b-d 75%, T4a-b 25%. No pts with dMMR/MSI-H tumour. Overall 33 pts completed LCCRT, 31 pts (83%) completed all 4 cycles of AV and 32 pts (86%) had planned surgery (22, ULAR, 2 APR, 2 AR, 6 Other). ORR Pelvic MRI presurgery (N = 33): 2 CR, 14 PR, and 31 DCR. FDG PET/CT (N = 31): 10 CMR, 18 PMR and 3 SMD. Overall 10 pts Grade 3/4 AEs, with 15 G3/4 events. Only 3 pts with treatment-related G3 and no G4 AEs. No specific immune-related G3 AEs observed. Post-operative complications were not unexpected. **Conclusions:** Consolidation Avelumab post LCCRT for pts with LARC, was well tolerated with promising activity. Pathological response, biological substudies (immunological and ctDNA) and survival endpoints to be reported. Clinical trial information: NCT03299660. Research Sponsor: Merck Australia.

**Organ preservation and total neoadjuvant therapy for rectal cancer: Investigating long course chemoradiation versus short course radiation therapy.**

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**Background:** The efficacy of long course chemoradiation (LCRT) vs. short course radiation therapy (SCRT) relative to organ preservation (OP) in rectal cancer (RC) is unknown. We compared OP rates between SCRT and LCRT total neoadjuvant therapy (TNT) strategies. **Methods:** During the COVID-19 pandemic we established an institutional SCRT mandate with no exceptions. For comparison, we identified RC patients treated with LCRT immediately before and after the mandate period. After completion of TNT, patients were restaged by clinical exam, endoscopy, and MRI. A watch and wait (WW) approach was recommended for patients with a clinical complete response (cCR), defined by the MSK regression schema. Total mesorectal excision (TME) was recommended for non-cCR patients. OP was defined as remaining TME-free with no evidence of disease in the pelvis. We performed survival analysis for: local regrowth rate, OP, disease-free survival (DFS), and overall survival (OS). The validated low anterior resection syndrome (LARS) questionnaire (LARS-Q) was used to determine percentage of patients with major LARS. **Results:** We identified 332 consecutive patients with non-metastatic rectal adenocarcinoma treated with TNT (Jan. 2018-Jan. 2021). Patient and tumor characteristics were similar in the LCRT (n = 256) and SCRT (n = 76) cohorts. No significant differences in high-risk features were noted. Most patients had clinical stage III disease (82% in LCRT vs. 83% in SCRT). Induction chemotherapy followed by consolidative radiation was the most common treatment order (78% (LCRT) vs. 70% (SCRT)). The median interval from end of TNT to clinical restaging was 8 weeks (LCRT) and 9 weeks (SCRT). The cCR rate was 46% in both cohorts. Among patients with a cCR, the likelihood of WW management was similar (98% (LCRT) vs. 94% (SCRT)). From start of TNT, the median follow-up was 32 and 28 months respectively for LCRT and SCRT. The 2-year OS (95% vs. 92%), DFS (78% vs 70%), and distant recurrence (20% vs. 21%) rates were similar. Among all patients, the 2-year OP rate was 40% (95% CI 35-47%) for LCRT and 29% (95% CI 20-42%) with SCRT. In those patients managed by WW, the 2-year local regrowth rate was 20% (95% CI 12-27%) with LCRT vs. 36% (95% CI 16-52%) with SCRT. Percentage of patients reporting major LARS symptoms were similar among WW patients post TNT: baseline (23% (LCRT) vs. 18% (SCRT)), 6-months (41% (LCRT) vs. 42% (SCRT)), and 12-months (25% (LCRT) vs. 15% (SCRT)). **Conclusions:** In this nonrandomized comparison, while cCR rates were similar, we observed a numerically higher local regrowth rate with SCRT-TNT than with LCRT-TNT. Rate of major-LARS symptoms were similar with LCRT-TNT than with SCRT-TNT. The ongoing ACO/ARO/AIO-18.1 trial, hypothesizing that LCRT-TNT will increase OP rates relative to SCRT-TNT, will definitively address this question. Research Sponsor: U.S. National Institutes of Health.

# A prospective evaluation of pelvic radiation vs. no radiation among patients with young-onset rectal cancer: Impact on patient-reported outcomes.

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**Background:** The rise in young-onset rectal cancer (YORC) calls for better understanding of the long-term impact of radiation therapy (RT) on gastrointestinal (GI) toxicity and pelvic organ function. We aimed to prospectively capture the longitudinal trajectories of patient-reported outcomes (PROs) in YORC patients who received RT vs. no RT and to identify factors associated with unfavorable PROs.

**Methods:** We prospectively enrolled 120 YORC patients undergoing curative-intent treatment. The validated EORTC QLQ-CR29 was self-administered at time intervals grouped as: 0-11 months, 12-23 months, and 24+ months post-resection. Responses were stratified by receipt of neoadjuvant RT (yes vs. no). The longitudinal change in PROs was described by a linear mixed effects model. Multivariate linear regression was used to determine the impact of treatment factors on long-term PROs. **Results:** The median age at diagnosis was 44. The majority (N = 92, 77%) presented with cT3,4/N+ disease. Preoperative therapy included: no RT (N = 38, 32%; 8 [7%] who received chemotherapy alone, and 30 [25%] who received no neoadjuvant therapy), vs. RT (N = 82, 68%; where 59 [72%] also received concurrent capecitabine). More patients in the RT group had advanced T stage (3 or 4; 94% vs. 56%,  $P < 0.001$ ), distal tumor (median 7 vs. 12.5 cm from the anal verge,  $P < 0.01$ ), and underwent abdominal perineal resection (19 vs. 0%,  $P < 0.001$ ). After a median follow-up of 70 months, all were alive: 103 (86%) were disease-free, 9 (8%) had recurrence with successful salvage, and 8 (7%) had disease progression. In the RT group, sore skin improved at 12-23 and 24+ months (Estimate [B]: -16.5,  $P = 0.03$  and B: -14.4,  $P = 0.03$ ), dyspareunia improved at 12-23 months (B: -31.8,  $P < 0.01$ ), and blood/mucus in stool improved at 24+ months (B: -8.01,  $P < 0.01$ ) vs. 0-11 months. At 24+ months, RT receipt was associated with worse stool frequency (B: 26.4,  $P < 0.01$ ), urinary frequency (B: 18.4,  $P = 0.04$ ), and flatulence (B: 23.0,  $P = 0.02$ ). **Conclusions:** YORC often require multimodality therapy including RT. Sore skin, dyspareunia, and blood/mucus in stool improved, but flatulence and frequency can persist beyond 2 years post RT. Proactive counseling and supportive measures are needed to inform treatment choices and mitigate long-term impact. Research Sponsor: University of Texas MD Anderson G.S.Hogan Gastrointestinal Research Fund.

EORTC QLQ-CR29 scores stratified by receipt of neoadjuvant RT at each time-point post-resection (range from 0-100).

|                      | Mean (SD)<br>0-11 months |         | Mean (SD)<br>12-23 months |         | Mean (SD)<br>24+ months |         |
|----------------------|--------------------------|---------|---------------------------|---------|-------------------------|---------|
|                      | No RT                    | RT      | No RT                     | RT      | No RT                   | RT      |
| Urinary frequency    | 43 (28)                  | 28 (23) | 32 (23)                   | 27 (24) | 18 (20)                 | 27 (25) |
| Blood/mucus in stool | 10 (22)                  | 10 (13) | 3 (7)                     | 6 (11)  | 4 (13)                  | 4 (8)   |
| Stool frequency      | 37 (32)                  | 30 (20) | 35 (23)                   | 31 (25) | 21 (23)                 | 35 (25) |
| Flatulence           | 27 (43)                  | 33 (32) | 36 (10)                   | 31 (27) | 21 (22)                 | 36 (28) |
| Sore skin            | 27 (15)                  | 43 (30) | 21 (31)                   | 25 (26) | 13 (19)                 | 26 (26) |
| Dyspareunia          | 67 (47)                  | 67 (30) | 33 (0)                    | 42 (36) | 24 (36)                 | 46 (34) |

Higher score indicates greater symptom burden.

**Prophylactic hyperthermic intraperitoneal chemotherapy in T4 colorectal cancer: Can it improve the oncologic prognosis?**

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**Background:** Previous studies have shown that T4 stage was one of the risk factors for peritoneal metastasis (PM) of colorectal cancer (CRC). However, there is paradoxical data to prove that prophylactic hyperthermic intraperitoneal chemotherapy (HIPEC) treatment is beneficial for CRC patients. **Methods:** In this cohort study, 220 consecutive CRC patients with T4N0-2M0 were enrolled and recorded from November 2014 to November 2018 with a 3-year follow-up following surgery. A propensity score matching (PSM) approach was applied to balance the clinicopathological characteristics. Finally, 180 patients were matched successfully to further analyze. The 3-year disease-free survival (DFS) was set as the primary outcome, and the PM rate between the two groups after PSM was analyzed. **Results:** Before PSM, of 220 CRC patients enrolled, the 3-year DFS was 83.9% in the HIPEC group, which was better than 67.2% in the control group. The cumulative PM incidence was 2.2% in the HIPEC group and 12.6% in the control group ( $p = 0.047$ ). After PSM, 180 matched patients were formed for further analysis. The DFS and the cumulative PM rate of the patients in the HIPEC group were also better than that in the control group ( $p = 0.034$  and  $0.047$ , respectively). For laparoscopic surgery subgroup analyses, PM rate of the patients with laparoscopic surgery was 13.8% in the No HIPEC group, and 2.6% in the HIPEC group ( $p = 0.070$ ). Through multivariable analysis, it was found that prophylactic HIPEC correlated to better DFS [hazard ratio (HR) 0.43, 95% confidence interval (CI) 0.19-0.95;  $p = 0.037$ ], and N2 stage correlated to worse DFS [HR 1.97, 95% CI 1.09-3.56;  $p = 0.025$ ]. Besides, no post-operative death occurred, the anastomotic leakage rate was 2.2% in the HIPEC group and 0.7% in the control group ( $p = 0.439$ ). **Conclusions:** Prophylactic HIPEC may improve the prognosis in the patients with T4N0-1M0 CRC, but not in the T4N2M0 CRC, and it does not significantly increase the surgery-related complications. Laparoscopic surgery followed by HIPEC for T4 stage CRC is relatively safe, which does not increase the risk of PM. Research Sponsor: the National Natural Science Foundation of China [81472819, 81672342]; Zhejiang Provincial Key R&D Program of China [2019C03018], and Zhejiang Provincial Natural Science Foundation of China [LY20H160038].

