Published in final edited form as:
Semin Oncol. 2011 April ; 38(2): 284–308. doi:10.1053/j.seminoncol.2011.01.001.

Personalized Colon Cancer Care in 2010

Daniel V.T. Catenacci[1], **Mark Kozloff**[1], **Hedy L. Kindler**[1], and **Blase Polite**[1]
[1]Department of Medicine, Section of Hematology/Oncology, Center for Gastrointesinal Oncology. University of Chicago, Chicago, IL. 60637.

Abstract

Colon cancer therapies have improved patient outcomes significantly over the last decades in both the adjuvant and metastatic settings. With the introduction of a number of novel agents, both traditional chemotherapies and biologically targeted agents, the need to identify subgroups that are likely and not likely to respond to a particular treatment regimen is paramount. This will allow patients who are likely to benefit to receive optimal care, while sparing those unlikely to benefit from unnecessary toxicity and cost. With the identification of several novel biomarkers and a variety of technologies to interrogate the genome, we are already able to rapidly study patient tumor or blood samples and normal tissues to generate a large dataset of aberrations within the cancer. How to digest this complex information to obtain accurate, reliable, and meaningful results that will allow us to provide truly personalized care for colon cancer patients is just starting to be addressed. In this article, we briefly review the history of colon cancer treatment, with an emphasis on current clinical standards that incorporate a 'personalized medicine' approach. We then review strategies which will potentially improve our ability to individualize therapy in the future.

II. The Promise of Personalized Cancer Care

Colon cancer (CC) is the fourth most common cancer, and is the second leading cause of cancer deaths in the United States.[1] In 2009 there were 106,100 new cases and an estimated 49,920 deaths. The main prognostic factor for survival or relapse after surgery of localized disease is tumor stage. [23] While stage I CC is usually cured by surgery alone, adjuvant chemotherapy is currently recommended for stage III and high risk stage II cancers. About 75% of patients with stages I–III CC, can be cured with surgical intervention alone, however. In stage III CC, 40–50% of patients are cured by surgery, while approximately 35% of patients will relapse, despite adjuvant chemotherapy. [4] Thus, in the stage III setting, most patients who are candidates for adjuvant chemotherapy are treated, though the majority either do not require adjuvant treatment or do not benefit from it. The role of adjuvant chemotherapy is even more difficult to define in stage II CC as 60–70% of stage II patients are cured with surgery alone, and 15–20% relapse despite adjuvant chemotherapy. [5] The QUASAR study randomized 3239 CC patients at a low risk for disease recurrence to observation or 5-fluorouracil/folininc acid (5-FU/FA), 92% of these patients had stage II colorectal cancer (CRC). The benefit of 5-FU/FA was only 3.6% at 5 years, indicating that 96% of patients received chemotherapy unnecessarily. [6]

Corresponding author: **Daniel V.T. Catenacci**, dcatenac@medicine.bsd.uchicago.edu.

In the metastatic setting, patients are treated with the standard first- and second-line chemotherapy regimens, 5FU/LV with oxaliplatin (FOLFOX) and 5FU/LV with irinotecan (FOLFIRI), in either order, [7] combined with the anti-VEGF monoclonal antibody bevacizumab, and the anti-EFGR antibodies, cetuximab or panitumumab. [8] Given our inability to predict those who will and will not respond to these therapies, all patients typically receive all of these agents throughout the course of their management, with the exception of the EGFR inhibitors that are now omitted in patients with tumors that harbor mutated *KRAS* [9].

Although significant improvements have been made in CC outcomes over the past few decades, we need better tools to identify which poor prognosis early-stage patients will benefit from adjuvant therapy, and we require more robust predictive markers to help us tailor therapies for each specific patient with more advanced disease. We are currently poised to do this, by using novel technologies and computer software programs that provide the tools to unravel the complexities of CC biology, allowing for the development of personalized colon cancer care. [10]

In this review, we briefly discuss historical aspects that have led to the current standard CC treatments. We then discuss the current clinical circumstances and biomarkers that have already become incorporated into the personalization of CC care. We then review novel potential biomarkers that are showing promise in this arena, and discuss the integration of high-throughput genome wide studies and systems biology as a means to enhance our assessment of prognosis and tailor our interventions, in order to optimize clinical benefit, reduce toxicity, and minimize cost.

III. The History of Colon Cancer Care – the previous millennium

Evaluating 5-Fluorouracial/Leucovorin – Metastatic and Adjuvant Settings

Until the turn of the century, treatment options were limited for CC patients, both in the metastatic and adjuvant settings. [11] For more than 40 years, 5-fluorouracil/leucovorin (5-FU/LV) was the standard of care for mCC, and the results of 25 years of clinical trials in the adjuvant setting led to the acceptance of 5-FU/LV as the standard of care for patients with node-positive CC.[512] Many of the clinical trials that were conducted in the 1980s and 1990s were designed to address the schedule-dependent mode of action of 5-FU and evaluated differences in efficacy and toxicity of different dosing schedules.[13–15] Several trials and meta-analyses determined that infusional administration of 5-FU over several days or continuously was at least as effective as bolus 5-FU/LV, achieving similar median survival outcomes, and causing fewer severe toxicities.[51216–22] Due to better response rates and perceived convenience, bolus 5-FU/LV became the American standard of care for mCC patients, and remained so until 2000. In Europe, infusional regimens were preferred, particularly, the de Gramont regimen of every-2-week 5-FU/LV combining bolus with infusional 5-FU/LV (5FULV2).[22] These short infusional regimens became the backbone for combination with the next generation of chemotherapy agents, oxaliplatin and irinotecan (Table 1).

IV. Next Generation Cytotoxics and Biologics – the last 10 years

Metastatic Colorectal Cancer: Oxaliplatin and Irinotecan

The topoisomerase I inhibitor irinotecan was introduced as monotherapy for patients with mCC refractory to 5-FU/LV,[2324] and soon became standard of care in the second-line setting, improving median overall survival (OS) from 6.5–8.5 months for best supportive care (BSC) to 9.2–10.8 months for irinotecan.[2425] The addition of irinotecan to the 5-FU/LV backbone in the first-line setting improved outcomes in two randomized trials, one using

bolus 5-FU (IFL)[26], and the other with combined bolus and infusional 5-FU (FOLFIRI)[27]. In both trials, the addition of irinotecan improved the response rate and the median overall and progression-free survival time, thus the combination of irinotecan with a 5-FU based regimen replaced 5FU/LV as the standard first-line therapy for mCC.

The platinum derivative oxaliplatin has a similar mechanism of action to other platinum agents, although the anti-tumor profile differs from cisplatin.[28] When oxaliplatin was initially combined with 5FU-LV for first-line treatment of mCC, the combination yielded higher response rates and a longer PFS compared with 5-FU/LV, though because of crossover and a small sample size, it did not lead to an improvement in overall survival.[29] A phase III trial demonstrating a higher response rate and longer time to progression (TTP) in the second line setting with oxaliplatin plus infusional 5FU/LV versus either alone, led to FDA approval of this regimen for mCC refractory to irinotecan plus 5FU/LV.[30] It was subsequently shown, in the a large Intergroup trial, NCCTG 9741, that first-line FOLFOX was superior in efficacy and safety to IFL or the combination of irinotecan and oxaliplatin (IROX), and it had a more favorable toxicity profile, except for increased peripheral neuropathy.[31] Although there were several nuances in trial design that have been controversial,[11] FOLFOX became a standard of care for first line therapy for mCC.

The combination of 5FU/LV, oxaliplatin, and irinotecan, (FOLFOXIRI) was compared to FOLFIRI in the first line setting, but did not show any difference in OS, PFS, or response rates, but FOLFOXIRI did have a significantly higher incidence of neuropathy, diarrhea, and alopecia.[32] However, in a separate trial, Falcone *et al* reported that PFS and OS were both significantly improved in the FOLFOXIRI arm (median PFS, 6.9 *v* 9.8 months; hazard ratio [HR], 0.63; $P = .0006$; median OS, 16.7 *v* 22.6 months; HR, 0.70; $P = .032$).[33] A meta-analysis of these two trials reported that FOLFOXIRI conferred significant benefit in PFS, survival, response and oligometastatic R0 resection rates but was more toxic compared with FOLFIRI.[34] Long-term followup of patients treated with FOLFOXIRI showed that of 196 patients treated, 19% went on to have oligometastectomy, and after a median follow up of 67 months, 5-year and 8-year survival were 42% and 33% respectively.[35] Given the higher toxicity and lack of consensus on its superiority over standard therapy for the majority of patients, this regimen is used sparingly in practice. These trials are summarized in Table 1.

Metastatic Colorectal Cancer: Oral Fluoropyrimidines

Oral fluoropyrimidines became available at the same time as the studies which evaluated the incorporation of irinotecan and oxaliplatin into the treatment of mCC. Capecitabine is an oral pro-drug of 5-FU that is preferentially metabolized within tumor cells and the liver to 5-FU.[3637] Two randomized trials demonstrated the non-inferiority of capecitabine to both infusional and bolus 5FU/LV in mCC patients.[3839] Reversible grade 3 hand-foot syndrome and grade 3/4 hyperbilirubinemia were more frequent in the capecitabine arms. Single-agent capecitabine was thus FDA approved in the U.S. in 2002 for first-line therapy where combination therapy is not recommended.

A number of clinical trials, summarized in Table 1, have demonstrated the non-inferiority of the combination of capecitabine and oxaliplatin (CapeOx) to FOLFOX.[40–43] The combination of capecitabine and irinotecan (CAPIRI), however, resulted in intolerable toxicities and a shorter PFS in 2 randomized trials, effectively discouraging use of this regimen, especially in the U.S. where capecitabine is less well tolerated at standard doses.[54144–47] Replacing 5FU/LV with capecitabine and evaluating the sequential efficacy of combination versus single-agent therapy did not show any differences in OS in the CAIRO or MRC-FOCUS trials.[4849] This additional option of either an oral or intravenous fluoropyrimidine in combination with oxaliplatin, or as a single-agent, allows for further

individualization of care of CC patients, depending on patient performance status and if tumor response is the desired outcome.

Metastatic Colorectal Cancer: Sequence of Therapy

The optimal sequence of therapy for mCC has been addressed in randomized trials.[74849] Tournigand *et al* evaluated FOLFIRI then FOLFOX or the reverse sequence and reported no significant differences in OS and first-line response rates between the two approaches.[7] The toxicity profiles of the two regimens were significantly different: FOLFIRI caused more grade 3/4 mucositis, nausea/vomiting, and diarrhea, while neutropenia and neurotoxicity were more common with FOLFOX. These differences in toxicity profiles between FOLFOX and FOLFIRI, and the ability to choose between two relatively equivalent regimens, provided yet another opportunity (after choosing between infusional, bolus, or oral fluoropyrimidine) that allowed for the personalization of mCC care, in this case, based on anticipated side-effect profiles and patient preferences and co-morbidities.

Metastatic Colorectal Cancer: Duration of Therapy

Optimal treatment duration was addressed by the OPTIMOX-1 and OPTIMOX-2 trials.[5051] In OPTIMOX-1, FOLFOX given until disease progression was compared to FOLFOX7 (which uses a higher dose of oxaliplatin and omits of the myelopsuppressive bolus 5-FU) given for 12 weeks, with planned interruption of the oxaliplatin and continued 5FU/LV alone as maintenance, and planned re-introduction of oxaliplatin at 6 months or at disease progression, whichever came first. There were no differences in response rates, PFS, or OS, validating the intermittent use of oxaliplatin. Moreover, neurotoxicity was significantly reduced with planned interruption of oxaliplatin treatment.

OPTIMOX-2 went a step further, giving planned chemotherapy breaks, using an otherwise similar trial design. This trial was planned as a phase III but converted to a smaller phase II trial after bevacizumab was introduced, so the survival data should be interpreted with caution. Nevertheless, the arm with planned chemotherapy breaks had an inferior OS, suggesting that this is not the best approach, but it can be an option to individualize patient treatment based on patient preferences, particularly if there is a strong early response that is durable beyond 6 months.

The recently reported MACRO phase III trial randomized 480 first-line patients to either CapeOx-bevacizumab until progression (n=239) or CapeOx-bevacizumab for 6 cycles then single-agent bevacizumab (n=241), and observed no statistical differences in RR, PFS, or OS between the groups, but failed to meet its non-inferiority PFS primary endpoint (11 vs 10.3 months, p=0.59; HR 1.07 95% CI 0.84–1.36). (J Clin Oncol 28:15s,2010(suppl; abstr 3501). The 4-arm DREAM-OPTIMOX-3 phase III study evaluated FOLFOX or CapeOx and bevacizumab, with or without erlotinib. Given the increased toxicity, the combination of a fluoropyrimidine, oxaliplatin, bevacizumab, and erlotinib was deemed not feasible, and this ongoing study was amended to evaluate bevacizumab plus erlotinib as maintenance after completing 6 cycles of chemotherapy with bevacizumab. (J Clin Oncol 25(18S):187s, 2007;absr 4097) The GISCAD FOLFIRI trial reported that alternating 2 months of FOLFIRI treatment with 2 months of chemotherapy break obtained the same survival as a continuous treatment, thus reducing patient toxicity as well as the economic costs.(*Journal of Clinical Oncology*, 2006 ASCO Annual Meeting Proceedings Part I. Vol 24, No. 18S, 2006: 3505). The MRC-COIN 3-arm trial (CapeOx or FOLFOX with or without cetuximab) had an intermittent chemotherapy arm that showed a 9% increase in relative risk of death with a significant hazard ratio (HR) of 1.084, when compared to the two continuous chemotherapy arms. (*Journal of Clinical Oncology*, 2010 ASCO Annual Meeting Proceedings, Abstr. 3525) The results of these trials evaluating optimal therapy duration provide more flexibility

and personalization of mCC care enabling physicians to incorporate individual patient circumstances throughout the course of therapy, allowing for intermittent maintenance or complete breaks, with little to no adverse effect on overall clinical outcomes.

