Explaining the unexplainable: discrepancies in results from the CALGB/SWOG 80405 and FIRE-3 studies

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We propose a working hypothesis that integrates data from the CALGB/SWOG 80405 and FIRE-3 studies to explain apparent discrepancies in their results. Both trials assessed the combination of either cetuximab or bevacizumab with a different chemotherapy backbone: irinotecan in all patients in the FIRE-3 study, or oxaliplatin in 75% of the patients in the CALGB/SWOG 80405 study. The hypothesis is divided into three parts. Firstly, in addition to the biology or microenvironment of the tumour and the selection of the biologically targeted agents common to both trials, chemotherapy itself is an important variable that determines treatment efficacy because of a complex interplay between the biological therapy, the chemotherapy, and the microenvironment. Secondly, the tumour microenvironment, as defined by the Consensus Molecular Subtypes (CMS) classification, determines the interaction of chemotherapeutic agents with biologically targeted agents such as bevacizumab and cetuximab. Whereas irinotecan synergises with cetuximab across all CMS subtypes, oxaliplatin might have variable effects, synergising with cetuximab in fibroblast-poor microenvironments, such as CMS2 and CMS3, but activating fibroblast-rich microenvironments, such as CMS1 and CMS4, to release cytokines that might antagonise some of the cetuximab effects. Thirdly, the previous assumptions integrate into a final concept, which is that overall survival is determined not only by the biological therapy or the first-line treatment, but specifically by the sequence of first-line and second-line regimens, and the degree of synergism between them. In a clinical setting, the optimal first-line combination of biological therapy and chemotherapy predetermines the crossover to a specific second-line treatment, which affects the overall survival of a patient with a specific tumour subtype. Our working hypothesis suggests that the CALGB/SWOG 80405 and FIRE-3 studies are complementary rather than discrepant, and it provides an explanation for their opposing interpretations. In conclusion, proper interpretation of the CALGB/SWOG 80405 and FIRE-3 results requires an in-depth examination of the complex interplay, not only between the targeted biological agents and chemotherapeutic drugs, but also between therapies and the tumour biology and microenvironment, for each line of treatment.

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Introduction

In the past decade, there has been considerable debate as to whether bevacizumab or cetuximab should be the preferred first-line biological therapy for treatment of patients with *KRAS* wild-type metastatic colorectal cancer. Two large randomised studies, the CALGB/SWOG 80405 study[1] (referred to throughout as the CALGB study) and the FIRE-3 study,[2] were done to clarify this question. However, the studies reached opposing conclusions. The CALGB study found that the median overall survival was identical for the two biological therapies,[3] whereas the FIRE-3 study found a significant overall survival benefit for patients who were given cetuximab compared with bevacizumab as first-line therapy.[4] In subgroup analyses of the overall survival data according to tumour sidedness, both studies found that cetuximab was more effective than bevacizumab for left-sided tumours, whereas bevacizumab was preferable for right-sided tumours.

In 2015, Guinney and colleagues[5] published the Consensus Molecular Subtypes (CMS) classification of colorectal cancer. This classification subdivides colorectal cancer into four subgroups (CMS1–4) according to the gene expression of the tumour cells, and that of cells in the tumour microenvironment.[5] CMS1 tumours are characterised by high microsatellite instability, hypermutation, and an immune hot microenvironment; CMS2 tumours display an epithelial phenotype, and are chromosomally unstable with WNT and MYC signalling activation; CMS3 tumours display an epithelial phenotype but have evident metabolic dysregulation; and CMS4 tumours display a mesenchymal phenotype with transforming growth factor-β (TGF-β) activation, stromal invasion, and angiogenesis. With use of the CMS classification, the two studies did unplanned, subgroup analyses, which led to disparate and, at first glance, inconclusive results.[6,7] The findings suggested that with the same first-line biological therapy, a different median overall survival could be obtained for tumours with a similar biology and microenvironment. We propose that a third variable, which differed between the studies, could be responsible for the discrepancies in the results. A major difference between the studies was the choice of backbone chemotherapy that was used for first-line treatment; oxaliplatin was given to 75% of the CALGB study patients, whereas irinotecan was given to all patients in the FIRE-3 study.

With reference to the chemotherapy backbone, and with insights from preclinical and clinical investigations, we have examined the interplay between biological therapies, chemotherapy, tumour cells, and the micro-environment to form a working hypothesis that could explain the discrepancies between the CALGB and FIRE-3 studies.