### Treatments and clinical outcomes in stage II colon cancer (CC) patients (pts) with 12-gene Oncotype DX Colon Recurrence Score assay-guided therapy: Real-world data.

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**Background:** The role of adjuvant chemotherapy (CT) in stage II CC is debated. The validated 12-gene Oncotype DX Colon Recurrence Score test provides a Recurrence Score (RS) result (range, 0-100) which estimates recurrence risk (RR) in stage II/III pts. We studied treatment and clinical outcomes in CC pts in whom treatment decisions incorporated the RS result. **Methods:** This prospectively designed cohort study included all stage II, MMR-P, CC pts who underwent the 12-gene Oncotype DX testing through Clalit between 1/2011 and 12/2016 and had available data with minimum 3-yr follow-up. Kaplan-Meier (KM) estimates and log-rank tests were used to compare RR and CC specific mortality (CCSM) between RS categories. Multivariable analysis (MVA) identified variables associated with RR/CCSM. **Results:** The analysis included 938 pts. Median age, 68 (IQR, 60-76) yrs; 96% had T3 tumors, and 89% had  $\geq 12$  nodes examined. Median RS was 26 (IQR, 19-33). The 3 RS categories (0-29, 30-40, and 41-100) included 65%, 24%, and 11% of pts, respectively. The overall CT use rate was 24%, with a significant difference between the 3 categories (14%, 36%, and 60%, respectively,  $P < .0001$ ). Pts with very low RS (0-15) comprised 14% of the cohort (CT use rate, 11%). Younger pts, and those with invasion/perforation/obstruction were more likely to receive CT. Clinical outcomes with a median follow up of 6.9 (IQR, 5.5-8.6) yrs are presented (Table). Among untreated pts, KM estimates for RR and CCSM differed significantly between the 3 RS categories ( $P < .0001$ ). Outcome of untreated RS 0-15 pts was excellent. In an MVA model, male sex, presence of invasion/perforation/obstruction and higher RS category (RS 41-100 vs 0-29 and vs 30-40, but not RS 30-40 vs 0-29) were associated with increased RR. For CCSM, the results were similar, but this time age  $\geq 70$  yrs replaced sex as a significant prognostic variable. Clinical outcomes within each RS group did not differ significantly between treated and untreated pts, but were numerically better with CT in the RS 41-100 group. **Conclusions:** This real-world analysis showed that the RS results provide independent prognostic information in stage II CC. Further studies are needed to investigate the potential role of the RS result as a predictor of CT benefit, but the data suggest that this benefit may be limited to pts with high RS results. Research Sponsor: Exact Sciences.

| RS group           | N         |                   | N CT- | 5-yr recurrence risk (95% CI) |                    | 5-yr CCSM (95% CI) |                   |
|--------------------|-----------|-------------------|-------|-------------------------------|--------------------|--------------------|-------------------|
|                    | N (%)     | untreated treated |       | Untreated                     | CT                 | Untreated          | CT                |
| Very low: 0-15     | 136 (14%) | 121               | 15    | 6.2% (3.0-12.4%)              | 13.3% (3.4-40.5%)  | 3.4% (1.3-8.8%)    | 13.3% (3.4-40.5%) |
| Low: 0-29          | 606 (65%) | 524               | 82    | 13.0% (10.3-16.2%)            | 15.7% (9.3-25.1%)  | 4.7% (3.1-6.9%)    | 6.1% (2.5-13.7%)  |
| Int.: 30-40        | 225 (24%) | 143               | 82    | 15.8% (10.6-22.9%)            | 18.3% (11.3-28.1%) | 5.0% (2.4-10.2%)   | 3.7% (1.2-10.9%)  |
| High: 41-100 (11%) | 107       | 43                | 64    | 30.6% (18.2-46.6%)            | 24.3% (15.4-36.0%) | 19.0% (9.3-34.9%)  | 17.0% (9.7-28.2%) |

### Efficacy and safety of KN026 in combination with KN046 in patients with locally advanced unresectable or metastatic HER2-positive other solid tumors.

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**Background:** Besides breast cancer (BC) and gastric cancer, HER2 is also widely expressed in other solid tumors, such as colorectal cancer (CRC), NSCLC, gallbladder cancer (GBC), renal pelvis cancer (RPC), pancreatic ductal adenocarcinoma (PDAC), etc. The reports of immunotherapy combined with HER2-targeted therapy are limited. KN026 is a novel bispecific antibody that simultaneously binds to two distinct HER2 epitopes. KN046 is a novel bispecific antibody that blocks both PD-L1 interaction with PD-1 and CTLA-4 interaction with CD80/CD86. **Methods:** KN026-203 study (NCT04521179) is an open-label, phase II, multi-center study to evaluate the efficacy and safety of KN026 (30mg/kg, Q3W, C1D1 and C1D8 loading) combined with KN046 (5mg/kg, Q3W) in patients (pts) with HER2-positive (defined as HER2 IHC 3+ or HER2 gene amplification) solid tumors. The study consisted of 3 cohorts, including HER2 positive gastric or gastroesophageal cancer (GC/GEJ), BC and other solid tumors. Pts received treatment until disease progression or unacceptable toxicity. Tumor assessments were scheduled every 6 weeks. The primary endpoints were ORR and DOR. The efficacy and safety of KN026 combined with KN046 were reported. **Results:** As of 10 November 2022, 26 pts with HER2 positive tumor other than BC and GC/GEJ were enrolled, including 15 CRC, 5 NSCLC, 4 GBC, 1 RPC and 1 PADC pts. All those pts had previous systemic treatment. The median age was 56 (range 37 ~ 67) years. 15 (57.7%) pts had liver metastasis. 92.3% of pts and all CRC pts had received  $\geq 2$  lines prior treatment. 5 (19.2%) and 6 (23.1%) pts had received prior anti-HER2 and anti-PD-(L)1 therapy, respectively. All 26 pts were assessed according to RECIST v1.1. The confirmed ORR was 53.8% (95% CI: 33.4, 73.4) with mDOR 6.8 months (95% CI: 2.9, 15.3). The mPFS was 5.6 months (95% CI: 2.9, 16.5) and 12-month OS rate was 80.4% (95% CI: 59.1, 91.4) with median follow-up time 16.6 months. For 15 CRC pts, the ORR was 53.3% (95% CI: 26.6, 78.7) with mDOR 11.7 months (95% CI: 3.2, NE). The mPFS was 12.2 months (95% CI: 2.7, NE). 12-month OS rate was 80.0% (95% CI: 50.0, 93.1) with median follow-up time 16.0 months. Common TRAEs were infusion related reaction (38.5%), AST increased (34.6%), ALT increased (26.9%), conjugated bilirubin increased (26.9%), rash (26.9%), anemia (26.9%) and blood bilirubin increased (26.9%). Most of them were Grade 1 or 2. The most common  $\geq$  Gr3 TRAEs were conjugated bilirubin increased (7.7%) and AST increased (7.7%). There was no treatment related death. **Conclusions:** KN026 combined with KN046 treatment had demonstrated favorable efficacy and safety profile in HER2 positive other solid tumors (non-GC/GEJ and non-BC). Especially very promising efficacy were observed in  $\geq 3$  lines HER2 positive CRC. Based on these results, a pivotal study was planned to verify the efficacy and safety of KN026 and KN046 combo. Clinical trial information: NCT04521179. Research Sponsor: Alphamab.