Metastatic Colorectal Cancer: Novel Biologic Therapeutics – anti-angiogenics

Bevacizumab is a monoclonal antibody that binds to and sequesters vascular endothelial growth factor (VEGF). Given the absence of benefit as a single-agent, bevacizumab was evaluated in combination with the standard chemotherapy regimens discussed above. The first phase III trial to demonstrate front line efficacy was a phase III trial of IFL with bevacizumab or placebo, which led to an improvement in OS of 4.7 months along with a statistically significant improvement in PFS in the bevacizumab arm.[52] Since IFL was replaced with FOLFOX and FOLFIRI, these regimens plus bevacizumab (FOLFOX-bev, FOLFIRI-bev) were widely adopted for first-line use in the U.S.[45]

The N016966 trial evaluated bevacizumab in the front-line setting with either FOLFOX or CapeOx in a placebo-controlled 2 by 2 design.[53] Bevacizumab improved PFS in this trial, but response rates were not improved, and the differences in OS were not statistically significant (p=0.077). Since FOLFIRI-bev and FOLFOX/CapeOx-bev have not been directly compared in the first-line setting, either is currently considered acceptable, with patient preference and the side-effect profile contributing to the decision making.

The continued use of bevacizumab in the second-line setting beyond progression (BBP) was addressed in the BRiTE prospective, observational study, which showed that patients who received BBP (n=642) had a median OS of 31.8 months compared to 19.9 months for those who did not (n=531), suggesting that continued BBP might be beneficial.[54] This question is currently under prospective investigation in the iBET SWOG 0600 randomized phase III trial which compares irinotecan-cetuximab and bevacizumab to irinotecan- cetuximab alone,[55] and in the SPIRITT randomized phase II trial,[5657] in which *KRAS* wild type patients receive second-line FOLFIRI and are randomized to either panitumumab or bevacizumab.

No other angiogenesis inhibitors have shown efficacy in mCC, including sunitinib as a single-agent,[58] or in combination with FOLFIRI or FOLFOX. The small molecule VEGFR inhibitor cediranib (AZD2171) was evaluated in the Horizon-2 (FOLFOX +/− cediranib) and Horizon-3 trials (FOLFOX-bev vs. FOLFOX-cediranib); both studies were negative. PTK/ZK 7787, another oral VEGFR inhibitor, failed to improve OS in the first (CONFIRM-1) or second (CONFIRM-2) line studies. (*Journal of Clinical Oncology*, 2007 ASCO Annual Meeting Proceedings Part I. Vol 25, No. 18S (June 20 Supplement), 2007: 4033) However, PTK/ZK 7787 reportedly improved PFS significantly in the overall population, and showed improved PFS and OS in patients with high baseline serum LDH. While encouraging, there are no prospectively validated predictive markers in routine use to help identify those patients most likely to benefit from bevacizumab or other anti-angiogenic therapies.

Metastatic Colorectal Cancer: Novel Biologic Therapeutics – anti-EGFR agents

Cetuximab and panitumumab are monoclonal antibodies (chimeric human-mouse, and fully human, respectively) that block the ligand-binding site of the epidermal growth factor receptor (EGFR), consequently inhibiting the transduction of this signal intracellularly.[5960]

The BOND trial evaluated patients with mCC that was irinotecan (100%) and oxaliplatin (63%) -refractory CC with single single-agent cetuximab or combination cetuximab and irinotecan.[61] The combination achieved a response rate of 22.9% compared to 10.8% for single-agent cetuximab; median TTP was 4.1 and 1.5 months, respectively. The NCIC evaluated cetuximab versus best supportive care (BSC) in unselected patients with chemo-

refractory (fluoropyrimidine, oxaliplatin, irinotecan) mCC; cetuximab improved PFS (HR 0.68, p<0.001) and OS (HR 0.77, p=0.005) in this setting.[62] Panitumumab demonstrated similar anti-tumor activity as a single-agent in patients with chemotherapy refractory mCC, showing an overall response rate of 10% and a very modest improvement in PFS compared with BSC (8 vs. 7.3 weeks).[63] These trials established the utility of these antibodies in the second and third-line setting in patients with chemo-refractory mCC.

The CRYSTAL trial evaluated FOLFIRI plus or minus cetuximab in the front-line setting and achieved a statistically significant improvement in PFS (only 27 days), but it increased toxicity, specifically diarrhea and skin rash.[6465] (J Clin Oncol 28:15s, 2010 (suppl; abstr 3570)) There was a survival advantage for patients with *KRAS* wild-type tumors in the cetuximab arm (HR 0.796, p=0.0093). The OPUS phase II trial, which looked at front-line FOLFOX with or without cetuximab, reported a higher overall response rate favoring the cetuximab arm (45.6% versus 35.7%).[66] PFS was not statistically superior in the intention-to-treat arm, but it was better in the *KRAS* wild-type subgroup analysis.[66]

Similarly, the PRIME trial (FOLFOX +/− panitumumab) showed an improved PFS in wild-type KRAS tumors (p=0.02), although the OS endpoint did not reach significance. *J Clin Oncol 28:15s, 2010 (suppl; abstr 3528)* In contrast, the MRC-COIN (CapeOx or FOLFOX +/− cetuximab) phase III trial reported no significant differences in PFS and OS when anti-EGFR antibodies were added to first line therapy in *KRAS* wild-type patients. *(J Clin Oncol 28:15s, 2010 (suppl; abstr 3502)) (*Table 1,2*)*

Metastatic Colon Cancer: EGFR inhibition and KRAS mutation

KRAS is a GDP/GTP binding protein that facilitates ligand-dependent receptor tyrosine kinase (RTK) intracellular signaling, and is a known oncogene that is mutated in codons 12, 13, and 61 of exon 2 in approximately 29% of all human cancers.[67] In colon cancer, the mutation frequency is approximately 35–45%. It is now known that these mutations lead to independent activation downstream of several RTKs, including EGFR.[967]

In the CRYSTAL trial discussed above[65] (Table 2), a retrospective analysis reported a *KRAS* mutation in 35.6% of the 540 patient samples available for testing. There was no benefit from the addition of cetuximab to FOLFIRI in PFS (p=0.47) or overall response rate (p=0.46) for those patients with *KRAS* mutations. This contrasts with *KRAS* wild-type tumors, where the addition of cetuximab improved PFS from 8.7 months to 9.9 (p=0.017) and the response rate from 43.2% to 59.3% (p=0.0025). The OPUS randomized phase II trial showed similar findings, with an improved response rate from 37% to 61% (p=0.0163) but a minimal difference in PFS from 7.3 to 7.7 months.[67] CAIRO-2, randomizing CapeOx-bev +/− cetuximab, also substantiated these findings in 528 patients, with *KRAS* mutant tumors having a worse PFS, 9.4 months, compared with 10.7 months in the wild-type group with the addition of cetuximab (p=0.01).[68] The EVEREST trial (*J Clin Oncol* 26: 2008 (May 20 suppl; abstr 4001) randomized patients with grade 0/1 rash to escalated cetuximab versus standard dosing. The 37% of patients with a *KRAS* mutation did not benefit from either arm.[67]

The effect of *KRAS* mutation on the efficacy of panitumumab was evaluated in 427 of the 463 (92%) of patients in the phase III trial discussed above.[63] Responses were only observed in patients with wild-type *KRAS* (p<0.0001).[69] In a 747 patient cohort, 40% of samples were *KRAS* mutated. Treatment with panitumumab acheived response rates of 6.7% in *KRAS* mutants versus 35.8% in wild type (p<0.0001), median PFS was 12 weeks and 24 weeks, respectively (HR 1.98, p<00001).[70]

Based on these studies, *KRAS* testing has become routine to exclude treatment with EGFR inhibitory antibodies for those CC patients with a *KRAS* mutation. Testing has been standardized to assess for 7 common point mutations in codons 12 and 13 using a number of methods, including dideoxysequencing, pyrosequencing, allele-specific amplification techniques, allele discrimination, multiplex PCR, and PCR-restriction fragment length polymorphism, reviewed elsewhere.[67] These studies are summarized in Table 2. There is a suggestion that different *KRAS* mutations have differing sensitivities to cetuximab, and prospective randomized trials may be warranted to evaluate cetuximab therapy in the G13D mutant alleles.[71]

Metastatic Colorectal Cancer: Combined Biologic Therapeutics

The BOND-2 randomized phase II trial (Table 1) evaluated the concurrent administration of bevacizumab and cetuximab with or without irinotecan in 83 patients with irinotecan-refractory mCC.[72] The response rate in the irinotecan arm was encouraging, at 37%, compared with 20% in the dual antibody alone arm. The much larger phase III PACCE study assigned patients to FOLFOX-bev or FOLFIRI-bev, and then randomized them to either receive or not receive panitumumab. This trial completed accrual but was terminated after a pre-planned efficacy analysis at 231 events showed a shorter PFS in the panitumumab arm.[73] The CAIRO-2 trial confirmed the results of the PACCE trial, by demonstrating that CapeOx-bev plus cetuximab produced inferior response rates and PFS than CapeOx-bev alone.[68]

The data from the PACCE and CAIRO-2 trials indicate that combination bevacizumab and EGFR inhibitory monoclonal antibody cannot be recommended in the front-line metastatic setting as a standard of care.

Stage II and III Colon Cancer – oxaliplatin

Given the improvements observed in metastatic CC using oxaliplatin and 5-FU combinations, similar strategies were pursued in the adjuvant setting. Data from the MOSAIC and NSABP C-07 trials (Table 3) confirmed the superiority of the oxaliplatin-5FU/LV arm over 5FU/LV alone in.[74–76] The MOSAIC trial showed 5-year DFS rates of 73.3% and 67.4% for the FOLFOX-4 and 5FU/LV2 arms, respectively (HR = 0.8; p=0.023); 6-year OS rates were 78.5% and 76% respectively (p=0.046). The stage III subgroup (60% of patients) had an even larger benefit, 72.9% versus 68.7% for addition of oxaliplatin. The C-07 trial showed a similar improvement in DFS in the FLOX regimen (oxaliplatin added to weekly bolus of 5-FU/LV) (HR of 0.8, p<0.004) to that of the MOSAIC trial. FOLFOX thus became the standard of care for adjuvant treatment of stage III patients. In the stage II setting, it was recommended that patients and physicians have a discussion of the risks and benefits of therapy. The N016968 trial evaluating CapeOx versus 5FU/LV in stage III patients was recently reported to improve 3, 4, and 5 yr DFS (5 yr 66.1% vs 59.8%, p<0.0045). (J Clin Oncol (Meeting Abstracts) 2010 28: 3521) The appropriate duration of therapy in the adjuvant setting, 3 versus 6 months, is currently being evaluated.[77]

Stage II and III Colon Cancer - irinotecan

Unfortunately, the benefits observed with irinotecan in the advanced setting did not translate to similar benefits in the adjuvant setting (Table 3). Three studies of irinotecan added to 5FU/LV failed to show improvement compared with the control arm. CALGB 89803 studied IFL versus bolus 5FU/LV in stage III colon cancer patients, and failed to demonstrate improvement in DFS or OS for IFL.[78] The ACCORD-2 trial and the PETACC-3 studies, which compared infusional 5FU/LV with or without irinotecan,

similarly did not meet their primary end points of superiority over the 5FU/LV control arm.[7980]

Stage II and III Colon Cancer – oral fluoropyrimidines

The X-ACT trial evaluated the equivalence of capecitabine at a dose of 1250mg/m^2 BID to bolus 5-FU/LV in stage III CC, and confirmed that it is a reasonable alternative to infusional 5-FU, analogous to the mCC setting.[81] As above, the N016968 trial evaluating CapeOx versus 5FU/LV in stage III patients recently reported an improved 3, 4, and 5 yr DFS for the CapeOx regimen.

Stage II and III Colon Cancer: anti-angiogenics and EGFR inhibitors

Despite its proven benefit in mCC, the addition of bevacizumab to FOLFOX6 recently failed to demonstrate benefit in the NSABP C-08 adjuvant trial.[8283] (J Clin Oncol 27:18s, 2009 (suppl; abstr LBA4)) The effect of prolonged bevacizumab maintenance will be addressed in the NSABP C-12 trial. The formal results of the AVANT trial, which compared FOLFOX4 to FOLFOX4-bev and CapeOx-bev, are expected shortly.

Similarly, the N0147 trial, which evaluated cetuximab in combination with FOLFOX, versus FOLFOX alone in the adjuvant setting, failed to show an advantage in DFS, in either *KRAS* wild type or mutant tumors. [78] (J Clin Oncol 28:15s, 2010 (suppl; abstr 3508); J Clin Oncol 28:18s, 2010 (suppl; abstr CRA3507)). The ongoing PETACC-8 trial is also testing the question of the value of adding cetuximab to chemotherapy in *KRAS* wild type tumors. At present, the use of *KRAS* testing to guide therapy in the adjuvant setting has not resulted in any definitive guidelines, as *KRAS* status has not had any prognostic or predictive impact in a number or retrospective analyses.[84]

It is important to note that even in mCC, only about 40% of the *KRAS* wild type patients derive benefit from cetuximab.[6483] Other molecular aberrations that are likely to further define EGFR inhibitor sensitivity may shortly become part of standard personalized clinical care, and are discussed further below.

The need to personalize stage II adjuvant therapy further

Stage II tumors have a wide range of 5 year OS rates, ranging from 87.5% (IIA) to 58.4% (IIC).[3] Meta-analyses are conflicting in terms of reported benefit in OS or DFS in patients who receive adjuvant chemotherapy.[164385] The QUASAR study, which enrolled 3239 patients, of which 92% of patients were stage II, included 29% who had rectal cancer.[6] Patients were randomized to receive 5FU/LV or observation, and in an analysis of only those patients with stage II disease CRC (including those with rectal cancer), a small reduction in the risk of death at 5 years (p=0.04) was still present. The available data support recommending adjuvant treatment in high-risk stage II patients. The definition of ‘high-risk’ varies in different studies, however. Some, but not all studies have included in this definition T4 tumors, pericolonic tumor deposits (N1c -which now upstages otherwise node negative tumors to IIIA or IIIB, depending on T),[3] mismatch repair status (see below), occlusion/ perforation at presentation, poorly differentiated histology, lymphovascular invasion, and <12 lymph nodes sampled. It is clear that criteria other than pathologic stage and these high-risk features are necessary to improve prognosis, so that truly high-risk patients can receive therapies that offer a benefit, while patients with a low risk of recurrence are spared from unnecessary treatment.

Tailoring Adjuvant Therapy in Stage II Disease: Microsatellite Instability (MSI)

Microsatellite (MS) DNA consists of short nucleotide repeats and is abundant throughout the human genome. Microsatellite instability (MSI), where these repeats are aberrantly

lengthened or shortened, has a recognized contribution to cancer pathobiology.[86] MSI is a consequence of deficient mismatch repair (dMMR) of DNA due to loss of function of the *MLH1, MSH2, MSH6*, and *PMS2* genes. Loss of these genes, usually as germline mutation, occurs in 80% of hereditary nonpolyposis colon cancers (Lynch syndrome) that typically occur at young ages. MSI is also associated with 15–20% of sporadic colon cancers.

dMMR sporadic colon cancers result primarily from aberrant genome-wide epigenetic methylation that results in decreased transcription of MMR genes, particularly *MLH1*. When MMR function is lost, either through germline loss-of-function mutation or via somatic promoter methylation, then MSI occurs throughout the genome in prone regions of the DNA. When MSI occurs in critical proteins that contain MS loci, namely tumor suppressors (eg. TGFB1, TGFB2, BAX), this leads to their inactivity, and hence contribution to tumorigenesis.[86] The MMR system is capable of recognizing certain DNA adducts caused by exogenous alkylation damage.[86] MMR can also recognize 5-FU, despite its inability to deform DNA, and it is postulated that tumor cells with intact or proficient MMR (pMMR) can recognize the incorporation of a fluoropyrimidine into DNA with consequent activation of the signaling cascade leading to apoptosis, whereas cells with dMMR cannot.[8687]

MSI can be detected by standardized testing of 5–10 MS loci using PCR and/or immunohistochemical (IHC) stains of tissues to detect decreased protein expression[88]. PCR results are reported as MSI-high (unstable in ≥30% of markers), MSI-low (unstable in 10–30%) and MS stable (MSS) in cases with <10% instability. Complete lack of MMR protein (particularly MLH1) expression by IHC is an alternative to PCR testing.[88]

Clinically, dMMR status is associated with lower stage, better prognosis, right-sided tumor location and lack of benefit from 5-FU based adjuvant chemotherapy, making it a prognostic as well as an apparent predictive marker, as determined from retrospective tissue analyses of the PETACC-3, QUASAR and CALGB 89803 trials.[689–91]

MSI was evaluated as a prognostic factor in the PETACC-3 study. Patients with both stage II and III disease and MSI-H had an improved DFS and OS when compared to the MSS and MSI-L groups.[89] (J Clin Oncol 27:15s, 2009 (suppl; abstr 4001)) Restricting the analysis to stage II patients, the improved prognosis was even more apparent (HR 0.159, p=0.011) while there was a non-significant trend toward improved prognosis in stage III patients (HR 0.699, P=0.12). Recent reports showed a significantly improved OS in patients with MSI-H tumors who relapsed (HR 0.51 [0.28–0.95], p<0.034). (J Clin Oncol 28:15s, 2010 (suppl; abstr 3504)) Similarly, the QUASAR study showed MSI-H to have an improved DFS (HR 0.31, p<0.001), and it was an independent prognostic marker for stage II patients.

A recent meta-analysis of 1,027 patients pooled from 5 trials (FFCD 8802, NCCTG 79–48–52, NCCRG 87–46–51, INT0035, and GIVIO) who were randomized to 5-FU based chemotherapy with levimasole or LV or surgery alone showed that approximately 15% of patients had dMMR.[91] In those stage II and stage III patients with dMMR who were treated with surgery alone (79/515), an improved DFS (HR 0.51, p=0.009) and OS (HR 0.47, p=0.004) were observed compared to those with proficient MMR (pMMR). In subgroup analyses, stage II patients with dMMR appeared to do worse with 5-FU treatment (NS p=0.9), and stage III patients did not benefit. In 5-FU treated patients, there was a clear benefit for pMMR patients (n= 426), as the poor prognosis that was seen in the untreated patients was nullified by 5-FU treatment. Subgroup analysis of the pMMR patients treated with 5-FU showed an improved DFS in stage III (surgery alone, N = 222, 5-FU-treated, N = 212) patients (HR 0.64, p=0.001), while stage II patients (surgery alone, N = 214, 5-FU-treated, N = 214) trended towards an improved DFS, which did not reach statistical significance (HR 0.84, p=0.38).

Based on these collective results, it is now strongly recommended to test all stage II CC patients for dMMR by MSI DNA analysis, as well as to consider excluding those stage II patients with MSI-H tumors from receiving adjuvant chemotherapy. It is important to keep in mind, however, that the predictive value of MSI testing in the adjuvant setting,was developed from clinical trials using older chemotherapy regimens and does not address the question of the potential benefit of FOLFOX in this setting. Also, the recently suspended ECOG 5202 addresses the question of FOLFOX +/− bevacizumab in pMMR stage II higher risk tumors.[92]

The value of MMR as a predictive marker for cytotoxics other than 5-FU,is unclear. The CALGB 89803 reported a higher 5 year DFS rate in stage III dMMR CC treated with irinotecan/5-FU compared to those with pMMR.[90] This difference was not observed in patients treated with 5-FU/LV alone. However, the PETACC-3 study failed to confirm these results.[84] It is possible that the differences seen in the two trials are due to selection of different stage patients and different MSI-H definitions; 89803 used a higher cutoff of ≥5 MSI loci to qualify as MSI-H compared to the PETACC-3 analysis.[84] The issue of MSI status and its ability to predict the potential benefit of irinotecan in the adjuvant setting is not resolved to date because of these discrepant retrospective analyses. Given that MSI-H patients have relatively good clinical outcomes and given the three negative trials of adjuvant irinotecan discussed above,[78–80] definitive prospective results may be difficult to acquire.

V. Current Practices that Personalize Colon Cancer Therapy

Despite the recently obtained insights described above, oncologists are still limited in their ability to individualize therapy for their patients with colon cancer. In the metastatic setting, personalized care includes a choice between oral or infusional 5-FU/LV and a choice of the cytotoxic chemotherapies irinotecan or oxaliplatin, based on the acceptable toxicity profile, as each may be preferred in specific circumstances. Integrated anatomical-metabolic imaging with FDG PET/CT has an established role in selected patients, leading to improved staging and restaging, to assist in accurately assessing potentially resectable disease, and thus directly impacting patient management in these cases.[9394] Treatment of the elderly, usually defined as those >70 years old, has been debated without development of definitive guidelines; recommendations are left to the clinical experience of the treating oncologist.[9596] More specific to tailoring therapy with molecular markers, *KRAS* testing for mutation to determine eligibility for EGFR monoclonal antibody therapy is now routinely recommended, as it has been demonstrated that *KRAS* mutant tumors do not respond to these inhibitors, in the trials discussed above (CRYSTAL, PRIME, COIN, CAIRO-2; Table 2); this was also confirmed more recently in a large cohort of 773 tumor samples.[70] EGFR expression by IHC has not accurately predicted response to EGFR monoclonal antibodies, and is not recommended for routine use.[9] There are currently no predictive markers in routine use that identify those most likely to benefit from bevacizumab.

In the adjuvant setting, all eligible stage III patients receive FOLFOX without available tools to further categorize these patients into subgroups of those likely and unlikely to benefit from this regimen. In the stage II setting, microsatellite instability testing has become more common outside of clinical trials to assist in treatment decision-making.

VI. More Personalization - Almost There?

A number of biological markers and studies are on the verge of making major contributions to personalizing care for patients with CC. The following is a review of the most promising areas of research attempting to advance the armamentarium of personalized colon cancer care.

ONCOTYPE DX Colon Cancer Assay and ColoPrint

The Onco*type* DX Colon Cancer Assay is a 12-gene assay that provides an individualized score predictive of the risk of colon cancer recurrence for patients with stage II disease.[97] Current NCCN guidelines do not routinely recommend adjuvant chemotherapy for all stage II patients, but recommend considering treatment for those with high risk of recurrence based on clinical and pathologic parameters. The Onco*type* DX Colon Cancer Assay is a standardized multi-gene RT-PCR assay conducted on formalin fixed paraffin embedded (FFPE) primary colon tumor tissue, designed, validated and currently available as a commercial test.[97] The test was validated in the large, independent, multcenter QUASAR trial.[7] The recurrence score (RS) is based on the quantitative expression of 7 cancer genes, normalized to 5 reference genes. Of the 2,146 QUASAR patients with stage II disease, 1436 samples were available to evaluate the primary endpoint, recurrence-free interval (RFI), and secondary endpoints DFS, and OS. The continuous RS was significantly associated with recurrence risk (p=0.004), with a near-linear relationship between RS and risk of recurrence. Recurrence risk at 3 years ranged from 9–11% at low RS, and 25–27% at high RS. In the pre-specified multivariate analysis, this association remained significant (p=0.008) as an independent predictor of recurrence, in addition to tumor T stage and MMR status. Tumor grade and number of nodes sampled were also significant but not as important. Lymphovascular invasion was not significant in this multivariate analysis. As a continuous variable, the RS score per 25 units, had a HR of 1.61 (p=0.008). Tumors with dMMR comprised 13% of the study population with a HR 0.32 (p=<0.008). T4 tumors comprised 15% of samples with HR 1.83 (p=0.005). High tumor grade comprised 29% of samples with HR 0.62 (p=0.028). Number of nodes examined (<12) comprised 62% of patients with HR 1.47 (p=0.04). Finally, lymphovascular invasion comprised 13% of patients with HR 1.4 (NS p=0.175).

The RS score was also analyzed for 3 groups of pre-specified cutpoints (low <30, 47% of patients; intermediate 30–40, 30.7% of patients; and high ≥41, 25.6% of patients). (J Clin Oncol 27:15s, 2009 (suppl; abstr 4000)) The recurrence risk of the ‘high’ group versus the ‘low’ group had a HR of 1.47. The low group risk of recurrence was 12% (9–16%), intermediate was 18% (13–24%) and high 22% (16–29%). The company suggests that T-stage and MMR status should be integrated into the overall decision algorithm for stage II patients: low risk - dMMR and T3 tumors, consider no treatment; high risk – T4 and pMMR, consider adjuvant treatment; and intermediate – T3 and pMMR, consider Onco*type* DX Colon Assay to assist decision-making. Others have also proposed this algorithm.[83]

It must be acknowledged that the utility of the Oncotype Dx Colon Assay has not been validated prospectively in a clinical trial as an integrated biomarker, like the ongoing adjuvant TAILORx (Trial Assigning Individualized Options for Treatment (Rx)) and MINDACT (Microarray In Node negative Disease May Avoid ChemoTherapy) prospective trials in breast cancer designed to validate the Onco*type* Dx and MammaPrint assays.[9899] Prospective clinical trials designed to validate this test are expected to assist in determining the appropriate incorporation of this assay into standard care for stage II patients. Also, it should be noted that the Treatment Predicitve score (TS) did not help predict which patients would benefit from 5-FU based adjuvant therapy in this study.

The ColoPrint Assay is a gene expression array consisting of 18 genes (J Clin Oncol 28:15s, 2010 (suppl; abstr 3513), and was used to evaluate 137 stage II patients. The assay identified most patients (74%) as low risk of recurrence. The 5-year distant-metastasis-free survival was 95% for low risk patients and 79.9% for high risk patients as defined by this assay. In the univariate analysis, ColoPrint was the only significant parameter to predict the development of distant metastasis with a HR of 4.3 (95% CI 1.36–13.56, p = 0.007).

Other Biomarkers for Available Biologics

Reports of other molecular aberrations in CC are further refining our ability to predict sensitivity or resistance to EGFR inhibitors, in addition to *KRAS* status.[9100–105] *BRAF*, a serine-threonine protein kinase downstream effector of *KRAS* signaling, is mutated (in exon 15 V600E) in approximately 5–12% of colon cancers, and appears to be mutually exclusive of *KRAS* mutation.[7084] In a study evaluating pooled stage II and III patients from three trials (PETACC-3, EORTC 40993, and SAKK 60-00), 1,564 samples showed that 7.9% of tumors harbored *BRAF* mutations (versus 37% with *KRAS* mutations), and *BRAF* mutation was associated with MSI-H dMMR ($p<0.0001$).[100] *BRAF* mutation was not associated with relapse-free survival, but was related to OS (HR 2.2, p=0.003). The CRYSTAL trial also reported *BRAF* mutation as a negative prognostic factor (Table 2).