### Molecular profiling and characterization of the tumor immune microenvironment (TME) in appendiceal carcinoma (AC).

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**Background:** The rarity of AC presents challenges in understanding disease pathogenesis. We previously showed that AC has higher rates of mutations in *KRAS* and *GNAS* and lower rates of *TP53*, *APC*, and *PIK3CA* than CRC. The appendix also has many lymphoid clusters and regulates IgA production in the large bowel, suggesting that AC may be subject to more lymphocytic regulation than CRC. We sought to characterize the molecular profile and TME across AC histopathological types. **Methods:** AC samples were analyzed by DNA sequencing (592 genes, NextSeq, or whole exome sequencing, NovaSeq), whole transcriptome sequencing (WTS, NovaSeq), and immunohistochemistry (IHC) for molecular profiling, including microsatellite instability (MSI), mismatch repair (MMR), PD-L1 (SP142), and tumor mutational burden (TMB). Microenvironment Cell Population-counter Quantiseq was used to quantify tumor immune contexture using WTS. AC histopathology was derived from pathology reports. Mann-Whitney U and ChiSquare tests were applied as appropriate, with P-values adjusted for multiple comparisons using Benjamini-Hochberg. **Results:** AC (N = 731) were grouped by histology: 5% goblet cell (GC), 6% high-grade adenocarcinoma (HGA), 14% low grade mucinous (LGM), 33% mucinous adenocarcinoma (MA), 1% pseudomyxoma peritonei (PMP), 6% signet ring cell carcinoma (SRC), and 35% adenocarcinoma not otherwise specified (NOS) (see Table). Age at collection and PD-L1+ (0-5.2%) did not differ by histopathology. Seven patients had dMMR (4 NOS and 3 MA). Median TMB was significantly higher in NOS vs. GC (4 mutations/megabase vs 2,  $q < 0.001$ ), NOS vs. LGM (4 vs 3,  $q = 0.048$ ), MA vs. GC (4 vs 2,  $q < 0.001$ ), and MA vs. LGM (4 vs 3,  $q = 0.037$ ). Distinct TME patterns were observed in NOS vs. MA (median cell fraction: dendritic cells 0.07 vs 0,  $q < 0.01$ ; M2 macrophages 0.055 vs 0.042,  $q = 0.030$ ; natural killer cells 0.034 vs 0.031,  $q = 0.011$ ; CD4 T cells 0.006 vs 0,  $q = 0.044$ ) and GC vs. MA (M1 macrophages 0.039 vs 0.057,  $q = 0.021$ ; dendritic cells 0.012 vs 0,  $q < 0.01$ ; B cells 0.061 vs 0.050,  $q = 0.013$ ; M2 macrophages 0.074 vs 0.042,  $q < 0.01$ ; natural killer cells 0.041 vs 0.031,  $q = 0.01$ ; and neutrophils 0.017 vs 0.038  $q = 0.045$ ). T cell inflamed signature (TIS) and immune-oncology (IO) marker gene expression were higher in HGA and lower in LGM and MA. Somatic mutation rates differed by histopathology (see Table). **Conclusions:** There is significant heterogeneity in TMB, TME, and mutational profiles across AC histologies. MA has a particularly immune-cold TME shown by lower infiltration of lymphocytes, TIS, and IO gene expression. These findings are critical to identify novel biomarkers and develop new therapeutic strategies for AC. Research Sponsor: None.

| Gene        | AC Subtype     |                 |                  |                 |                 |                 |                  |
|-------------|----------------|-----------------|------------------|-----------------|-----------------|-----------------|------------------|
|             | GC<br>(N = 36) | HGA<br>(N = 43) | LGM<br>(N = 104) | MA<br>(N = 239) | PMP<br>(N = 10) | SRC<br>(N = 46) | NOS<br>(N = 253) |
| <i>GNAS</i> | 2.8%           | 31%             | 77.4%            | 43%             | 100%            | 6.8%            | 5.6%             |
| <i>KRAS</i> | 5.7%           | 56.1%           | 94.5%            | 78.3%           | 100%            | 16.3%           | 37.4%            |
| <i>TP53</i> | 14.7%          | 26.2%           | 9.9%             | 47.2%           | 0%              | 21.4%           | 56%              |
| <i>APC</i>  | 2.8%           | 7.1%            | 3.2%             | 5.2%            | 0%              | 0%              | 23.2%            |

## Comparison of synchronous neoplasms at colonoscopic diagnosis of early-onset versus average-onset colorectal cancer.

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**Background:** Colorectal cancer (CRC) incidence has steadily increased in adults younger than 50 years (EO-CRC) since the 1990s, with a predominance in the left colon and rectum. Precursor lesions of CRCs are adenomas, including advanced adenomas, and sessile serrated polyps. Given that neoplastic development is age-related, we hypothesized that patients with early-onset CRC (EO-CRC) may have fewer adenomas compared to patients with average-onset CRC (AO-CRC) at diagnosis. **Methods:** We performed a retrospective review of electronic health records at Mayo Clinic or Mayo Health System [2012 to 2021] and randomly selected 150 patients with EO-CRC who met eligibility criteria and matched them with 150 AO-CRC patients based on gender and colonoscopy indication. Known hereditary syndromes or inflammatory bowel disease were excluded. At colonoscopy where CRC was diagnosed, we recorded findings of adenomas, advanced adenomas (> 1 cm, villous histology, or high-grade dysplasia), and sessile serrated polyps (SSP). **Results:** Median age at diagnosis was 43 years for EO-CRC and 67 years for AO-CRC. No significant differences in BMI, family history of CRC, MMR, *BRAF*<sup>V600E</sup> *KRAS* status or SSPs were found between groups (all  $p > 0.05$ ). Among patients with colon cancer, no difference was found for adenoma number yet patients with EO-colon cancer (EO-CC) had larger polyps [median of 10 mm vs 5 mm,  $p = 0.001$ ] (Table) compared with AO-colon cancer (AO-CC). At least 1 adenoma was found in 56% and 47% of patients with EO-CC and AO-CC, respectively. Moreover, advanced adenomas were significantly more common in the EO-CC group compared with the AO-CC group (42% and 14%,  $p < 0.001$ ) [Table]. Among patients with rectal cancer, non-advanced adenomas were less frequent and although advanced adenoma were similar in frequency, they were more commonly located in the rectum in EO-rectal compared to AO-rectal cancers (Table). **Conclusions:** Patients with EO-colon cancer had a significantly higher likelihood of a synchronous advanced adenoma in the colon compared to those with AO-CRC. Together, these data suggest accelerated carcinogenesis throughout the colorectum in early onset patients, with increased risk for development of synchronous and metachronous CRC. Research Sponsor: None.

Synchronous neoplasia characteristics stratified by age group at cancer diagnosis.

| Characteristics <sup>b</sup> |                      | EO-CC <sup>a</sup><br>N = 75 | AO-CC <sup>a</sup><br>N = 75 | P-value              | EO-RC <sup>a</sup><br>N = 75 | AO-RC <sup>a</sup><br>N = 75 | P-value            |
|------------------------------|----------------------|------------------------------|------------------------------|----------------------|------------------------------|------------------------------|--------------------|
| Adenomas                     | Detected             | 42 (56%)                     | 35 (47%)                     | 0.327 <sup>c</sup>   | 21 (28%)                     | 36 (48%)                     | 0.018 <sup>c</sup> |
|                              | Right                | 27 (64%)                     | 28 (78%)                     | 0.292 <sup>c</sup>   | 8 (38%)                      | 27 (75%)                     | 0.013 <sup>d</sup> |
|                              | Left                 | 31 (74%)                     | 22 (59%)                     | 0.265 <sup>c</sup>   | 14 (67%)                     | 19 (53%)                     | 0.455 <sup>c</sup> |
| Advanced Adenomas            | Detected             | 31 (42%)                     | 10 (14%)                     | < 0.001 <sup>c</sup> | 11 (15%)                     | 14 (19%)                     | 0.661 <sup>c</sup> |
|                              | High grade dysplasia | 15 (20%)                     | 0 (0%)                       | < 0.001 <sup>c</sup> | 2 (3%)                       | 3 (4%)                       | 1 <sup>d</sup>     |

<sup>a</sup> Early onset (EO), average onset (AO), colon cancer (CC), rectal cancer (RC) <sup>b</sup> n (%), median (IQR) <sup>c</sup> Chi-square <sup>d</sup> Fisher's exact <sup>e</sup> Mann Whitney U.

# Atezolizumab plus tiragolumab in combination with chemoradiotherapy in localized squamous cell carcinoma of the anal canal: TIRANUS (GEMCAD-2103) trial.

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**Background:** Radical chemoradiotherapy (CRT) is the standard of care in patients with localized anal squamous cell carcinoma; however, around 30% of patients do not achieve a complete clinical response (CCR) and require salvage surgery. Approximately 84% of anal carcinoma is associated with high risk types of human papilloma virus (HPV), primarily HPV 16 that generates high frequencies of tumor-infiltrating lymphocytes and inflammatory responses that have been linked with upregulation of PD-L1 (up to 74% of patients with squamous cell anal cancer). Additionally, poliovirus receptor (PVR) expression has been described in several squamous cell carcinomas, and has been correlated with PD-L1 expression and poorer prognosis, suggesting dual inhibition of PVR and PD-L1 as a potential mechanism of overcome the resistance to immune checkpoint monotherapy. Moreover, CRT induces the generation of tumor-neoantigens and could act in synergy with immunotherapy in this setting. The trial hypothesizes that the addition of atezolizumab (anti-PD-L1) and tiragolumab (anti-TIGIT) to chemoradiotherapy may lead to enhanced and more durable responses. **Methods:** TIRANUS is a Phase II, single-arm, open-label, non-randomized, multicenter clinical trial of atezolizumab and tiragolumab in concomitance with standard CRT as definitive therapy in treatment-naïve, localized squamous cell carcinoma of the anal canal. Patients receive atezolizumab (1200mg) plus tiragolumab (600 mg) for 2 cycles (Q3W) in concomitance with the 6 weeks of CRT (cisplatin: 60 mg/m<sup>2</sup> on days 1 and 29; 5-FU: 1000 mg/m<sup>2</sup> per day on days 1-4 and 29-32; radiotherapy: 1.8 Gy per day / total dose 54 Gy). After the concomitant phase, patients receive atezolizumab (1200mg) and tiragolumab (600 mg) Q3W for 6 additional cycles (consolidation phase). The primary endpoint is CCR rate, defined as the percentage of patients who achieve radiological complete response (CR), disappearance of all lesions according to RECIST 1.1 criteria (locally assessed) and no presence of residual disease assessed by biopsy at the end of consolidation phase (week 26). Secondary endpoints include Locoregional failure rate (LFR), Disease-free survival (DFS), Colostomy-free survival (CFS), Overall survival (OS), quality of life, safety and the determination of immune biomarkers potentially predictors of response in blood and tumor samples. Using a precision analysis by Clopper-Pearson method (asymptotic 95% confidence interval) and an expected CCR rate of 85%, a total of 45 evaluable patients are needed to obtain a precision estimation of ±10.4%. Accrual started in February 2023. EudraCT: 2021-005887-24 Clinical Trial identifier: NCT05201612. Clinical trial information: NCT05661188. Research Sponsor: GEMCAD though industry partner Roche.

### Phase II/III study of circulating tumor DNA as a predictive biomarker in adjuvant chemotherapy in patients with stage II colon cancer: NRG-GI005 (COBRA).

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**Background:** Detection of circulating tumor DNA (ctDNA) shed into the bloodstream represents a highly specific and sensitive approach for identifying microscopic or residual tumor cells after surgical resection. For patients (pts) with colon cancer (CC), the detection of ctDNA is associated with persistent disease after resection and outperforms traditional clinical and pathological features in prognosticating risk for recurrence. However, for pts with stage II CC, there are currently no validated biomarkers predicting benefit in identifying pts whose residual disease cancer be cleared by adjuvant chemotherapy. We hypothesize that for pts whose stage II colon cancer has been resected and who have no traditional high-risk features, a positive ctDNA status may identify those who will benefit from adjuvant chemotherapy. **Methods:** In this prospective phase II/III clinical trial, pts (N = 1,408) with resected stage II CC without traditional high-risk features and whom the evaluating oncologist deems suitable for active surveillance (i.e., not needing adjuvant chemotherapy) will be randomized 1:1 into 2 arms: standard-of-care/observation (Arm A), or prospective testing for ctDNA (Arm B). Postoperative blood will be analyzed for ctDNA with the Guardant Reveal assay, covering CC-relevant mutations and CC-specific methylation profiling. Pts in Arm B with ctDNA detected will be treated with 6 months of adjuvant (FOLFOX) chemotherapy. For all pts in Arm A, ctDNA status will be analyzed retrospectively at the time of endpoint analysis. The primary endpoints are clearance of ctDNA with adjuvant chemotherapy (phase II) and recurrence-free survival (RFS) for “ctDNA-detected” pts treated with or without adjuvant chemotherapy (phase III). Secondary endpoints will include time-to-event outcomes (OS, RFS, TTR) by ctDNA marker status and treatment, prevalence of detectable ctDNA in stage II CC, and rates of compliance with assigned intervention. Archived normal and matched tumor and blood samples will be collected for exploratory correlative research. Enrollment continues across North America to the 1408-patient phase III endpoint. NCT#: 04068103. Support: U10CA180868, -180822; UG1CA189867; GuardantHealth. Clinical trial information: NCT04068103. Research Sponsor: U.S. National Institutes of Health; GuardantHealth.

**The Ohio State University Guardant Shield Colorectal Cancer Screening Project.**

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**Background:** Asymptomatic screening is an effective method for detecting early cancers when prognosis strongly depends on stage at time of diagnosis. Unscreened individuals often have a basic understanding of the importance and availability of colorectal cancer (CRC) screening but have concerns regarding the collection method of the currently available stool-based options or the invasiveness of direct visualization options that deter test completion. We hypothesize that offering a blood-based CRC screening test during a routine healthcare visit can increase screening adherence, especially within hard-to-reach populations and those hesitant about completing CRC screening. **Methods:** The Ohio State University Guardant Shield Colorectal Cancer Screening Project (NCT05716477) is a prospective study in the United States designed to recruit individuals who are currently engaging with mobile health clinics and are not up to date with CRC screening, with the aim of improving health in hard-to-reach populations. The study's primary objective is to evaluate the acceptance of a commercially available blood-based CRC screening test (Shield, Guardant Health, USA) in individuals aged 45-84, who have existing appointments with a mobile health clinic, and are not up to date with guideline recommended CRC screening. The study team screens for eligibility criteria before the appointment and reaches out to the individual to begin the informed consent process. Positive blood-based test results will be triaged to colonoscopy for further evaluation. A post-study survey will be administered to consented individuals aimed to collect data on CRC screening knowledge which will be correlated to patient demographics and uptake. Acceptance will be defined as those approached and with completed specimen collection. **Progress:** The study initiated on October 25, 2022, with the first patient enrolled on December 20, 2022. Enrollment is expected to continue with a target enrollment of 300 individuals. **Clinical trial information:** NCT05716477. **Research Sponsor:** Guardant Health.

# **BREAKWATER: An open-label, multicenter, randomized, phase 3 study, with a safety lead-in (SLI), of first-line (1L) encorafenib (E) + cetuximab (C) ± chemotherapy (CT) vs standard-of-care (SOC) CT for BRAF V600E-mutant metastatic colorectal cancer (mCRC).**

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**Background:** Globally, 8-12% of patients (pts) with mCRC have BRAFV600E mutations, which confer poor prognosis. Based on BEACON (NCT02928224), EC was approved for the treatment (tx) of previously treated pts with BRAF V600E-mutant mCRC. BREAKWATER (NCT04607421) is evaluating EC ± CT vs SOC CT in pts with BRAF V600E-mutant mCRC. In the BREAKWATER SLI (N = 57), EC + CT showed encouraging antitumor activity. Confirmed ORR by BICR (95% CI) with EC + mFOLFOX6 and EC + FOLFIRI, respectively, was 68.4% (46.0, 84.6) and 75.0% (46.8, 91.1) in 1L, and 37.5% (13.7, 69.4) and 44.4% (24.6, 66.3) in 2L. mPFS by BICR (95% CI) with EC + mFOLFOX6 and EC + FOLFIRI, respectively, was 11.1 months [mo] (8.5, NE) and NE (13.8, NE) in 1L, and 10.8 mo (4.3, NE) and 12.6 mo (6.9, NE) in 2L. These results support the continued evaluation of EC + CT. Based on the SLI, EC + mFOLFOX6 was selected for the phase 3 portion. Here we present the updated study design. **Methods:** BREAKWATER is an ongoing, open-label, multicenter, randomized, phase 3 study evaluating 1L EC ± CT vs SOC CT alone in pts with BRAF V600E-mutant mCRC. Approximately 620 pts will be enrolled in the phase 3 portion and an additional 135 in cohort 3 (Table). Phase 3 and cohort 3 inclusion criteria are age ≥16 (or ≥18 based on country); no prior systemic tx for metastatic disease; measurable disease (RECIST 1.1); BRAF V600E-mutant mCRC (blood or tumor tissue); ECOG PS 0 or 1; and adequate bone marrow, hepatic, and renal function. Pts who received prior BRAF or EGFR inhibitors, those with symptomatic brain metastases (unless stable for ≥4 weeks prior to randomization), or with MSI-H/dMMR tumors (unless ineligible to receive immune checkpoint inhibitors due to a pre-existing medical condition) are excluded. Study treatments and endpoints are shown in the Table. Phase 3 enrollment began in November 2021. Clinical trial information: NCT04607421. Research Sponsor: Pfizer Inc.

| Study treatments and endpoints. |                                                                                                                                                                                                                           |                                                                                         |
|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
|                                 | Phase 3                                                                                                                                                                                                                   | Cohort 3 <sup>b</sup>                                                                   |
| <b>Randomization</b>            | 1:1 <sup>a</sup>                                                                                                                                                                                                          | 2:1                                                                                     |
| <b>Stratification</b>           | · ECOG PS (0 vs 1)<br>· Regions (US/Canada vs Europe vs Rest of World)                                                                                                                                                    | · ECOG PS (0 vs 1)                                                                      |
| <b>Treatment (1L)</b>           | · <b>Arm A (n≈150):</b> EC <sup>a</sup><br>· <b>Arm B (n = 235):</b> EC + mFOLFOX6<br>· <b>Arm C (control; n = 235):</b> investigator's choice of mFOLFOX6 ± bevacizumab, FOLFOXIRI ± bevacizumab, or CAPOX ± bevacizumab | · <b>Arm D (n = 90):</b> EC + FOLFIRI<br>· <b>Arm E (n = 45):</b> FOLFIRI ± bevacizumab |
| <b>Endpoints</b>                |                                                                                                                                                                                                                           |                                                                                         |
| <b>Primary</b>                  | PFS <sup>c</sup> and ORR <sup>c</sup> (Arm B vs C)                                                                                                                                                                        | ORR <sup>c</sup> (Arm D vs E)                                                           |
| <b>Key secondary</b>            | OS (Arm B vs C)                                                                                                                                                                                                           | PFS <sup>c</sup> (Arm D vs E)                                                           |

<sup>a</sup>Randomization to Arm A ceased upon site institutional review board/ethics committee and competent authority approval of protocol amendment 5. <sup>b</sup>Cohort 3 to begin after phase 3 enrollment is complete. <sup>c</sup>By BICR. BICR, blinded independent central review; CAPOX, capecitabine/oxaliplatin; FOLFIRI, fluorouracil/leucovorin/irinotecan; FOLFOX, fluorouracil/leucovorin/oxaliplatin; FOLFFOXIRI, fluorouracil/leucovorin/oxaliplatin/irinotecan.