In the largest cohort to date (n=773) of chemotherapy-refractory mCC treated with cetuximab plus chemotherapy in the pre-*KRAS* selection era, *BRAF* mutations were observed in 4.7% (36/761).[70] Other genes in the *KRAS* signaling pathway, including *N-RAS* and *PIK3CA* (encodes PI3-kinase) were also evaluated. *N-RAS* mutation occurred in 2.6% (17/644) and PIK3CA mutation occurred in 14.5% of tumors (108/743), with 68.5% (74/108) of mutations observed in exon 9 and 20.4% (22/108) in exon 20. In addition to confirming the negative effect of *KRAS* mutation on cetuximab activity, it was observed that *BRAF, N-RAS and PIK3CA* exon 20 mutations were significantly associated with a low response rate, and worse PFS and OS in multivariate analyses.[70]

Amphiregulin (AREG) and epiregulin (EREG) are EGFR ligands that activate the EGFR pathway. AREG and EREG expression levels were quantified by collection of mRNA and reverse transcriptase polymerase chain reaction in primary tumors, to determine if expression levels were able to predict the outcome in patients with mCC treated with the combination of cetuximab and irinotecan.[106] In *KRAS* wild type patients, there was a significant association between log-transformed ligand expression and response for EREG (odds ratio for objective response, 1.90; 95% CI, 1.27 to 2.83; p = 0.0005) and for AREG (odds ratio for objective response, 1.862; 95% CI, 1.22 to 2.72; p = 0.0017). In a Cox regression model, dichotomized ligand expression was significantly associated with progression-free survival (PFS) and overall survival (OS). EREG PFS had a HR of 0.41 (95% CI, 0.274 to 0.609; p = 0.001), and AREG PFS HR was 0.43 (95% CI, 0.29 to 0.64; p = 0.001). EREG OS HR was 0.42 (95% CI, 0.28 to 0.63; p = 0.0001), and AREG OS HR was 0.40 (95% CI, 0.27 to 0.64; p= 0.0001). There was no predictive power of ligand expression in patients with *KRAS* mutation.

EGFR copy number by FISH, both clustered amplification and by high polysomy, as described by the Cappuzzo and Moroni methods,[105107] has been reported to be an independent predictor of EFGR inhibitor sensitivity.[105108]

Recently, it has been suggested that a comprehensive molecular analysis of the entire EGFR pathway be calculated into an integrated score, in order to enhance the predictive ability of individual makers.[108109] A prospective evaluation of these markers is clearly needed.

Although there are currently no molecular predictors of response to bevacizumab,[110] there are some promising strategies on the horizon, such as 89Zr-bevacizumab PET of early antiangiogenic tumor response demonstrated i*n vivo* mice,[111] and gene expression signatures that are associated with response.[112] Perfusion imaging may also play a role in the future.[113]

AKT, MAPK,JNK, IGF1R, MET, Hedgehog(Hh) Signaling, Notch

A number of other oncogenic pathways have shown to be aberrantly activated in CC, including AKT, MAPK, JNK, IGF1R, MET, Hedgehog (Hh) and Notch.[114–118] Each of

these pathways have preclinical evidence that targeted inhibition of key molecules in the pathways abrogate malignant phenotypes, and a number of inhibitory small molecules and monoclonal antibodies are currently being evaluated in preclinical and early phase clinical trials for these targets. Results from these trials may allow selection of the most promising agents to bring into larger trials in CC in the adjuvant and metastatic settings. An example is the phase II trial evaluating perifosine (a combined AKT, MAPK and JNK kinase inhibitor) with capecitabine in chemo-refractory mCC (J Clin Oncol 27:15s, 2009 (suppl; abstr 4081)), where it more than doubled median TTP over capecitabine alone. This trial is of particular interest given the relative poor prognosis of chemorefractory mCC patients that have exhausted all standard therapies (fluoropyrimidine, oxaliplatin, irinotecan, bevacizumab +/− cetuximab/panitumumab for *KRAS* wild type).[115] It is often difficult to consistently define the number of lines of prior treatment for patients with mCC, as they often switch between the above agents due to toxicity and other issues not related to disease progression. Clinical trials evaluating promising novel inhibitors in this chemorefractory or ‘third line’ setting are desperately needed.

Chemotherapy Metabolism and Personalized Delivery

Guidance in personalizing delivery of specific chemotherapy drugs depending on patient or tumor characteristics may be on the horizon. The thymidylate synthase gene, *TYMS* encodes an enzyme, thymidylate synthase (TYMS), involved in DNA synthesis; that is inhibited by 5-FU. The assessment of TYMS as a predictive (for fluoropyrimidines) or prognostic biomarker has produced conflicting reports, and the clinical significance of protein and mRNA levels, and between germline variation of *TYMS* and gene function remain to be elucidated, as do the importance of other enzymes in the pyrimidine biosynthesis pathway such as dihydropyrimidine dehydrogenase.[84] The ECOG 4203 trial is evaluating outcomes in patients with high TYMS treated with FOLFOX-bev or IROX-bev, while low TYMS expressing tumors receive only FOLFOX-bev.[119]

Genetic variation in the UGT1A1 enzyme, an enzyme involved in clearance of irinotecan, can predict risk of severe neutropenia.[120] The $^{*}28/^{*}28$ variant is only found in ~10% of patients in the U.S., but is associated with a high risk of severe neutropenia. Patients with the more common variants $^{*}1/^{*}1$ and $^{*}1/^{*}28$ may be able to safely tolerate higher than standard irinotecan doses that have the potential to improve the efficacy of treatment. Phase I trials evaluating higher dosing in these patients are underway, with the intention of testing whether improvements in clinical outcomes can be achieved with escalated doses in these selected patients.[56]

ERCC1 is a nucleotide excision repair gene associated with repair of DNA adducts induced by platinum-based chemotherapy. It is thought that ERCC1 levels might predict response to oxaliplatin in patients with mCC, and an exploratory study is ongoing in the adjuvant setting evaluating changes in ERCC1 levels before and after treatment with oxaliplatin.

Circulating Tumor Cells (CTCs)

Circulating tumor cells (CTCs) have been identified in patients with advanced CC and may play a role in hematogenous metastases.[121122] Using a commercially available assay, CTCs are identified by collecting 7.5ml of blood and enriching for cells with epithelial markers via cell-specific markers identified by antibodies[123124]. CTCs are positive for the epithelial cell adhesion molecule (EpCAM), often over-expressed in CC. CTCs are also positive for cytokeratin and negative for CD45 leukocyte marker. An alternative method of detection is the CTC chip.[125]

It was recently reported that 430 mCC patients could be stratified into favorable and unfavorable prognostic groups based on baseline CTC levels of <3 CTC/7.5ml or >3 CTCs/7.5ml.[126] Higher CTC levels was associated with a worse PFS (4.5 v 7.9 months, p=0.0002) and OS (9.4 v 18.5 months, p<0.0001). These differences persisted throughout various time points during therapy. Patients whose baseline CTCs converted from high to low after 3–5 weeks of therapy had a significantly longer PFS (6.2 v 1.6 months, p=0.02) and OS (11 v 3.7 months, p=0.0002), compared with patients with high CTCs at both time points. The investigators concluded that number of CTCs before and during treatment is an independent prognostic marker in mCC. The same authors showed that baseline levels of CTCs is an important prognostic factor within specific subgroups defined by treatment or patient chacteristics.[127]

Future prospective trials to validate these results may be warranted to confirm CTCs as an independent stratification factor. CTCs may have many potential future applications to enhance the personalization of colon cancer care, including for *KRAS* status testing without tissue biopsy,[128] as an independent marker for stratification after curative surgery,[121122129] as well as for global genome profiling of cancer,[130] all of which warrant further investigation.

Chromosomal Instability (CIN), DNA ploidy, Loss of Heterozygosity – 18q, APC, p53

It is notable that approximately 30% of human genes encode proteins that regulate DNA fidelity.[86] There are a variety of different mechanisms that can lead to the loss of genomic DNA stability, with four suggested categories: (1) subtle sequence changes, including base substitutions, deletions, or insertions, as well as MSI; (2) alterations in chromosome number (aneuploidy, also termed CIN), (3) chromosomal rearrangements or 'translocation instability', and (4) gene amplification.[86] As discussed, MSI, due either to germ-line mutation or aberrant CpG island methylation, occurs in 15% of colon cancers. CIN is thought to occur in 80–85% of CC, and although the mechanisms are not fully delineated, is believed to be a consequence of deregulation of the processes that mediate the mitotic spindle checkpoint, DNA damage checkpoints, chromosome metabolism, and centrosome function.[86] A number of recurring genetic abnormalities have been associated with the initiation of CIN, but also as a consequence of CIN, or occasionally both. These genes include *APC, KRAS, PIK3CA, SMAD4, TP53* amongst others. Hence, each of these genes and their proteins, as well as loss of chromosome regions, in particular 18q, have been evaluated for prognostic and/or predictive relevance in colon cancer.[84]

It has been debated whether or not loss of 18q heterozygosity (LOH) has prognostic significance in stage II and III colon cancer, since some studies but not others have supported this hypothesis.[84] The 18q region is rich in putative tumor suppressor genes, including *SMAD4, SMAD2, SMAD7, CABLES1*, and *DCC*.[83131] In order to explain the apparently contradictory data, questions have been raised about the different methodologies available to measure 18q loss, as well as what is exactly being measured.[84] In particular, given the large number of putative genes lost on 18q, it has been suggested that quantitative assays measuring these genes or proteins may have more relevance, as has been described for *SMAD4*.[132] It has also been postulated that 18q allelic imbalance is merely a surrogate marker for global genomic chromosomal instability that leads to aneuploidy and chromosomal gain and loss.[84] As such, other regions of the genome commonly lost in colon cancer, including 17p and 5q, are regions that harbor tumor suppressors important in colon cancer tumorigenesis, such as p53 (inactivated in 35–55% of CC) and APC (inactivated in 85% of CC), respectively.[133]

The conflicting data about the association of these various genes/proteins with treatment response and colon cancer prognosis, are reviewed elsewhere,[84131] but highlight a

fundamental problem that translational scientists currently face. The immense complexity of cancer, and the conditional nature of these various markers, depend on the global cellular context. For instance, dMMR is a relatively good prognostic indicator; however, *BRAF* mutations tend to occur more frequently in dMMR tumors,[84131] increasing the overall risk of recurrence for this subgroup of dMMR tumors.

Thus, it is the *aggregate* of gene or protein loss and gain, through a variety of biologic mechanisms that is the key to understanding colon cancer carcinogenesis and to advancing into the future of personalized cancer care. Evaluating each individual biomarker, analogous to a univariate analysis, leads to the generation of conflicting data, depending on the global cellular context (other aberrations in the cell),and on the study sample selection, method of biomarker detection, and investigator interpretation of results. An integrated approach that attempts to evaluate the aggregate of aberrations in a given cancer is showing great promise, and is discussed in more detail in below.

VII. The Future is Near – Integrated Global Analysis

The technology is now available to conduct high-throughput analyses of tumor cells in comparison to normal cells of the same patient, in order to differentiate germline from somatic alterations. Genome wide sequencing or single nucleotide polymorphism (SNP) platform analyses for point mutations/insertions/deletions/SNPs (mutome),[134] gene mRNA or protein expression (transcriptome, proteome),[135136] and gene copy number and DNA ploidy (amplicome),[137138] can each be determined from FFPE samples rapidly and reproducibly. The methods and applications of each are reviewed in the “Primer on the molecular profiling of cancer” (Stricker *et al*), elsewhere in this issue. These technologies are becoming more cost-effective, particularly as the cost of ordering each biomarker ‘a la carte’ for each patient sample (such as will be required, for example, to predict benefit of EGFR inhibitors discussed earlier, and particularly to identify rare mutations currently not routinely tested, such as KRAS codons 61 [2%], 146 [2%] and 59 [0.1%][70]) will eventually overtake the cost of performing a single global cancer cell assessment that will provide all of these results (and more) from a single test. Software programs are available to integrate each of these high throughput analyses in order to determine and filter data into meaningful prognostic information and to prioritize treatment strategies based on these immense datasets.[92]

The promise of personalizing care based on these novel technologies must overcome a number of technical difficulties and hurdles with respect to obtaining accurate, precise, meaningful and reliable results from human tissues. The development of assays and then incorporating them into clinical trials in order to prospectively validate them will be just as challenging, and it will be a long road before they become accepted for routine use in the clinical oncology community. A number of guidelines and roadmaps have been laid out detailing how to best accomplish these goals.[139–142;89] The promise of true personalization of cancer care, however, is getting closer.

VII. Conclusions

We are at the dawn of the era of truly personalized colon cancer care, both in the advanced and adjuvant setting. The days of one-size-fits-all treatment approaches, both in terms of selecting chemotherapy and biologic regimens, as well as actual drug doses, will one day be a thing of the past. It will be replaced with an individualized treatment plan that is designed based upon a number of baseline diagnostic tests performed on tumor and blood samples after discovering the cancer. We will recognize that each individual’s tumor, just as we acknowledge each other’s individuality, is unique. This will require high-throughput

strategies to provide us with details that enable us to refine our treatments to best address each clinical scenario.