**An open-label clinical trial of RP2 and RP3 oncolytic immunotherapy in combination with atezolizumab and bevacizumab for the treatment of patients with advanced colorectal carcinoma.**

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**Background:** Colorectal cancer (CRC) is the third most frequently diagnosed cancer and the second leading cause of cancer-related mortality worldwide. PD-1/PD-L1 inhibition, in combination with other modalities, has demonstrated significant benefit in patients (pts) with microsatellite instability-high or mismatch repair deficient CRC. However, these agents have limited, if any, clinical benefit in pts with microsatellite stable (MSS) or mismatch repair proficient (pMMR) CRC. RP2 is an enhanced potency oncolytic herpes simplex virus type 1 which expresses the fusogenic gibbon ape leukemia virus glycoprotein with the R sequence deleted (GALV-GP-R-), granulocyte-macrophage colony-stimulating factor (GM-CSF), and an anti-CTLA-4 antibody-like molecule; RP3 additionally expresses 4-1BB and CD40 activating ligands, but does not express GM-CSF. Both agents have demonstrated preliminary safety and efficacy in pts with solid tumors. This study will evaluate the safety and efficacy of RP2 and RP3 in combination with atezolizumab (Atezo) and bevacizumab (Bev) in pts with advanced MSS and pMMR CRC. **Methods:** Pts with a histologic diagnosis of unresectable and/or metastatic CRC, with documented MSS or pMMR status, and previously treated with up to 3 standard-of-care systemic regimens will be enrolled in the RP2 + Atezo + Bev or RP3 + Atezo + Bev treatment groups (30 pts per group). Further key inclusion criteria include Eastern Cooperative Oncology Group performance status 0–1 and adequate hepatic, renal, and hematologic function. RP2/RP3 will be injected into tumors by direct (including via colonoscope) or image-guided injection. Pts will receive 8 total doses of up to 10 mL of RP2 or RP3, with a first dose concentration of  $1 \times 10^6$  plaque-forming units (PFU)/mL, followed by 3 doses of  $1 \times 10^7$  PFU/mL every 2 weeks, and then 4 doses of  $1 \times 10^7$  PFU/mL every 3 weeks. Pts may receive a second course of up to 8 injections of RP2/RP3 if study criteria are met. Bev will be administered starting on day 1 (with first dose of RP2/RP3); Atezo will be administered starting week 7 on the same days as RP2/RP3. The primary endpoint is objective response rate; secondary endpoints are safety, overall survival, progression-free survival, duration of response, and complete response rate. Antitumor activity will be evaluated using Response Evaluation Criteria in Solid Tumors version 1.1 as modified for this study. Safety will be assessed by physical examination, clinical laboratory evaluations, vital signs, and monitoring for adverse events (AEs; including serious AEs). Clinical trial information: NCT05733611. Research Sponsor: Replimune Inc.

**Alliance A022101: A pragmatic, randomized phase III trial evaluating total ablative therapy for patients with limited metastatic colorectal cancer: Evaluating Radiation, Ablation, and Surgery (ERASur).**

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**Background:** For patients with oligometastatic colorectal cancer (CRC), aggressive local therapy of isolated metastases, particularly in the liver, has been associated with long-term progression-free survival and overall survival (OS) primarily based on retrospective evidence. However, in patients with limited metastatic CRC that is deemed inoperable or those with additional disease outside of the liver or lungs, the role of local ablative therapies, including microwave ablation (MWA) and stereotactic body radiation therapy (SBRT), to render patients disease free is less clear. Further, despite the long history of treating oligometastatic CRC with local therapy, which is provider biased and not evidence based, questions remain regarding the benefit of extending the paradigm of metastatic directed therapy to patients with more extensive disease. This trial seeks to use a pragmatic multimodality approach that mirrors the current clinical dilemma. This study is designed to evaluate the safety and efficacy of adding total ablative therapy (TAT) of all sites of disease to standard of care systemic treatment in those with limited metastatic CRC. **Methods:** A022101 is a National Clinical Trials Network randomized phase III study planned to enroll 364 patients with newly diagnosed metastatic CRC (BRAF wild-type, micro-satellite stable) with  $\leq 4$  sites of metastatic disease on baseline imaging. Liver-only metastatic disease is not permitted, and lesions must be amenable to any combination of surgical resection, MWA, and/or SBRT with SBRT required for at least one lesion. Patients receive first-line systemic therapy for 4-6 months and are then randomized 1:1, stratified by number of metastatic organ sites (1-2 vs. 3-4), timing of metastatic disease diagnosis (de novo vs. secondary), and presence of metastatic disease outside the liver and lungs in at least one site. Patients in Arm 1 will receive TAT which consists of treatment of all metastatic sites with SBRT  $\pm$  MWA  $\pm$  surgical resection followed by standard of care systemic therapy. Patients in Arm 2 will continue with standard of care systemic therapy alone. The primary endpoint is OS. Secondary endpoints include event-free survival, treatment-related toxicities, and local recurrence with exploratory biomarker analyses. The study needs 346 evaluable patients combined in the 2 arms to demonstrate an improvement in OS with a hazard ratio of 0.7 to provide 80% power with a one-sided alpha of 5%. The trial utilizes a group sequential design with two interim analyses (25% and 50% of events) for futility. The trial activated in January 2023 and recruitment is ongoing. Support: U10CA180821, U10CA180882; <https://acknowledgments.alliancefound.org>. U10CA180820 (ECOG-ACRIN); U10CA180868 (NRG); U10CA180888 (SWOG); Clinicaltrials.gov identifier: NCT05673148 Clinical trial information: NCT05673148. Research Sponsor: U.S. National Institutes of Health.

**STELLAR-303: A phase 3 study of XLO92 in combination with atezolizumab versus regorafenib in patients with previously treated metastatic colorectal cancer.**

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**Background:** Metastatic colorectal cancer (mCRC) is associated with poor prognosis and limited treatment options for patients who have progressed on first- and second-line chemotherapy. Regorafenib and trifluridine-tipiracil are approved in third- or later-line setting, but survival benefit is limited. Immune checkpoint inhibitor (ICI) therapy with nivolumab ± ipilimumab or single-agent pembrolizumab is approved in microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) mCRC; however, MSI-H mCRC is present in only ~5% patients. Phase 1/2 studies have evaluated the tyrosine kinase inhibitor (TKI) cabozantinib in combination with the ICIs atezolizumab and durvalumab and have demonstrated encouraging clinical activity, with data suggesting greater benefit in *RAS* wild-type (wt) versus *RAS* mutant (mut) mCRC (Abrams TA, et al. ASCO GI 2022:Abs 121; Saeed A, et al. ASCO GI 2022:Abs 135). XLO92 is a TKI that targets MET, VEGFR2, and the TAM kinases AXL and MER, which are implicated in tumor growth, metastasis, angiogenesis, and immunosuppression of the tumor microenvironment. XLO92 displays immunomodulatory properties that may promote an immunopermissive tumor microenvironment and its half-life of 16–22 hours is supportive of once daily (QD) dosing. The phase 3 STELLAR-303 study (NCT05425940) is evaluating the efficacy and safety of XLO92 in combination with atezolizumab versus regorafenib in patients with microsatellite stable or microsatellite instability-low mCRC who have progressed after or are intolerant to standard of care (SOC) therapy. **Methods:** STELLAR-303 is a global, randomized, open-label, phase 3 study. Eligible patients are aged ≥18 years with non-MSI-H/dMMR mCRC (determined by tissue-based analysis) that is measurable per RECIST v1.1 and have an ECOG performance status of 0–1 and adequate organ function. Patients must have progressed during/after or be intolerant to SOC therapies for mCRC; prior regorafenib, trifluridine-tipiracil, or anti-PD-L1/PD-1 ICIs are not allowed. Patients are randomized 1:1 to XLO92 100 mg PO QD + atezolizumab 1200 mg IV Q3W or to regorafenib 160 mg PO QD (21 days/28-day cycle); randomization is stratified by geographic region (Asia, other), *RAS* status (wt, mut), and liver metastases (yes, no). Enrollment of 600 patients is planned (400 *RAS*wt, 200 *RAS*mut). The primary endpoint is duration of overall survival (OS) in the *RAS*wt population, with other efficacy endpoints (progression-free survival, objective response rate, and duration of response per RECIST v1.1 by investigator and change in tumor markers) assessed in the *RAS*wt population and, with OS, in the *RAS*mut populations and in all randomized patients. Additional endpoints will assess safety, quality of life, changes in biomarkers, pharmacokinetics, immunogenicity, and healthcare utilization. Enrollment is ongoing. Clinical trial information: NCT05425940. Research Sponsor: Exelixis, Inc.

**MOUNTAINEER-03: Phase 3 study of tucatinib, trastuzumab, and modified FOLFOX6 as first line treatment in HER2+ metastatic colorectal cancer.**

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**Background:** The current standard of care (SOC) for the treatment of metastatic colorectal cancer (mCRC) is multi-agent chemotherapy, with or without a VEGF or EGFR inhibitor. Human epidermal growth factor receptor-2 (HER2) is a validated clinical target in breast and gastric cancers. HER2 amplification occurs in 3%–5% of patients with mCRC; the rate of HER2 amplification can increase to approximately 10% in patients with *RAS/BRAF* wild-type mCRC tumors. Tucatinib (TUC)—a highly selective, HER2-directed tyrosine kinase inhibitor—is approved in multiple regions for HER2-positive (HER2+) metastatic breast cancer, approved in the US for HER2+ metastatic colorectal cancer, and under investigation in gastrointestinal cancers. MOUNTAINEER (NCT03043313) evaluated the safety and efficacy of TUC and trastuzumab (Tras) in patients with treatment-refractory *RAS* wild-type, HER2+ mCRC. Results from the primary endpoint analysis showed clinically meaningful activity, with a confirmed objective response rate (ORR) per blinded independent central review (BICR) of 38.1% (95% confidence interval [CI] 27.7, 49.3) and median duration of response per BICR of 12.4 months (95% CI 8.5, 20.5). Results also demonstrated TUC + Tras was well tolerated, with a low discontinuation rate (5.8%) and no deaths due to adverse events. MOUNTAINEER-03 will further investigate TUC in combination with modified FOLFOX (mFOLFOX) and Tras in patients with *RAS* wild-type HER2+ mCRC. **Methods:** MOUNTAINEER-03 (NCT05253651) is a global, open-label, randomized, phase III study for first-line treatment of HER2+ and *RAS* wild-type mCRC. Approximately 400 patients will be randomized 1:1 to the TUC experimental arm (TUC [300 mg orally twice daily] + Tras + mFOLFOX) or the SOC arm (mFOLFOX alone or in combination with either bevacizumab or cetuximab). HER2 status is determined centrally with tissue-based HER2 immunohistochemistry and in situ hybridization assays. Eligible patients must not have received prior treatment in the metastatic setting but may have received adjuvant treatment if completed > 6 months prior to enrollment. Patients must be ≥18 years old with an Eastern Cooperative Oncology Group Performance Status of ≤1 and *RAS* wild-type mCRC. Patients with treated stable central nervous system metastases are eligible. Randomization is stratified by primary tumor location (left-sided vs other) and liver metastases (presence/absence). The primary endpoint is progression-free survival per RECIST v1.1 assessed by BICR. Key secondary endpoints are overall survival and confirmed ORR per RECIST v1.1 assessed by BICR. Enrollment is ongoing in North America, Asia, Australia, and Europe. Clinical trial information: NCT05253651. Research Sponsor: Seagen, Inc.