References

1. Petrelli NJ, Winer EP, Brahmer J, Dubey S, Smith S, Thomas C, et al. Clinical Cancer Advances 2009: major research advances in cancer treatment, prevention, and screening--a report from the American Society of Clinical Oncology. J Clin Oncol. 2009; 27(35):6052–6069. [PubMed: 19901123]
2. Temple LK. The prognosis of colon cancer is dependent on accurate staging. J Surg Oncol. 2010; 102(1):1–2. [PubMed: 20578171]
3. SB, Edge; DR, Byrd; CC, Compton, et al., editors. AJCC Cancer Staging Manual. 7th ed. Philadelphia, PA: Springer Verlag; 2010.
4. Grothey A. Adjuvant chemotherapy in colon cancer - is it worth it? Eur J Cancer. 2010; 46(10): 1768–1769. [PubMed: 20434902]
5. Sargent D, Sobrero A, Grothey A, O'Connell MJ, Buyse M, Andre T, et al. Evidence for cure by adjuvant therapy in colon cancer: observations based on individual patient data from 20,898 patients on 18 randomized trials. J Clin Oncol. 2009; 27(6):872–877. [PubMed: 19124803]
6. Quasar Collaborative G, Gray R, Barnwell J, McConkey C, Hills RK, Williams NS, et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet. 2007; 370(9604):2020–2029. [PubMed: 18083404]
7. Tournigand C, Andre T, Achille E, Lledo G, Flesh M, Mery-Mignard D, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. J Clin Oncol. 2004; 22(2):229–237. [PubMed: 14657227]
8. Engstrom PF. Systemic therapy for advanced or metastatic colorectal cancer: National Comprehensive Cancer Network guidelines for combining anti-vascular endothelial growth factor and anti-epidermal growth factor receptor monoclonal antibodies with chemotherapy. Pharmacotherapy. 2008; 28(11 Pt 2):18S–22S. [PubMed: 18980548]
9. Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. J Natl Cancer Inst. 2009; 101(19):1308–1324. [PubMed: 19738166]
10. Schilsky RL. Personalizing cancer care: American Society of Clinical Oncology presidential address 2009. J Clin Oncol. 2009; 27(23):3725–3730. [PubMed: 19581526]
11. Venook A. Critical evaluation of current treatments in metastatic colorectal cancer. Oncologist. 2005; 10(4):250–261. [PubMed: 15821245]
12. O'neil, B.; Kelly, H.; Morse, M.; Goldberg, R. Colorectal Cancer: evidence-based chemotherapy strategies. In: Saltz, LB., editor. Adjuvant Chemotherapy for Colon Cancer. Totowa, NJ: Humana Press, Inc; 2007. p. 23
13. Lokich JJ, Ahlgren JD, Gullo JJ, Philips JA, Fryer JG. A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: a Mid-Atlantic Oncology Program Study. J Clin Oncol. 1989; 7(4):425–432. [PubMed: 2926468]
14. Fraile RJ, Baker LH, Buroker TR, Horwitz J, Vaitkevicius VK. Pharmacokinetics of 5-fluorouracil administered orally, by rapid intravenous and by slow infusion. Cancer Res. 1980; 40(7):2223–2228. [PubMed: 7388790]
15. Milano G, Ferrero JM, Francois E. Comparative pharmacology of oral fluoropyrimidines: a focus on pharmacokinetics, pharmacodynamics and pharmacomodulation. Br J Cancer. 2004; 91(4): 613–617. [PubMed: 15280932]
16. Gill S, Loprinzi CL, Sargent DJ, Thome SD, Alberts SR, Haller DG, et al. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? J Clin Oncol. 2004; 22(10):1797–1806. [PubMed: 15067028]
17. Haller DG, Catalano PJ, Macdonald JS, O'Rourke MA, Frontiera MS, Jackson DV, et al. Phase III study of fluorouracil, leucovorin, and levamisole in high-risk stage II and III colon cancer: final report of Intergroup 0089. J Clin Oncol. 2005; 23(34):8671–8678. [PubMed: 16314627]

18. Poplin EA, Benedetti JK, Estes NC, Haller DG, Mayer RJ, Goldberg RM, et al. Phase III Southwest Oncology Group 9415/Intergroup 0153 randomized trial of fluorouracil, leucovorin, and levamisole versus fluorouracil continuous infusion and levamisole for adjuvant treatment of stage III and high-risk stage II colon cancer. J Clin Oncol. 2005; 23(9):1819–1825. [PubMed: 15774775]

19. Kohne CH, Wils J, Lorenz M, Schoffski P, Voigtmann R, Bokemeyer C, et al. Randomized phase III study of high-dose fluorouracil given as a weekly 24-hour infusion with or without leucovorin versus bolus fluorouracil plus leucovorin in advanced colorectal cancer: European organization of Research and Treatment of Cancer Gastrointestinal Group Study 40952. J Clin Oncol. 2003; 21(20):3721–3728. [PubMed: 12963704]

20. Thirion P, Michiels S, Pignon JP, Buyse M, Braud AC, Carlson RW, et al. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: an updated metaanalysis. J Clin Oncol. 2004; 22(18):3766–3775. [PubMed: 15365073]

21. Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. Meta-analysis Group In Cancer. J Clin Oncol. 1998; 16(1):301–308. [PubMed: 9440757]

22. de Gramont A, Bosset JF, Milan C, Rougier P, Bouche O, Etienne PL, et al. Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with bimonthly high-dose leucovorin and fluorouracil bolus plus continuous infusion for advanced colorectal cancer: a French intergroup study. J Clin Oncol. 1997; 15(2):808–815. [PubMed: 9053508]

23. Rougier P, Bugat R, Douillard JY, Culine S, Suc E, Brunet P, et al. Phase II study of irinotecan in the treatment of advanced colorectal cancer in chemotherapy-naive patients and patients pretreated with fluorouracil-based chemotherapy. J Clin Oncol. 1997; 15(1):251–260. [PubMed: 8996150]

24. Cunningham D, Pyrhonen S, James RD, Punt CJ, Hickish TF, Heikkila R, et al. Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. Lancet. 1998; 352(9138):1413–1418. [PubMed: 9807987]

25. Rougier P, Van Cutsem E, Bajetta E, Niederle N, Possinger K, Labianca R, et al. Randomised trial of irinotecan versus fluorouracil by continuous infusion after fluorouracil failure in patients with metastatic colorectal cancer. Lancet. 1998; 352(9138):1407–1412. [PubMed: 9807986]

26. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med. 2000; 343(13):905–914. [PubMed: 11006366]

27. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet. 2000; 355(9209):1041–1047. [PubMed: 10744089]

28. Rixe O, Ortuzar W, Alvarez M, Parker R, Reed E, Paull K, et al. Oxaliplatin, tetraplatin, cisplatin, and carboplatin: spectrum of activity in drug-resistant cell lines and in the cell lines of the National Cancer Institute's Anticancer Drug Screen panel. Biochem Pharmacol. 1996; 52(12):1855–1865. [PubMed: 8951344]

29. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol. 2000; 18(16):2938–2947. [PubMed: 10944126]

30. Rothenberg ML, Oza AM, Bigelow RH, Berlin JD, Marshall JL, Ramanathan RK, et al. Superiority of oxaliplatin and fluorouracil-leucovorin compared with either therapy alone in patients with progressive colorectal cancer after irinotecan and fluorouracil-leucovorin: interim results of a phase III trial. J Clin Oncol. 2003; 21(11):2059–2069. [PubMed: 12775730]

31. Goldberg RM, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, et al. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol. 2004; 22(1):23–30. [PubMed: 14665611]

32. Souglakos J, Androulakis N, Syrigos K, Polyzos A, Ziras N, Athanasiadis A, et al. FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) vs FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): a multicentre

randomised phase III trial from the Hellenic Oncology Research Group (HORG). Br J Cancer. 2006; 94(6):798–805. [PubMed: 16508637]

33. Falcone A, Ricci S, Brunetti I, Pfanner E, Allegrini G, Barbara C, et al. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. J Clin Oncol. 2007; 25(13):1670–1676. [PubMed: 17470860]
34. Montagnani F, Chiriatti A, Turrisi G, Francini G, Fiorentini G. A systematic review of FOLFOXIRI chemotherapy for the first-line treatment of metastatic colorectal cancer: improved efficacy at the cost of increased toxicity. Colorectal Dis. 2010
35. Masi G, Loupakis F, Pollina L, Vasile E, Cupini S, Ricci S, et al. Long-term outcome of initially unresectable metastatic colorectal cancer patients treated with 5-fluorouracil/leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) followed by radical surgery of metastases. Ann Surg. 2009; 249(3):420–425. [PubMed: 19247029]
36. Miwa M, Ura M, Nishida M, Sawada N, Ishikawa T, Mori K, et al. Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. Eur J Cancer. 1998; 34(8):1274–1281. [PubMed: 9849491]
37. Schuller J, Cassidy J, Dumont E, Roos B, Durston S, Banken L, et al. Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. Cancer Chemother Pharmacol. 2000; 45(4):291–297. [PubMed: 10755317]
38. Van Cutsem E, Twelves C, Cassidy J, Allman D, Bajetta E, Boyer M, et al. Oral capecitabine compared with intravenous fluorouracil plus leucovorin in patients with metastatic colorectal cancer: results of a large phase III study. J Clin Oncol. 2001; 19(21):4097–4106. [PubMed: 11689577]
39. Hoff PM, Ansari R, Batist G, Cox J, Kocha W, Kuperminc M, et al. Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. J Clin Oncol. 2001; 19(8):2282–2292. [PubMed: 11304782]
40. Diaz-Rubio E, Tabernero J, Gomez-Espana A, Massuti B, Sastre J, Chaves M, et al. Phase III study of capecitabine plus oxaliplatin compared with continuous-infusion fluorouracil plus oxaliplatin as first-line therapy in metastatic colorectal cancer: final report of the Spanish Cooperative Group for the Treatment of Digestive Tumors Trial. J Clin Oncol. 2007; 25(27):4224–4230. [PubMed: 17548839]
41. Porschen R, Arkenau HT, Kubicka S, Greil R, Seufferlein T, Freier W, et al. Phase III study of capecitabine plus oxaliplatin compared with fluorouracil and leucovorin plus oxaliplatin in metastatic colorectal cancer: a final report of the AIO Colorectal Study Group. J Clin Oncol. 2007; 25(27):4217–4223. [PubMed: 17548840]
42. Cassidy J, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, et al. Randomized phase III study of capecitabine plus oxaliplatin compared with fluorouracil/folinic acid plus oxaliplatin as first-line therapy for metastatic colorectal cancer. J Clin Oncol. 2008; 26(12):2006–2012. [PubMed: 18421053]
43. Sakamoto J, Ohashi Y, Hamada C, Buyse M, Burzykowski T, Piedbois P. Efficacy of oral adjuvant therapy after resection of colorectal cancer: 5-year results from three randomized trials. J Clin Oncol. 2004; 22(3):484–492. [PubMed: 14752071]
44. Kohne CH, De Greve J, Hartmann JT, Lang I, Vergauwe P, Becker K, et al. Irinotecan combined with infusional 5-fluorouracil/folinic acid or capecitabine plus celecoxib or placebo in the first-line treatment of patients with metastatic colorectal cancer. EORTC study 40015. Ann Oncol. 2008; 19(5):920–926. [PubMed: 18065406]
45. Fuchs CS, Marshall J, Mitchell E, Wierzbicki R, Ganju V, Jeffery M, et al. Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: results from the BICC-C Study. J Clin Oncol. 2007; 25(30):4779–4786. [PubMed: 17947725]

46. Haller DG, Cassidy J, Clarke SJ, Cunningham D, Van Cutsem E, Hoff PM, et al. Potential regional differences for the tolerability profiles of fluoropyrimidines. J Clin Oncol. 2008; 26(13):2118–2123. [PubMed: 18445840]
47. Schmoll HJ, Cartwright T, Tabernero J, Nowacki MP, Figer A, Maroun J, et al. Phase III trial of capecitabine plus oxaliplatin as adjuvant therapy for stage III colon cancer: a planned safety analysis in 1,864 patients. J Clin Oncol. 2007; 25(1):102–109. [PubMed: 17194911]
48. Koopman M, Antonini NF, Douma J, Wals J, Honkoop AH, Erdkamp FL, et al. Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer (CAIRO): a phase III randomised controlled trial. Lancet. 2007; 370(9582):135–142. [PubMed: 17630036]
49. Seymour MT, Maughan TS, Ledermann JA, Topham C, James R, Gwyther SJ, et al. Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial. Lancet. 2007; 370(9582):143–152. [PubMed: 17630037]
50. Tournigand C, Cervantes A, Figer A, Lledo G, Flesch M, Buyse M, et al. OPTIMOX1: a randomized study of FOLFOX4 or FOLFOX7 with oxaliplatin in a stop-and-Go fashion in advanced colorectal cancer--a GERCOR study. J Clin Oncol. 2006; 24(3):394–400. [PubMed: 16421419]
51. Chibaudel B, Maindrault-Goebel F, Lledo G, Mineur L, Andre T, Bennamoun M, et al. Can chemotherapy be discontinued in unresectable metastatic colorectal cancer? The GERCOR OPTIMOX2 Study. J Clin Oncol. 2009; 27(34):5727–5733. [PubMed: 19786657]
52. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004; 350(23):2335–2342. [PubMed: 15175435]
53. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol. 2008; 26(12):2013–2039. [PubMed: 18421054]
54. Grothey A, Sugrue MM, Purdie DM, Dong W, Sargent D, Hedrick E, et al. Bevacizumab beyond first progression is associated with prolonged overall survival in metastatic colorectal cancer: results from a large observational cohort study (BRiTE). J Clin Oncol. 2008; 26(33):5326–5334. [PubMed: 18854571]
55. http://www.clinicaltrials.gov/ct/show/NCT00499369.
56. http://webres.uccrc.org/clinical_trials.
57. http://clinicaltrials.gov/ct2/show/NCT00418938.
58. Saltz LB, Rosen LS, Marshall JL, Belt RJ, Hurwitz HI, Eckhardt SG, et al. Phase II trial of sunitinib in patients with metastatic colorectal cancer after failure of standard therapy. J Clin Oncol. 2007; 25(30):4793–4799. [PubMed: 17947727]
59. Baselga J. Why the epidermal growth factor receptor? The rationale for cancer therapy. Oncologist. 2002; (7 Suppl 4):2–8. [PubMed: 12202782]
60. Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J Clin Oncol. 2010; 28(7):1254–1261. [PubMed: 20100961]
61. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med. 2004; 351(4):337–345. [PubMed: 15269313]
62. Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med. 2007; 357(20):2040–2048. [PubMed: 18003960]
63. Van Cutsem E, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol. 2007; 25(13): 1658–1664. [PubMed: 17470858]
64. Cercek A, Saltz L. Evolving treatment of advanced colorectal cancer. Curr Oncol Rep. 2010; 12(3):153–159. [PubMed: 20425074]

65. Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med. 2009; 360(14): 1408–1417. [PubMed: 19339720]
66. Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol. 2009; 27(5):663–671. [PubMed: 19114683]
67. Saif MW, Shah M. K-ras mutations in colorectal cancer: a practice changing discovery. Clin Adv Hematol Oncol. 2009; 7(1):45–53. 64. [PubMed: 19274041]
68. Tol J, Koopman M, Cats A, Rodenburg CJ, Creemers GJ, Schrama JG, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. N Engl J Med. 2009; 360(6):563–572. [PubMed: 19196673]
69. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008; 26(10):1626–1634. [PubMed: 18316791]
70. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010; 11(8):753–762. [PubMed: 20619739]
71. De Roock W, Jonker DJ, Di Nicolantonio F, Sartore-Bianchi A, Tu D, Siena S, et al. Association of KRAS p.G13D Mutation With Outcome in Patients With Chemotherapy-Refractory Metastatic Colorectal Cancer Treated With Cetuximab. JAMA. 2010; 304(16):1812–1820. [PubMed: 20978259]
72. Saltz LB, Lenz HJ, Kindler HL, Hochster HS, Wadler S, Hoff PM, et al. Randomized phase II trial of cetuximab, bevacizumab, and irinotecan compared with cetuximab and bevacizumab alone in irinotecan-refractory colorectal cancer: the BOND-2 study. J Clin Oncol. 2007; 25(29):4557–4561. [PubMed: 17876013]
73. Hecht JR, Mitchell E, Chidiac T, Scroggin C, Hagenstad C, Spigel D, et al. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. J Clin Oncol. 2009; 27(5):672–680. [PubMed: 19114685]
74. Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med. 2004; 350(23): 2343–2351. [PubMed: 15175436]
75. Kuebler JP, Wieand HS, O'Connell MJ, Smith RE, Colangelo LH, Yothers G, et al. Oxaliplatin combined with weekly bolus fluorouracil and leucovorin as surgical adjuvant chemotherapy for stage II and III colon cancer: results from NSABP C-07. J Clin Oncol. 2007; 25(16):2198–2204. [PubMed: 17470851]
76. Andre T, Boni C, Navarro M, Tabernero J, Hickish T, Topham C, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol. 2009; 27(19):3109–3116. [PubMed: 19451431]
77. http://clinicaltrials.gov/ct2/show/NCT00958737.
78. Saltz LB, Niedzwiecki D, Hollis D, Goldberg RM, Hantel A, Thomas JP, et al. Irinotecan fluorouracil plus leucovorin is not superior to fluorouracil plus leucovorin alone as adjuvant treatment for stage III colon cancer: Results of CALGB 89803. Journal of Clinical Oncology. 2007; 25(23):3456–3461. [PubMed: 17687149]
79. Ychou M, Hohenberger W, Thezenas S, Navarro M, Maurel J, Bokemeyer C, et al. A randomized phase III study comparing adjuvant 5-fluorouracil/folinic acid with FOLFIRI in patients following complete resection of liver metastases from colorectal cancer. Ann Oncol. 2009; 20(12):1964–1970. [PubMed: 19567451]
80. Van Cutsem E, Labianca R, Bodoky G, Barone C, Aranda E, Nordlinger B, et al. Randomized phase III trial comparing biweekly infusional fluorouracil/leucovorin alone or with irinotecan in the adjuvant treatment of stage III colon cancer: PETACC-3. J Clin Oncol. 2009; 27(19):3117–3125. [PubMed: 19451425]

81. Twelves C, Wong A, Nowacki MP, Abt M, Burris H 3rd, Carrato A, et al. Capecitabine as adjuvant treatment for stage III colon cancer. N Engl J Med. 2005; 352(26):2696–2704. [PubMed: 15987918]

82. Allegra CJ, Yothers G, O'Connell MJ, Sharif S, Colangelo LH, Lopa SH, et al. Initial safety report of NSABP C-08: A randomized phase III study of modified FOLFOX6 with or without bevacizumab for the adjuvant treatment of patients with stage II or III colon cancer. J Clin Oncol. 2009; 27(20):3385–3390. [PubMed: 19414665]

83. Rousseau B, Chibaudel B, Bachet JB, Larsen AK, Tournigand C, Louvet C, et al. Stage II and stage III colon cancer: treatment advances and future directions. Cancer J. 2010; 16(3):202–209. [PubMed: 20526097]

84. Tejpar S, Bertagnolli M, Bosman F, Lenz HJ, Garraway L, Waldman F, et al. Prognostic and predictive biomarkers in resected colon cancer: current status and future perspectives for integrating genomics into biomarker discovery. Oncologist. 2010; 15(4):390–404. [PubMed: 20350999]

85. Mamounas E, Wieand S, Wolmark N, Bear HD, Atkins JN, Song K, et al. Comparative efficacy of adjuvant chemotherapy in patients with Dukes' B versus Dukes' C colon cancer: results from four National Surgical Adjuvant Breast and Bowel Project adjuvant studies (C-01, C-02, C-03, and C-04). J Clin Oncol. 1999; 17(5):1349–1355. [PubMed: 10334518]

86. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology. 2008; 135(4):1079–1099. [PubMed: 18773902]

87. Meyers M, Wagner MW, Mazurek A, Schmutte C, Fishel R, Boothman DA. DNA mismatch repair-dependent response to fluoropyrimidine-generated damage. J Biol Chem. 2005; 280(7): 5516–5526. [PubMed: 15611052]

88. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004; 96(4):261–268. [PubMed: 14970275]

89. Bosman FT, Yan P, Tejpar S, Fiocca R, Van Cutsem E, Kennedy RD, et al. Tissue biomarker development in a multicentre trial context: a feasibility study on the PETACC3 stage II and III colon cancer adjuvant treatment trial. Clin Cancer Res. 2009; 15(17):5528–5533. [PubMed: 19690194]

90. Bertagnolli MM, Niedzwiecki D, Compton CC, Hahn HP, Hall M, Damas B, et al. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. J Clin Oncol. 2009; 27(11):1814–1821. [PubMed: 19273709]

91. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol. 2010; 28(20):3219–3226. [PubMed: 20498393]

92. Kreeger PK, Lauffenburger DA. Cancer systems biology: a network modeling perspective. Carcinogenesis. 2010; 31(1):2–8. [PubMed: 19861649]

93. Chowdhury FU, Shah N, Scarsbrook AF, Bradley KM. [18F]FDG PET/CT imaging of colorectal cancer: a pictorial review. Postgrad Med J. 2010; 86(1013):174–182. [PubMed: 20237012]

94. Herbertson RA, Scarsbrook AF, Lee ST, Tebbutt N, Scott AM. Established, emerging and future roles of PET/CT in the management of colorectal cancer. Clin Radiol. 2009; 64(3):225–237. [PubMed: 19185652]

95. Moore M, Gibbs P. Adjuvant chemotherapy use among older patients with stage III colon cancer. JAMA. 2010; 303(23):2353. author reply 53–4. [PubMed: 20551402]

96. Sargent DJ, Goldberg RM, Jacobson SD, Macdonald JS, Labianca R, Haller DG, et al. A pooled analysis of adjuvant chemotherapy for resected colon cancer in elderly patients. N Engl J Med. 2001; 345(15):1091–1097. [PubMed: 11596588]

97. 2010. http://www.oncotypedx.com/en/Colon/HealthcareProfessional/Overview.aspx.

98. http://www.cancer.gov/clinicaltrials/EORTC-10041.

99. http://www.ascopost.com/articles/july-2010/multiparameter-breast-cancer-gene-profiling-assays.

100. Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the

PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol. 2010; 28(3):466–474. [PubMed: 20008640]

101. Souglakos J, Philips J, Wang R, Marwah S, Silver M, Tzardi M, et al. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. Br J Cancer. 2009; 101(3):465–472. [PubMed: 19603024]
102. Baldus SE, Schaefer KL, Engers R, Hartleb D, Stoecklein NH, Gabbert HE. Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases. Clin Cancer Res. 2010; 16(3):790–799. [PubMed: 20103678]
103. Laurent-Puig P, Cayre A, Manceau G, Buc E, Bachet JB, Lecomte T, et al. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. J Clin Oncol. 2009; 27(35):5924–5930. [PubMed: 19884556]
104. Lieberman R. Personalized medicine enters the US marketplace: KRAS, anti-EGFR monoclonal antibodies, and colon cancer. Am J Ther. 2009; 16(6):477–479. [PubMed: 19829092]
105. Moroni M, Veronese S, Benvenuti S, Marrapese G, Sartore-Bianchi A, Di Nicolantonio F, et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. Lancet Oncol. 2005; 6(5):279–286. [PubMed: 15863375]
106. Jacobs B, De Roock W, Piessevaux H, Van Oirbeek R, Biesmans B, De Schutter J, et al. Amphiregulin and epiregulin mRNA expression in primary tumors predicts outcome in metastatic colorectal cancer treated with cetuximab. J Clin Oncol. 2009; 27(30):5068–5074. [PubMed: 19738126]
107. Varella-Garcia M, Diebold J, Eberhard DA, Geenen K, Hirschmann A, Kockx M, et al. EGFR fluorescence in situ hybridisation assay: guidelines for application to non-small-cell lung cancer. J Clin Pathol. 2009; 62(11):970–977. [PubMed: 19861557]
108. Moroni M, Sartore-Bianchi A, Veronese S, Siena S. EGFR FISH in colorectal cancer: what is the current reality? Lancet Oncol. 2008; 9(5):402–403. [PubMed: 18452847]
109. Sartore-Bianchi A, Bencardino K, Di Nicolantonio F, Pozzi F, Funaioli C, Gambi V, et al. Integrated molecular dissection of the epidermal growth factor receptor (EFGR) oncogenic pathway to predict response to EGFR-targeted monoclonal antibodies in metastatic colorectal cancer. Target Oncol. 2010; 5(1):19–28. [PubMed: 20383783]
110. Van Cutsem E. Progress with biological agents in metastatic colorectal cancer leads to many challenges. J Clin Oncol. 2006; 24(21):3325–3327. [PubMed: 16849745]
111. Nagengast WB, de Korte MA, Oude Munnink TH, Timmer-Bosscha H, den Dunnen WF, Hollema H, et al. 89Zr-bevacizumab PET of early antiangiogenic tumor response to treatment with HSP90 inhibitor NVP-AUY922. J Nucl Med. 2010; 51(5):761–767. [PubMed: 20395337]
112. Yang SX, Steinberg SM, Nguyen D, Wu TD, Modrusan Z, Swain SM. Gene expression profile and angiogenic marker correlates with response to neoadjuvant bevacizumab followed by bevacizumab plus chemotherapy in breast cancer. Clin Cancer Res. 2008; 14(18):5893–5899. [PubMed: 18794102]
113. Lassau N, Chebil M, Chami L, Bidault S, Girard E, Roche A. Dynamic contrast-enhanced ultrasonography (DCE-US): a new tool for the early evaluation of antiangiogenic treatment. Target Oncol. 2010; 5(1):53–58. [PubMed: 20379790]
114. Pitts TM, Tan AC, Kulikowski GN, Tentler JJ, Brown AM, Flanigan SA, et al. Development of an integrated genomic classifier for a novel agent in colorectal cancer: approach to individualized therapy in early development. Clin Cancer Res. 2010; 16(12):3193–3204. [PubMed: 20530704]
115. Reidy DL, Vakiani E, Fakih MG, Saif MW, Hecht JR, Goodman-Davis N, et al. Randomized, Phase II Study of the Insulin-LikeGrowth Factor-1 Receptor Inhibitor IMC-A12, With or Without Cetuximab, in Patients With Cetuximab- orPanitumumab-Refractory Metastatic Colorectal Cancer. J Clin Oncol. 2010
116. Stecca B, Ruiz IAA. Context-dependent regulation of the GLI code in cancer by HEDGEHOG and non-HEDGEHOG signals. J Mol Cell Biol. 2010; 2(2):84–95. [PubMed: 20083481]
117. Bagai R, Fan W, Ma PC. ARQ-197, an oral small-molecule inhibitor of c-Met for the treatment of solid tumors. IDrugs. 2010; 13(6):404–414. [PubMed: 20506063]