**A phase 1/2a, multicenter, open-label study of CNA3103 (LGR5-targeted, autologous CAR-T cells) in patients with metastatic colorectal cancer (mCRC).**

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**Background:** Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) is a cancer stem cell (CSC) marker that is abundant in solid tumors, highly expressed in colorectal cancer and involved in tumor initiation, growth, and metastasis. Its expression is linked to poor survival and poor patient response to therapy in mCRC, and presents an attractive opportunity for selectively targeting a highly specific, functional CSC receptor in this disease. CNA3103 is a first-in-class cell product consisting of autologous T cells transduced to express a CAR to target LGR5. It contains the antigen-binding domain of BNC101, a humanized monoclonal LGR5 antibody previously tested in Phase 1 in mCRC subjects and demonstrated to be safe and well tolerated with no off-target binding activity. **Methods:** This is a first in human, multicenter, open label, Phase 1/2a dose escalation and expansion study to determine the safety of and overall best response to CNA3103 in subjects with mCRC. Up to 44 subjects (24 in Phase 1 and 20 in Phase 2a) will be enrolled. The trial follows a BOIN design with a minimum of 3 consecutively enrolled subjects per cohort, and a 28-day DLT evaluation period. Four cell dose levels ( $5 \times 10^7$ ,  $1.5 \times 10^8$ ,  $4.5 \times 10^8$ ,  $1.5 \times 10^9$ ) of CNA3103 will be evaluated. A Safety Monitoring Committee (SMC) will oversee the conduct of the study. The pharmacokinetic (PK) profile of CNA3103 in blood will be assessed. Exploratory objectives include evaluation of LGR5+ cells and CNA3103 in tumor biopsies, immune cellular profile, cytokine levels and circulating tumor DNA in blood, and immunogenicity of CNA3103. Main inclusion criteria: mCRC subjects positive for LGR5 expression and failing prior treatment with 5-FU, oxaliplatin and irinotecan-based regimens for metastatic disease;  $>1$  radiologically measurable lesion per RECISTv1.1;  $>1$  biopsiable lesion at baseline; ECOG 0 or 1; serum albumin  $>3$  g/dL; and normal organ and marrow function. Main exclusion criteria: BRAF-mutated disease, clinical ascites/ pleural effusions; active autoimmune/ connective tissue disease; history of inflammatory bowel disease or active peptic ulcer disease. The trial is sponsored by Carina Biotech. Phase 1 enrolment is limited to Australian sites. Phase 2a enrolment will take place in Australia and the US. For information, contact Lina Jablonskis [lina@carinabiotech.com](mailto:lina@carinabiotech.com) or +61 437 891 563. Research Sponsor: Carina Biotech.

**Total neoadjuvant treatment without surgery for locally advanced rectal cancer: Prospective study to assess tumor response, circulating genetic, and epigenetic biomarkers, and stromal transcriptome to interpret clinical outcome (NO-CUT trial).**

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**Background:** Total neoadjuvant therapy (TNT) has recently become a standard therapy for locally advanced rectal cancer (LARC). All nonsurgical interventions, including chemotherapy and concurrent chemoradiotherapy (CRT), are administered before either surgical resection of the rectum or non-operative management (NOM), depending on response to TNT. In the case of NOM, the impact of distant relapse on survival remains to be elucidated and circulating tumor DNA (ctDNA) monitoring has been proposed as a tool for early detection of relapse. Furthermore, predictive biomarkers of response to TNT are warranted for the early identification of patient candidates for NOM. In this regard, stromal contribution to sensitivity to pre-operative CRT is investigated by a transcriptomic signature on baseline tumor samples, as previously proposed by Isella C et al., *Nature Genet*, 2015. **Methods:** NO-CUT is a multicenter one-stage phase II trial aimed at assessing if a TNT approach with CAPOX followed by CRT can safely spare demolitive surgery in patients with LARC achieving a clinical complete response without increasing the risk of distant metastatic relapse. A Brookmeyer and Crowley approach was chosen to test the null hypothesis that the true distant relapse-free survival at 2.5 years rate is  $\leq 75\%$  against a one-sided alternative. The NOM cohort of the trial needs to accrue 44 evaluable patients and to be followed for at least 2.5 years. Such design yields a type I error rate of 10% and power of 80% when the true 2.5 years distant RFS proportion is 87%. Since it is assumed that the proportion of patients entering in the NOM cohort of the trial is at least 25%, a total of 180 patients are to be enrolled in NO-CUT study. Patients with medium-low LARC undergo TNT (4 cycles of CAPOX: capecitabine 1000 mg/m<sup>2</sup> bid days 1-14 q 3 weeks; oxaliplatin 130 mg/m<sup>2</sup> day 1 q 3 weeks) and 5 weeks of standard pelvic CT-RT, then restaging with imaging (MRI, CT-scan, and ultrasound endoscopy), and tumor biopsy. According to an algorithm defined for assessing tumor response, patients enter either NOM or surgery cohorts. During the follow-up phase of the trial, the NOM cohort is followed with intensive pelvic imaging and periodic blood samplings for 5 years. Tumor samples and ctDNA are studied at baseline, after TNT, and during follow-up. As of January 2023, 161 of planned 180 patients in 4 centers have been enrolled. Supported by grants from Associazione Italiana Ricerca Cancro (AIRC IG 2017-20685) and Fondazione Oncologia Niguarda. Clinical trial information: EudraCT 2017-003671-60. Research Sponsor: AIRC IG; FON (Fondazione Oncologia Niguarda).

**Colon adjuvant chemotherapy based on evaluation of residual disease (CIRCULATE-US): NRG-G1008.**

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**Background:** Currently, there are no biomarkers validated prospectively in randomized studies for resected colon cancer (CC) to determine need for adjuvant chemotherapy (AC). However, circulating tumor DNA (ctDNA) represents a highly specific and sensitive approach (especially with serial monitoring) for identifying minimal/molecular residual disease (MRD) post-surgery in CC patients (pts), and may outperform traditional clinical and pathological features in prognosticating risk for recurrence. CC pts who do not have detectable ctDNA (ctDNA-) are at a much lower risk of recurrence and may be spared the toxicities associated with AC. Furthermore, for CC pts with detectable ctDNA (ctDNA+) who are at a very high risk of recurrence, the optimal AC regimen has not been established. We hypothesize that for pts whose CC has been resected, ctDNA status may be used to risk-stratify for making decisions about AC. **Methods:** In this prospective phase II/III trial, up to 1,912 pts with resected stage III A, B (all pts) and stage II, IIIC (ctDNA+ only) CC will be enrolled. Based on the post-operative ctDNA status using personalized and tumor-informed assay (Signatera™, bespoke assay), those who are ctDNA- (Cohort A) will be randomized to immediate AC with fluoropyrimidine (FP) + oxaliplatin (Ox) for 3-6 mos per established guidelines vs. serial ctDNA monitoring. Patients who are ctDNA+ post-operatively or with serial monitoring (Cohort B) will be randomized to FP+Ox vs. more intensive AC with addition of irinotecan (I) for 6 mos. The primary endpoints for Cohort A are time to ctDNA+ status (phase II) and disease-free survival (DFS) (phase III) in the immediate vs. delayed AC arms. The primary endpoint for Cohort B is DFS in the FP+Ox vs FP+Ox+I arms for both phase II and phase III portions of the trial. Secondary endpoints include prevalence of detectable ctDNA post-operatively, time-to-event outcomes (overall survival and time to recurrence) by ctDNA status, and the assessment of compliance to adjuvant therapy. Biospecimens including archival tumor tissue, as well as post-operative plus serial matched/normal blood samples, will be collected for exploratory correlative research. Active enrollment across the NCTN started in June 2022. NCT#: NCT05174169. Support: U10-CA-180868, -180822; UG1CA-189867; Natera, Inc. Clinical trial information: NCT05174169. Research Sponsor: U.S. National Institutes of Health; Natera, Inc.

### The CIRCULATE Spain study: Circulating tumor DNA based decision for adjuvant treatment in localized colon cancer.

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**Background:** A substantial proportion of colon cancer (CC) patients do not benefit from adjuvant chemotherapy, and almost 40% of them will develop metastases despite initial optimal treatment. We have designed the phase 2 CIRCULATE-SPAIN trial (EudraCT Number: 2021-000507-2) to prove the feasibility of using liquid biopsy detection of minimal residual disease to guide the post-surgical clinical management of localized CC patients. Moreover, it is important to define if conventional versus intensive adjuvant chemotherapy could convert plasma circulating tumor DNA (ctDNA) positive into a ctDNA negative status. **Methods:** CIRCULATE-SPAIN is a trial to prove the feasibility of using liquid biopsy detection of MRD to guide the post-surgical clinical management of early colon cancer patients. It is a multicenter, randomized, open-label phase II “Single-To-double ARm Transition (START)” study in which patients are firstly enrolled into a single arm (Phase IIa) study expandable to a randomized double arm (Phase IIb). The CIRCULATE-SPAIN trial involves two primary endpoints, one for the Phase IIa and a second one for the Phase IIb. The primary objective for Phase IIa is to analyze the potential effect of FOLFOXIRI as an intensive adjuvant treatment for ctDNA clearance as a surrogate biomarker of treatment efficacy. Only if the pre-fixed result of 14 out of 40 patients achieves ctDNA clearance, the trial will be expanded to a phase IIb in which patients will be enrolled to randomly receive intensive adjuvant treatment (FOLFOXIRI) versus conventional adjuvant therapy (CAPOX). The primary objective in this phase is to study differences on the conversion rate between both groups. An academic assay based on tumor informed approach is used for ctDNA determination. Tumor and germline samples analysis are carried out by an in-house-developed bioinformatics pipeline specialized in variant-calling analysis using a custom 29-gene panel. Somatic variants are identified by the germline mutations extraction. Different annotation databases and an internal pathological mutation database are used to rule out potential benign somatic variants. Those candidate somatic mutations with the highest allelic frequency are selected and a specific panel of amplicons is designed to monitor their presence in ctDNA using NGS. Plasma samples analysis is carried out by an in-house pipeline specialized in reducing potential background noise. To assess panel performance in both sample types, the bioinformatics pipelines include a set of methods to calculate and filter according to different quality control metrics. Recruitment for the Phase IIa is ongoing with a total of 67 pre-screened out of which two patients were eligible. Clinical trial information: 2021-000. 507-20. Research Sponsor: Instituto de Salud Carlos III.

# The FOxTROT platform: Clinical trials and exploratory research for personalization of neoadjuvant (NA) therapy in locally advanced colon cancer (LACC)—ISRCTN83842641.

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**Background:** The FOxTROT1 trial demonstrated a significant reduction in 2-year (yr) recurrence with 6 weeks (wks) of oxaliplatin and fluoropyrimidine (OxPp) NA chemotherapy (NAC) in patients (pts) with LACC, establishing NAC as a new therapeutic option. The ongoing FOxTROT platform will refine and personalize NA therapy in LACC. FOxTROT2 is testing NAC in older pts and those with frailty, who are poorly represented in clinical trials. FOxTROT3 is assessing intensified NAC with modified FOLFOXIRI (mFOLFOXIRI; 5-fluorouracil, oxaliplatin, irinotecan) in fit pts. The FOxTROT platform will also test targeted treatments with established safety and efficacy in defined molecular subgroups. FOxTROT4 is investigating the efficacy and safety of NA encorafenib and cetuximab in *BRAF*-mutant pMMR LACC. Other arms in setup are shown in Table. **Methods:** FOxTROT platform trials are international, multicenter, open-label, phase II/III RCTs. Pts with resectable, cT3-4 N0-2 M0, biopsy-proven colon adenocarcinoma are eligible for recruitment. Pts whose age or frailty precludes mFOLFOXIRI can be considered for FOxTROT2. Pts suitable for mFOLFOXIRI can be considered for FOxTROT3. *BRAF*-mutant pMMR pts can be considered for FOxTROT4. Pts are randomized 2:1 between intervention and control arms. FOxTROT2 randomizes between 6 wks OxPp NAC (choice of 100% or 80% dose) vs proceeding straight to surgery. Adjuvant chemotherapy (AC) is per physician choice. Primary outcome is 3-yr DFS. 759 pts will be recruited over 5 yrs. FOxTROT3: 6 wks mFOLFOXIRI NAC vs 6 wks OxPp NAC. All pts receive AC but physicians may de-escalate mFOLFOXIRI to OxPp. Hierarchical co-primary endpoints: tumor regression grade (TRG) (blinded central assessment) and 3-yr DFS. 873 pts over 5 yrs. FOxTROT4: 6 wks NA encorafenib and cetuximab vs 6 wks OxPp NAC. AC is per physician choice. Primary outcome: TRG. 45 pts over 3 yrs. Secondary outcomes include: histopathological endpoints, downstaging, minimal residual disease by ctDNA, safety, toxicity, OS, surgical outcomes and patient-reported outcomes; and DFS in FOxTROT4. The FOxTROT platform opened 7<sup>th</sup> Feb 2022, funded by Yorkshire Cancer Research. At submission, 50 pts had been randomized at 23 centers. Trial design and management is conducted in partnership with patient representatives. A program of translational and radiomics research is running in parallel. International recruitment in France and India will commence in 2023 with additional international partners joining from 2024. Clinical trial information: ISRCTN83842641. Research Sponsor: Yorkshire Cancer Research.

| Current FOxTROT arms.    |                     |                 |                       |               |               |
|--------------------------|---------------------|-----------------|-----------------------|---------------|---------------|
| FOxTROT                  | 2                   | 3               | 4                     | 5             | 6             |
| Pt group                 | Older, frailty      | Fit for triplet | <i>BRAF</i> mutant    | dMMR, frailty | HER2          |
| MMR status               | pMMR                | pMMR            | pMMR                  | dMMR          | pMMR          |
| Neoadjuvant intervention | OxPp                | mFOLFOXIRI      | Encorafenib/cetuximab | Anti-PD1      | HER2-directed |
| Control                  | Straight to surgery | OxPp NAC        | OxPp NAC              | Single arm    | Single arm    |
| Recruiting               | Yes                 | Yes             | Yes                   | In setup      | In setup      |

**PACE: A phase III trial of CAPOX versus anti-PD-1 inhibitor as adjuvant therapy for patients with dMMR/MSI-H stage III colon cancer.**

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**Background:** Approximately 12% of stage III colon cancer are deficient mismatch repair (dMMR), leading to high levels of microsatellite instability (MSI-H). In the KEYNOTE-177 study, the anti-PD-1 inhibitor Pembrolizumab alone significantly improved progression free survival (PFS) versus chemotherapy as first-line therapy for metastatic colorectal cancer (mCRC) patients with MSI-H/dMMR. We are conducting a phase III randomised trial to determine whether anti-PD-1 inhibitor Sintilimab monotherapy can improve disease free survival (DFS) compared with standard adjuvant chemotherapy in stage III colon cancer patients with dMMR. **Methods:** This is an open-label, multicentre, randomised phase III study. Patients aged  $\geq 18$  years with completely resected, stage III colon cancer confirmed to have dMMR/MSI-H and ECOG performance status 0-1 are eligible. Approximately 323 patients will be randomly assigned 1:1 to either Sintilimab monotherapy (200 mg IV every 3 weeks for 8 cycles) or CAPOX regimen (capecitabine, oxaliplatin for 4 or 8 cycles according to current standard practice). Adverse events (AEs) will be assessed throughout treatment and for 30 days thereafter (90 days for serious AEs) and graded per NCI CTCAE v4.0. Primary endpoint is 3-year DFS. The study was estimated to provide approximately 80% power to detect a hazard ratio of 0.56 for DFS at a two-sided alpha of 0.05. Secondary endpoints include OS, safety profile, toxicity, and quality of life. Archived tumor tissue and blood samples will be collected for correlative studies. Study enrollment is ongoing. Clinical trial information: NCT05236972. Research Sponsor: None.

**Neoadjuvant short-course radiotherapy followed by chemotherapy plus camrelizumab versus long-course chemoradiotherapy followed by chemotherapy for patients with locally advanced rectal cancer: A randomized, multicenter, open-label phase 3 trial (Union).**

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**Background:** Short-course preoperative radiotherapy (SCPRT) and long-course chemoradiotherapy (LCCRT) are standard neoadjuvant treatments for locally advanced rectal cancer (LARC). Further, the RAPIDO trial found that SCPRT combined with consolidation chemotherapy produced better short- and long-term outcomes than SCPRT alone for patients with high-risk LARC. A phase 2, single-arm trial of neoadjuvant SCPRT followed by camrelizumab (an anti-PD-1 antibody) plus chemotherapy demonstrated promising short-term outcomes in patients with LARC. The Union trial is evaluating the efficacy and safety of neoadjuvant SCPRT followed by camrelizumab plus chemotherapy versus LCCRT followed by chemotherapy in patients with LARC. **Methods:** The Union trial is enrolling patients aged 18-75 with histologically confirmed clinical stage T3-4/N<sup>+</sup> adenocarcinoma of the rectum, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and a tumor inferior margin less than 10 cm above the anal verge. Eligible patients are randomized in a 1:1 ratio to either arm A or arm B. Patients in arm A receive neoadjuvant treatment with SCPRT (a total of 25 Gy in 5 days) followed by camrelizumab (200 mg/m<sup>2</sup> intravenous drip on day 1, every 3 weeks for 2 cycles) plus CAPOX (oxaliplatin 130 mg/m<sup>2</sup> intravenous infusion over 2 hours on day 1, capecitabine 1000 mg/m<sup>2</sup> oral twice daily, days 1-14, every 3 weeks for 2 cycles), surgery, and adjuvant treatment with camrelizumab plus CAPOX (6 cycles) followed by camrelizumab until the medication duration of camrelizumab reaches one year. Patients in arm B receive neoadjuvant treatment with LCCRT (capecitabine 825 mg/m<sup>2</sup> twice daily, 5 days a week, combined with a total dose of 50.4 Gy in 28 days) followed by CAPOX (2 cycles), surgery, and adjuvant treatment with CAPOX (6 cycles). Randomization is stratified by clinical T stage ( $\leq$ T3 vs T4) and clinical N stage (N0 vs N<sup>+</sup>). The primary endpoint is pathologic complete response (pCR). The secondary endpoints are 3-year event-free survival rate, overall survival, R0 resection rate, completion rate of neoadjuvant treatment, 3-year disease-free survival rate, tumor regression grading, safety, and quality of life. pCR is evaluated per the AJCC 8th Edition TRG scoring system as assessed by the blinded independent central review. Adverse events are graded by the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0. Quality of life is assessed by the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30. This study began enrollment in July 2021 with a planned enrollment of 230 patients. Clinical trial information: NCT04928807. Research Sponsor: Jiangsu Hengrui Pharmaceuticals Co, Ltd, Shanghai, China.