118. Zhang Y, Li B, Ji ZZ, Zheng PS. Notch1 regulates the growth of human colon cancers. Cancer. 2010
119. https://www.protonet.fccc.edu/cgi-bin/protocol/proto_detail.cgi?protocol_id=ECOG4203&hosp_id=24. https://www.protonet.fccc.edu/cgi-bin/protocol/proto_detail.cgi?protocol_id=ECOG4203&hosp_id=24.
120. Cecchin E, Innocenti F, D'Andrea M, Corona G, De Mattia E, Biason P, et al. Predictive role of the UGT1A1, UGT1A7, and UGT1A9 genetic variants and their haplotypes on the outcome of metastatic colorectal cancer patients treated with fluorouracil, leucovorin, and irinotecan. J Clin Oncol. 2009; 27(15):2457–2465. [PubMed: 19364970]
121. Nelson NJ. Circulating tumor cells: will they be clinically useful? J Natl Cancer Inst. 2010; 102(3):146–148. [PubMed: 20107163]
122. Kim MY, Oskarsson T, Acharyya S, Nguyen DX, Zhang XH, Norton L, et al. Tumor self-seeding by circulating cancer cells. Cell. 2009; 139(7):1315–1326. [PubMed: 20064377]
123. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res. 2004; 10(20):6897–6904. [PubMed: 15501967]
124. http://www.veridex.com/cellsearch/CellSearchHCP.aspx.
125. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature. 2007; 450(7173): 1235–1239. [PubMed: 18097410]
126. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol. 2008; 26(19):3213–3221. [PubMed: 18591556]
127. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, et al. Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. Ann Oncol. 2009; 20(7):1223–1229. [PubMed: 19282466]
128. Yang MJ, Chiu HH, Wang HM, Yen LC, Tsao DA, Hsiao CP, et al. Enhancing detection of circulating tumor cells with activating KRAS oncogene in patients with colorectal cancer by weighted chemiluminescent membrane array method. Ann Surg Oncol. 2010; 17(2):624–633. [PubMed: 19937133]
129. Papavasiliou P, Fisher T, Kuhn J, Nemunaitis J, Lamont J. Circulating tumor cells in patients undergoing surgery for hepatic metastases from colorectal cancer. Proc (Bayl Univ Med Cent). 2010; 23(1):11–14. [PubMed: 20157496]
130. Smirnov DA, Zweitzig DR, Foulk BW, Miller MC, Doyle GV, Pienta KJ, et al. Global gene expression profiling of circulating tumor cells. Cancer Res. 2005; 65(12):4993–4997. [PubMed: 15958538]
131. Gangadhar T, Schilsky RL. Molecular markers to individualize adjuvant therapy for colon cancer. Nat Rev Clin Oncol. 2010; 7(6):318–325. [PubMed: 20440283]
132. Tanaka T, Watanabe T, Kazama Y, Tanaka J, Kanazawa T, Kazama S, et al. Chromosome 18q deletion and Smad4 protein inactivation correlate with liver metastasis: A study matched for T- and N- classification. Br J Cancer. 2006; 95(11):1562–1567. [PubMed: 17088901]
133. Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. Gastroenterology. 2010; 138(6):2059–2072. [PubMed: 20420946]
134. Fumagalli D, Gavin PG, Taniyama Y, Kim SI, Choi HJ, Paik S, et al. A rapid, sensitive, reproducible and cost-effective method for mutation profiling of colon cancer and metastatic lymph nodes. BMC Cancer. 2010; 10:101. [PubMed: 20233444]
135. Smith JJ, Deane NG, Wu F, Merchant NB, Zhang B, Jiang A, et al. Experimentally derived metastasis gene expression profile predicts recurrence and death in patients with colon cancer. Gastroenterology. 2010; 138(3):958–968. [PubMed: 19914252]

136. Rho JH, Qin S, Wang JY, Roehrl MH. Proteomic expression analysis of surgical human colorectal cancer tissues: up-regulation of PSB7, PRDX1, and SRP9 and hypoxic adaptation in cancer. J Proteome Res. 2008; 7(7):2959–2972. [PubMed: 18549262]

137. Lagerstedt KK, Staaf J, Jonsson G, Hansson E, Lonnroth C, Kressner U, et al. Tumor Genome Wide DNA Alterations Assessed by Array CGH in Patients with Poor and Excellent Survival Following Operation for Colorectal Cancer. Cancer Inform. 2007; 3:341–355. [PubMed: 19455253]

138. Ashktorab H, Schaffer AA, Daremipouran M, Smoot DT, Lee E, Brim H. Distinct genetic alterations in colorectal cancer. PLoS One. 2010; 5(1):e8879. [PubMed: 20126641]

139. Yee D. How to train your biomarker. Clin Cancer Res. 2010; 16(12):3091–3093. [PubMed: 20530694]

140. Dancey JE, Dobbin KK, Groshen S, Jessup JM, Hruszkewycz AH, Koehler M, et al. Guidelines for the development and incorporation of biomarker studies in early clinical trials of novel agents. Clin Cancer Res. 2010; 16(6):1745–1755. [PubMed: 20215558]

141. Simon R. Roadmap for developing and validating therapeutically relevant genomic classifiers. J Clin Oncol. 2005; 23(29):7332–7341. [PubMed: 16145063]

142. Mandrekar SJ, Sargent DJ. Clinical trial designs for predictive biomarker validation: theoretical considerations and practical challenges. J Clin Oncol. 2009; 27(24):4027–4034. [PubMed: 19597023]

TABLE 1

Pivotal advanced mCC clinical trials

Author Trial Name[a]	Journal	Regimen Tested	Number of Patients	Line of Therapy	Previous Treatments	Primary Endpoint[b]	Significance
IRINOTECAN TRIALS							
Cunningham et al[24]	Lancet, 1998	Irinotecan vs BSC	189 Vs 90	Second	5-FU	**OS** 9.2 vs 6.5	p<0.0001
Rougier et al[25]	Lancet, 1998	Irinotecan vs 5-FU/LV	133 vs 134	Second	5-FU	**OS** 10.8 vs 8.5	p<0.035
Saltz et al[26]	NEJM, 2000	Irinotecan vs IFL vs 5-FU/LV	226 vs 231 vs 226	First	-	**PFS** 7 (IFL) vs 4.3 (5-FU) vs 4.2 (irinotecan) *OS 14.8 (IFL) vs 12.6 (5-FU) vs 12 (irinotecan)*	p<0.04
Douillard et al[27]	Lancet, 2000	FOLFIRI vs 5-FU/LV	199 vs 188	First	-	**RR** 35% v 22% *OS 17.4 vs 14.1*	p<0.005
OXALIPLATIN TRIALS							
de Gramont et al[29]	JCO, 2000	Oxali+ LV5FU2 vs LV5FU2	210 vs 210	First	-	**PFS** 9 vs 6.2 *OS 16.2 vs 14.7 NS*	p<0.0001
Rothenberg et al[30]	JCO, 2003	Oxali vs FOLFOX4 vs LV5FU2	156 vs 152 vs 151	Second	5FU Irinotecan (IFL)	**RR** 1.3% vs 9.9% vs 0% *OS not reported*	p<0.0001
Goldberg et al[31] NCCTG 9741	JCO, 2004	FOLFOX vs IFL vs IROX	267 vs 264 vs 264	First	-	**TTP** 8.7 vs 6.9 vs 6.5 *OS 19.5 vs 15 vs 17.4*	p<0.0014
FOLFOXIRI TRIALS							
Souglakos et al[32]	Br J Cancer, 2006	FOLFOXIRI vs FOLFIRI	137 vs 146	First	-	**OS** 21.5 vs 19.5	p=0.337 NS
Falcone et al[33]	JCO, 2007	FOLFOXIRI vs FOLFIRI	122 vs 122	First	-	**RR** 66% v 41% *OS 22.6 v 16.7*	p<0.0002
CAPECITABINE							
Van Cutsem et al[38]	JCO, 2001	Cape vs 5-FU/LV	301 vs 301	First	-	**RR non-inferiority** 18.9% vs 15% *OS 13.2 vs 12.1 NS*	NS 95% CI, 14.7%–23.8% vs 95% CI, 11.1%–19.5%
Hoff et al[39]	JCO, 2001	Cape vs 5-FU/LV	302 vs 303	First	-	**RR non-inferiority** 25.8% vs 11.6% *OS 12.5 vs 13.3 NS*	NS 95% CI, 21.0%–31.2% vs 95% CI, 8.2%–15.7%

Author Trial Name[a]	Journal	Regimen Tested	Number of Patients	Line of Therapy	Previous Treatments	Primary Endpoint[b]	Significance
Diaz-Rubio et al[40]	JCO, 2007	CapeOx vs FUOX	174 vs 174	First	-	**TTP non-inferiority** 8.9 vs 9.5 *OS* *18.1 vs 20.8 NS*	p=0.153 NS
Porschen et al[41]	JCO, 2007	CapeOx vs FUFOX	242 vs 234	First	-	**PFS non-inferiority** 7.1 vs 8 *OS* *16.8 vs 18.8 NS*	p=0.117 NS
Cassidy et al[42]	JCO, 2008	CapeOx vs FOLFOX4 CapeOx-bev vs FOLFOX-bev	767 vs 351 vs 349	First	-	**PFS non-inferiority** 8 vs 8.5 *OS* *19.8 vs 19.6 NS*	NS HR = 1.04; 97.5% CI, 0.93 to 1.16
Kohne et al[44] EORTC 40015	Ann Oncol, 2008	CapeIRI vs FOLFIRI	44 vs 41	First	-	**PFS non-inferiority** Early termination	Early Termination
Fuchs et al[45] BICC-C	JCO, 2007	CapeIRI vs FOLFIRI vs mIFL	145 vs 144 vs 141	First	-	**PFS** 5.8 vs 7.6 vs 5.9 *OS* *18.9 vs 23.1 vs 17.6 NS*	FOLFIRI vs mIFL p<0.004
OPTIMAL SEQUENCE TRIALS							
Tournigand et al[7]	JCO, 2004	FOLFIRI then FOLFOX vs Reverse	113 vs 113	First, then Second	-	**Second PFS** 14.2 vs 10.9 *OS* *21.5 vs 20.6 NS*	p=0.64
Koopman et al[48] CAIRO	Lancet, 2007	Cape then irinotecan, then CapeOX vs CapeIRI then CapeOX	336 339	First, Second +/− Third	-	**OS** 16.3 vs 17.4	p<0.3281 NS
Seymour et al[49] MRC-FOCUS	Lancet, 2007	A. 5FU/LV then Irinotecan vs B. 5FU/LV then Combo -FOLFIRI -FOLFOX vs C. Combo -FOLFIRI -FOLFOX	710 (712) 356 356 (713) 356 357	First, then Second		**OS** A. 13.9 vs B. FOLFIRI 15.0 FOLFOX 15.2 vs C. FOLFIRI 16.7 FOLFOX 15.4	p=NS
OPTIMAL DURATION TRIALS							
Tournigand et al[50] OPTIMOX-1	JCO, 2006	FOLFOX4 vs Drop oxali FOLFOX7	310 vs 310	First	-	**OS** 19.3 vs 21.2	p=NS
Chibaudel et al[51] OPTIMOX-2	JCO, 2009	FOLFOX4 vs Chemo-free FOLFOX7	108 vs 108	First	-	**DDC** 13.1 vs 9.2 *OS 23.8 vs 19.5*	p=0.046

Author Trial Name[a]	Journal	Regimen Tested	Number of Patients	Line of Therapy	Previous Treatments	Primary Endpoint[b]	Significance
Labianca et al GISCAD-FOLFIRI	JCO, 2006 abstr. 3505	FOLFIRI vs On off every 2 months	Total = 336	First	-	**OS** 16.9 vs 17.6	p=NS
Tabernero et al MACRO	JCO, 2010 abstr. 3501	CapeOX-bev vs CapeOX-bevX6 then bev alone	239 vs 231	First	-	**PFS non-inferiority** 11 vs 10.3 *OS 25.3 vs 20.7 NS*	p=0.59 HR 1.07 95% CI, 0.84–1.36*
Adams et al MRC-COIN	JCO, 2010 Abstr. 3525	ARM A: OX-Fp vs ARM C: Stop and go	Total= 1640	First	-	**OS** 15.6 vs 14.3	HR 1.084 80% CI, 1.008–1.165
DREAM-OPTIMOX-3		FOLFOX or CapeOX with bev X6 then bev+ erlotinib maintenace		First	-	ongoing	
ANTI-ANGIOGENESIS: BEVACIZUMAB							
Hurwitz et al[52]	NEJM, 2005	IFL-bev vs IFL	402 vs 411	First	-	**OS** 20.3 vs 15.6	p<0.001
Saltz et al[53] N016966	JCO, 2008	FOLFOX or XELOX + /− bev	Total= 1401	First	-	**PFS** 9.4 vs 8 *OS 21.3 vs 19.9 NS*	p<0.0023
Grothey et al[54] BRiTE **Observational**	JCO, 2008	BBP vs No BBP vs No therapy	642 vs 531 vs 253	First, then with Second	-	**OS** 31.8 vs 19.9 vs 12.6	P<0.001
ANTI-EGFR: CETUXIMAB AND PANITUMUMAB							
Cunningham et al[61] BOND	NEJM, 2004	Irinotecan-cetuximab vs cetuximab	218 vs 111	Second and Third	Irinotecan (100%) Oxali (63%)	**RR** 22.9% vs 10.8% *OS 8.6 v 6.9 NS*	P=0.007
Jonker et al[62]	NEJM, 2007	Cetuximab vs BSC	287 vs 285	Third	5-FU irinotecan oxaliplatin	**OS** 6.1 vs 4.6	p<0.001
Van Cutsem et al[63]	JCO, 2007	p-MAb vs BSC	231 vs 232	Third	5-FU irinotecan oxaliplatin	**PFS** 8wk vs 7.3 wk *OS NS – cross over design*	0.0001
Bokemeyer et al[66] OPUS **Phase II**	JCO, 2009	FOLFOX-cetuximab vs FOLFOX	169 vs 168	First	-	**RR** 46% v 36% *OS not reported*	p=0.64
Maughan et al MRC-COIN	JCO, 2010 Abstr. 3502	FOLFOX/ CapeOX +/− cetuximab	Total= 1640 KRAS wt + mt	First	-	**OS** *Not reported for total population (only KRAS wt)*	
Douillard et al PRIME	JCO, 2010 Abstr. 3528	FOLFOX+ p-MAb vs FOLFOX	593 vs 590	First	-	**PFS** *Not reported for total population (only KRAS wt)*	

Author Trial Name[a]	Journal	Regimen Tested	Number of Patients	Line of Therapy	Previous Treatments	Primary Endpoint[b]	Significance
Van Cutsem et al[65] CRYSTAL	NEJM, 2009	FOLFIRI+ cetuximab vs FOLFIRI	599 vs 599	First	-	**PFS** 8.9 vs 8 *OS* *19.9 vs 18.6 NS*	p=0.048
COMBINED BIOLOGICS: BEVACIZUMAB and ANTI-EGFR							
Saltz et al[71] BOND-2 **Phase II**	JCO, 2007	Irinotecan Bev and cetuximab vs Bev + Cetuximab	43 vs 40	Second and Third	Irinotecan (100%) Oxaliplatin (84–93%)	**TTP** 7.3 vs 4.9 *OS* *14.5 vs 11.4 NS*	not reported
Hecht et al[72] PACCE	JCO, 2009 ARM A	FOLFOX and bev + p-MAb vs FOLFOX and bev	413 vs 410	First	-	**PFS** 10 vs 11.4 *OS 19.4 vs 24.5*	p=0.004
Hecht et al[72] PACCE	JCO, 2009 ARM B	FOLFIRI and bev + p-MAb vs FOLFIRI and bev	115 vs 115	First	-	**PFS (2nd endpt)** 10.1 vs 11.9 *OS* *20.7 VS 20.5 NS*	p=NS
Tol et al[68] CAIRO-2	NEJM, 2009	CapeOx-bev + cetuxmab vs CapeOx-bev	378 vs 377	First	-	**PFS** 9.4 vs 10.7 *OS* *20.4 vs 20.3 NS*	p=0.01

[a] Randomized Phase III trials unless otherwise specified.

[b] Units indicated in months unless otherwise indicated.

* MACRO trial failed to meet primary endpoint of noninferiority (CI with upper limit HR<1.32)

BSC, best supportive care; OS, overall survival; PFS, progression free survival; RR, response rate; TTP, time to progression; DDC, duration of disease control; Cape, capecitabine; NS, not significant; Bev, bevacizumab; BBP, bevacizumab beyond progression; p-MAb, panitumumab; wt, wild type; mt, mutant.

TABLE 2

Personalization of mCC care based on molecular markers

PREDICTORS OF RESPONSE TO EGFR INHIBITION - KRAS								
Author Trial Name[a]	**Journal**	**Regimen Tested**	**Number of Patients**	**Number of KRAS wt**	**Number of KRAS mt**	**Primary Endpoint[b]**		**Significance**
						KRAS wt	**KRAS mt**	
De Roock et al BOND **Subgroup analysis**	ASCO 2007 Abstr. 4132	Irinotecan-cetuximab vs cetuximab	N=37	N=20	N=17	**RR**		KRAS mt p<0.01 Responders vs Nonresponders
						45.8%	1.3%	
Van Cutsem et al EVEREST **Subgroup analysis**	ASCO 2008, Abstr. 4001	Irinotecan-cetuximab vs Same + cetuximab escalation to rash	38 39	19 28	19 11	**RR**		Not reported
						4/19 (21.1%) 13/28 (46.4%)	0/19 0/11	
Amado et al[69] **Subgroup analysis**	JCO, 2008	p-MAb vs BSC	208 vs 219	124 vs 119	84 vs 100	**PFS**		KRAS wt HR 0.59 (0.48 to0.72)
						12.3 vs 7.3	7.4 vs 7.3	
Bokemeyer et al[66] OPUS **Phase II Subgroup analysis**	JCO, 2009	FOLFOX-cetuximab vs FOLFOX	113 vs 120	61 vs 73	52 vs 47	**RR**		KRAS wt p= 0.011 KRAS mt p=0.106 NS
						37/61 (61%) 27/73 (37%)	17/61 (31%) 23/47 (49%)	
Tol et al[68] CAIRO-2 **Subgroup analysis**	NEJM, 2009	CapeOx-bev + cetuxmab vs CapeOx-bev	256 vs 264	158 vs 156	98 vs 108	**PFS**		KRAS wt p=0.3 NS KRAS mt p=0.003
						10.5 vs 10.6	8.1 vs 12.5	
Hecht et al[72] PACCE **Subgroup analysis**	JCO, 2009 ARM A	FOLFOX and bev + p-MAb vs FOLFOX and bev	336 vs 328	201 vs 203	135 vs 125	**PFS**		KRAS wt HR 1.36 (1.04 to 1.77) KRAS mt HR 1.25 (0.91 to 1.71)
						9.8 vs 11.5	10.4 vs 11	
	ARM B	FOLFIRI and bev + p-MAb vs FOLFIRI	104 vs 97	57 vs 58	47 vs 39	**PFS**		KRAS wt HR 1.50 (0.82 to 2.76) KRAS mt HR 1.25 (0.65 to 2.21)
Maughan et al MRC-COIN **Subgroup analysis**	JCO, 2010 Abstr. 3502	FOLF OX/ CapeOX +/− cetuximab	N=1316	N=729	N=587	10 **O**	**S** 8.3	KRAS wt HR 1.50 (0.82 to 2.76) KRAS mt HR 1.25 (0.65 to 2.21)
						1 7 vs 17.9 12.5	- vs 11.9	
Douillard et al PRIME **Subgroup analysis**	JCO, 2010 Abstr. 3528	FOLFOX+ p-MAb vs FOLFOX	N=1096	N=656	N=440	**PFS**		KRAS wt p= 0.02 KRAS mt p=0.019
						9.6 vs 8	7.6 vs 5.7	

PREDICTORS OF RESPONSE TO EGFR INHIBITION - KRAS								
Author Trial Name[a]	**Journal**	**Regimen Tested**	**Number of Patients**	**Number of KRAS wt**	**Number of KRAS mt**	**Primary Endpoint[b]**		**Significance**
						KRAS wt	**KRAS mt**	
Van Cutsem et al CRYSTAL **Subgroup analysis**	GI ASCO 2010 Abstr. 281	FOLFIRI+ cetuximab vs FOLFIRI	530 vs 533	316 vs 350	214 vs 183	**PFS**		KRAS wt p= 0.0012 KRAS mt p=0.2661 NS
						9.9 vs 8.4	7.4 vs 7.7	
De Roock et al[70] **Subgroup analysis**	Lancet Oncol, 2010	p-MAb or cetuximab or cetuximab + chemo	N=747	448	299	**OS**		P<0.0001
						50 wks	32 wks	
N0147 **Prospective Adjuvant**	2010 Abstr. 3508, CRA3507	FOLFOX+ cetuximab vs FOLFOX	902 vs 858	902 vs 858	318 vs 340	**3 yr DFS**		p=0.33 NS KRAS mt p=0.02
						73.3 vs 74.1	62.3 vs 70.3	
PETACC-8 **Prospective Adjuvant**	Ongoing	FOLFOX+ cetuximab vs FOLFOX	N=>6000	-	-	**3 yr DFS**		First interim analysis planned 2011
PREDICTORS OF RESPONSE TO EGFR INHIBITION - OTHER								
Van Cutsem et al EVEREST **Subgroup analysis**	GI ASCO 2007, Abstr. 237 **RASH**	Irinotecan-cetuximab vs Same + cetuximab escalation to rash	45 44	-	-	**RR**		CI 5%–27% vs 17%–48%
						13%	30%	
Van Cutsem et al CRYSTAL **Subgroup analysis**	ASCO, 2010 Abstr. 3570 KRAS wt BRAF analysis	FOLFIRI+ cetuximab vs FOLFIRI	303 vs 322	BRAF wt 277 vs 289	BRAF mt 26 vs 33	**PFS**		BRAF wt p=0.0016 BRAF mt p=0.8656 NS
						10.9 vs 8.8	8 vs 5.6	
De Roock et al[70] **Subgroup analysis**	Lancet Oncol, 2010 KRAS wt BRAF analysis	p-MAb cetuximab cetuximab + chemo	N= 761	BRAF wt 725	BRAF mt 36	**OS**		p<0.0001
						54 wks	26 wks	
De Roock et al[70] **Subgroup analysis**	Lancet Oncol, 2010 PIK3A Exon 20 analysis	p-MAb cetuximab cetuximab + chemo cetuximab + chemo	N= 743	NRAS wt 635	NRAS mt 108 (22 exon 20 74 exon 9 12 other)	**OS**		p<0.0057
						51 wks	34 wks	
De Roock et al[70] **Subgroup analysis**	Lancet Oncol, 2010 KRAS wt NRAS Analysis	p-MAb cetuximab cetuximab + chemo	N= 644	NRAS wt 627	NRAS mt 17	**RR**		p<0.013
						38.1%	7.7%	

[a] Randomized Phase III trials unless otherwise specified.

[b] Units indicated in months unless otherwise indicated.

wt, wild type; mt, mutant; p-MAb, panitumumb; BSC, best supportive care; NS, not significant; bev, bevacizumab; RR, response rate; PFS, progression free survival; OS, overall survival; DFS, disease free survival.

TABLE 3

Pivotal adjuvant CC clinical trials

Author Trial Name[a]	Journal	Regimen Tested	Number of Patients	Stage	Primary Endpoint[b]	Significance
OXALIPLATIN TRIALS						
Kuebler et al[75] NSABP- C-07	JCO, 2007	FLOX vs 5FULV	1207 vs 1200	II – 28.9% III-70.9% vs II-28.8% III-70.5%	**4 yr DFS** 73.2% vs 67% *4 yr OS 78.5% vs 76%*	p<0.0034
Andre et al[74,76] MOSAIC	JCO, 2009	FOLFOX4 vs LV5FU2	1108 vs 1111	II - 40.2% III-59.8% vs II-39.9% III-60.1%	**6 yr DFS** 73.3% vs 67.4% *6 yr OS 78.5% vs 76%*	p<0.003
Haller et al XELOXA NO16968	GI ASCO, 2010 Abstr. 284	CapeOx vs 5FULV	N= 1886	III only	**5 yr DFS** 66.1% vs 59.8% *OS Not reported*	p<0.0045
RINOTECAN TRIALS						
Saltz et al[78] CALGB 89803	JCO, 2007	IFL vs 5FULV	635 vs 629	III only	**5 yr OS** 68% vs 71%	p=0.85 NS
Van Cutsem et al[80] PETACC-3	JCO, 2009	FOLFIRI vs LV5FU2	1485 vs 1497	II – 29.3% III-70.3% vs II-29.7% III-70%	**5 yr DFS** 56.7% vs 54.3% *5 yr OS 73.6% vs 71.3% NS*	p=0.106 NS
Ychou et al[79] ACCORD-2	Ann Oncol, 2009	FOLFIRI vs LV5FU2	161 vs 160	Resected IV	**DFS** 24.7 vs 21.6 *3 yr OS 73% vs 72% NS*	p=0.44 NS
CAPECITABINE						
Twelves et al[81] X-ACT	NEJM, 2005	Cape vs 5FULV	1004 vs 983	III only	**3 yr DFS non-inferiority** 64.2% vs 60.6% *3 yr OS 81.3% vs 77.6% NS*	Superiority p=0.12 NS Equivalence p<001
Haller et al XELOXA NO16968	GI ASCO, 2010 Abstr. 284	CapeOx vs 5FULV	N= 1886	III only	**5 yr DFS** 66.1% vs 59.8% *OS Not reported*	p<0.0045
ANTI-ANGIOGENESIS: BEVACIZUMAB						
Wolmark et al NSABP C-08	ASCO, 2009 Abstr. LBA4	FOLFOX6 + bev vs FOLFOX6	1334 vs 1338	II – 24.9% III –75.1%	**3 yr DFS** 77.4% vs 75.5%	p=0.15 NS
AVANT	Ongoing	FOLFOX4 + bev or XELOX + bev vs FOLFOX4	1150 vs 1150	High risk II + III	**DFS**	
ANTI-EGFR: CETUXIMAB						

Author Trial Name[a]	Journal	Regimen Tested	Number of Patients	Stage	Primary Endpoint[b]	Significance
Alberts et al N0147	ASCO, 2010 Abstr. CRA-3507	FOLFOX6 + cetuximab vs FOLFOX6	902 vs 858	III only KRAS wt only	**3 yr DFS** 74.1% vs 73.3% *3 yr OS 87.3% vs 82.1% NS*	p=0.33 NS
PETACC-8	Ongoing	FOLFOX+ cetuximab vs FOLFOX	N= >6000	III only KRAS wt only	**3 yr DFS**	First interim analysis planned 2011

[a] Randomized Phase III trials unless otherwise specified.

[b] Units indicated in months unless otherwise indicated.

DFS, disease free survival; OS, overall survival; Cape, capecitabine; NS, not significant; Bev, bevacizumab; wt, wild type.