## Phase II, single-arm, open-label study of dostarlimab monotherapy in previously untreated patients with stage II/III dMMR/MSI-H locally advanced rectal cancer.

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**Background:** For patients (pts) with locally advanced rectal cancer (RC), organ-sparing non-operative management (NOM) following a clinical complete response (cCR) to treatment avoids the potential complications and quality of life impact associated with surgery/chemoradiotherapy. Although neo-adjuvant chemotherapy is standard treatment, pts with mismatch repair deficient (dMMR) tumors respond poorly. Efficacy of the programmed death (PD)-1 inhibitor dostarlimab in previously untreated pts with locally advanced dMMR RC was evaluated in a Phase II study. All 12 pts who completed 6 months of treatment had clinical complete response (cCR) and received NOM, with no Grade  $\geq 3$  adverse events reported. Further research with a larger multicenter population/longer follow-up is needed to confirm these findings. AZUR-1 will evaluate the efficacy and safety of dostarlimab in pts with previously untreated locally advanced dMMR/microsatellite instability-high (MSI-H) RC. **Methods:** AZUR-1 (NCT05723562) is a global, multicenter, single-arm, open-label, Phase II study. ~100 pts will be enrolled across 10 countries. Key eligibility criteria include age  $\geq 18$  years, no prior radiation/systemic therapy or surgery for RC, Eastern Cooperative Oncology Group performance status 0–1 and no tumor-caused symptomatic bowel obstruction. Pts must have a tumor with dMMR status or MSI-H phenotype, determined locally or by the central reference laboratory. Prescreening is available at sites without local dMMR/MSI-H testing; prescreening is not required if dMMR/MSI-H status has been previously determined. Dostarlimab (500 mg) will be administered intravenously every 3 weeks for  $\leq 9$  cycles. The primary endpoint is cCR by independent central review (ICR) at 12 months, defined as achieving and maintaining cCR for 12 months (the 12-month period starts from the first disease assessment after last dose of study intervention that demonstrates cCR by ICR). Secondary endpoints include cCR by ICR at 36 months, 3-year event-free survival (EFS3) by investigator assessment, 5-year disease-specific survival, and 5-year overall survival. Pts with cCR by end of treatment will be assessed (CT/MRI, rectal MRI, endoscopy, rectal primary biopsy) every 4–6 months for recurrent disease for 5 years (NOM). Efficacy and safety will be assessed in all pts who received  $\geq 1$  dose of dostarlimab. For a range of plausible observed cCR12 rates (60–95%) in the primary analyses, the planned sample size of 100 pts will provide a Clopper–Pearson exact binomial two-sided 95% confidence interval with lower confidence limit within ~10% of the observed cCR12 rate. No interim analyses are planned. References: [1] Cercek, A *et al.* *NEJM* 2022;386:2363–76. Funding: GSK (219369). Editorial support provided by Fishawack Health, funded by GSK. Clinical trial information: NCT05723562. Research Sponsor: GSK (219369).

**Alliance A022104/NRG-GI010: A randomized phase II trial testing the efficacy of triplet versus doublet chemotherapy to achieve clinical complete response in patients with locally advanced rectal cancer—The Janus Rectal Cancer trial.**

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**Background:** Recent data have demonstrated that in locally advanced rectal cancer (LARC), a total neoadjuvant therapy (TNT) approach improves compliance with chemotherapy and increases rates of tumor response compared to neoadjuvant chemoradiation (CRT) alone. They further indicate that the optimal sequencing of TNT involves consolidation (rather than induction) chemotherapy to optimize complete response rates. Data, largely from retrospective studies, have also shown that patients (pts) with clinical complete response (cCR) after neoadjuvant therapy may be managed safely with the watch and wait approach (WW) instead of preemptive total mesorectal resection (TME). However, the optimal consolidation chemotherapy regimen to achieve cCR has not been established, and a randomized clinical trial has not robustly evaluated it as a primary endpoint. Collaborating with a multidisciplinary oncology team and pt groups, we designed this NCI-sponsored study of chemotherapy intensification to address these issues and to drive up cCR rates, to provide opportunity for organ preservation and improve quality of life for pts. **Methods:** In this Alliance for Clinical Trials in Oncology randomized phase II trial (1:1), up to 312 pts with microsatellite stable LARC, clinical stage T4N0, any T, N+ or T3N0 requiring abdominoperineal resection or coloanal anastomosis and distal margin within 12 cm of anal verge will be enrolled. Stratification factors include tumor stage (T4 vs T1-3), nodal stage (N+ vs N0) and distance from anal verge (0-4; 4-8; 8-12 cm). Pts will be randomized to receive neoadjuvant long course CRT (LCRT) followed by consolidation doublet (mFOLFOX6 or CAPOX) or triplet chemotherapy (mFOLFIRINOX) for 4 months. LCRT in both arms involves 4500 cGy in 25 fractions over 5 weeks (w) + 900 cGy boost in 5 fractions with a fluoropyrimidine (capecitabine preferred). Pts will undergo assessment 8-12 w post-TNT completion for the primary endpoint of cCR per previously validated criteria and recommended WW and surveillance if cCR is achieved or TME if an incomplete response is noted. Secondary objectives include time-to event outcomes (disease-free and overall survival, organ preservation time and time to distant metastasis) and adverse effects. Statistical power, based on cCR, incorporates a one-sided alpha = 0.048 and power = 90% yielding 312 patients (156 per arm). Biospecimens including archival tumor tissue, matched / normal blood samples and serial rectal MRI will be collected for exploratory correlative research. This study, activated in late 2022, is open across the NCTN and is actively recruiting. Support: U10CA180821, U10CA180882, U24 CA196171; <https://acknowledgments.alliancefound.org>. U10CA180868 (NRG); U10CA180888 (SWOG); Clinicaltrials.gov ID: NCT05610163 Clinical trial information: NCT05610163. Research Sponsor: U.S. National Institutes of Health.

**A randomized trial of consolidation-targeted adjuvant therapy with encorafenib and cetuximab versus usual care for patients with stage II/III BRAF V600E colon cancer: Alliance for Clinical Trials in Oncology A022004.**

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**Background:** Patients with mismatch repair proficient (pMMR) BRAF V600E mutant high-risk stage II (T4) or stage III colon cancer have a substantial risk of recurrence despite standard adjuvant therapy. In patients with metastatic BRAF V600E mutant colorectal cancer, the combination of the RAF inhibitor encorafenib and the EGFR inhibitor cetuximab has been shown to improve overall and progression-free survival compared to standard therapy after at least one prior line of therapy. This study will evaluate if this combination improves disease-free survival (DFS) in patients with resected BRAF V600E mutant pMMR/microsatellite stable (MSS) high-risk stage II (T4) or stage III colon cancer after standard adjuvant therapy. **Methods:** The study has a seamless phase II/III design, enrolling a maximum of 394 patients. BRAF mutation status can be determined locally or submitted for central testing in trial pre-screening. Eligible patients will be randomized 1:1 to usual surveillance versus 6 months of treatment with encorafenib (300mg oral daily) plus cetuximab (500mg/m<sup>2</sup> intravenous every 14 days). Stratification factors are adjuvant chemotherapy regimen (FOLFOX versus CAPOX), duration of adjuvant therapy (3 months versus > 3 months), tumor stage (T4N0 versus T1-3N1 versus T4N1/TanyN2), and ctDNA marker status (detectable versus undetectable) at randomization. Adjuvant therapy must be completed at most 8 weeks prior to registration. For the phase II component, in the ctDNA detectable cohort, the primary endpoint will be ctDNA clearance rate at 6 months, and, in the ctDNA undetectable cohort, the primary endpoint will be 6-month ctDNA recurrence-free survival, where events consist of death, recurrence, or ctDNA positivity. The primary endpoint of the phase III component is DFS, and secondary endpoints are overall survival, adverse events, and alternative DFS calculated from the date of primary resection, rather than date of study randomization. Patient reported symptoms (PRO-CTCAE) will be collected and analyzed as an exploratory endpoint, and serial blood banking will support future correlative studies. The trial will proceed to the phase III portion if either of the two endpoints in the phase II cohorts is statistically superior in patients receiving encorafenib plus cetuximab. The phase III study sample size will provide 80% power at a one-sided  $\alpha$  of 0.05 to detect an approximately 10% improvement in the three-year DFS rate, assuming 68% in the control arm. Two interim analyses will be performed for DFS endpoint. Support: U10CA180821, U10CA180882; <https://acknowledgments.alliancefound.org>. U10CA180820 (ECOG-ACRIN); U10CA180868 (NRG); U10CA180888 (SWOG); Pfizer, Eli Lilly; Clinicaltrials.gov ID: NCT05710406. Clinical trial information: NCT05710406. Research Sponsor: U.S. National Institutes of Health; Pfizer, Eli Lilly.