CMS classification and biological therapy interactions

The CALGB study showed that left-sided colorectal cancer responds better to cetuximab-based therapy

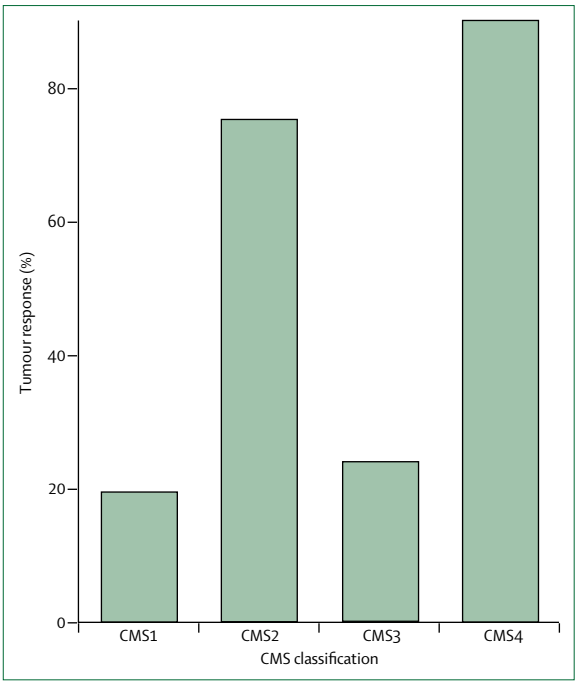

Figure 1: **Tumour response to cetuximab by CMS classification**
Data are reproduced from Fontana and colleagues.[15] CMS=Consensus Molecular Subtypes.

compared with bevacizumab-based therapy (overall survival 39·3 months [95% CI 32·9–42·9] *vs* 32·6 months [28·3–36·2], p<0·04), whereas right-sided tumours might respond better to bevacizumab.[8] These conclusions were also supported by the results of the FIRE-3 study.[9]

A large body of evidence is now available showing that right-sided and left-sided tumours have different biology and tumour microenvironments. The reduced effect of cetuximab on right-sided *KRAS* wild-type tumours[10] is explained by frequent *BRAF* and *PTEN* mutations[5,11,12] and CpG island methylator phenotype-high tumour status,[5] with reduced expression of *EGFR* or its ligands, epiregulin, and amphiregulin.[13,14] Differences in the responses of left-sided and right-sided tumours to cetuximab were also reported by Fontana and colleagues,[15] who determined the response to cetuximab according to the CMS distribution of the tumours (figure 1). Although all tumours were *KRAS* wild-type, only 20–23% responded to cetuximab in the CMS1 and CMS3 subtypes, compared with 76–88% in the CMS2 and CMS4 subtypes (predominant in left-sided tumours), supporting the notion that left-sided tumours respond better to cetuximab than right-sided tumours.[8,9]

The CALGB and FIRE-3 studies found that left-sided tumours are less responsive to bevacizumab than cetuximab.[5,6] A retrospective evaluation of the phase 3 AGITG MAX trial,[16] which evaluated archival primary tumours of 237 patients with metastatic colorectal cancer, found that patients with CMS4 tumours achieved a lower proportion of responses with bevacizumab, which partially supports the finding of the CALGB and FIRE-3 studies. Even with excessive VEGF pathway activation[7] and angiogenesis,[17] the response to bevacizumab in the CMS4 subgroup was surprisingly poor. This poor response in tumours with a high angiogenic phenotype might be explained by the predominance of cancer-associated fibroblasts and tumour-associated macrophages in the CMS4 microenvironment,[5,17] which release multiple proangiogenic factors that promote VEGF-independent angiogenesis,[18] making the tumours refrctory to anti-VEGF treatment.[19–21] By contrast, the *BRAF* and angiogenesis-related pathways explain the relative clinical benefit of anti-VEGF therapy on the overall survival and progression-free survival of right-sided tumours.[22]

CMS classification and chemotherapy interactions

Because patients categorised in different CMS subgroups have different responses to biological therapies,[15,16] it is important to consider whether patients with different CMS subtypes also respond differently to the chemotherapeutic agents oxaliplatin and irinotecan. Despite being considered equally effective in unselected patients with metastatic colorectal cancer,[23] differential responses to oxaliplatin[24] and irinotecan[25] were reported before the publication of the CMS classification.[5] Patients with different colorectal cancer subtypes seem to respond differently to oxaliplatin, with the best response observed in the equivalent CMS2 subgroup (hazard ratio 0·61, 95% CI 0·43–0·87, p=0·006), followed by the equivalent CMS1 (0·76, 0·46–1·29), CMS4 (0·87, 0·64–1·19), and CMS3 (1·17, 1·54–2·53) subgroups (figure 2).[24] Similarly, patients with the stem-like subtype (equivalent to CMS4)[25] benefited universally from irinotecan therapy, followed by a lower benefit for patients with the equivalent CMS1 subtype and a further reduced benefit for patients with the CMS2 and CMS3 subtypes (figure 2).[25]

Chemotherapy and biological therapy interactions

Preclinical reports[26–32] and clinical studies[33–36] strongly suggest a synergistic effect of irinotecan and cetuximab. Irinotecan upregulates EGFR,[26] facilitating the binding of cetuximab to tumour cells and promoting tumour cell apoptosis.[27] The improved tumour binding mediates the antibody-dependent cell-mediated cytotoxicity (ADCC) effect of the biological therapy,[26] which initiates interferon-γ release by the stimulated natural killer cells, resulting in activation of dendritic cells, macrophages, and T cells.[26,28] Irinotecan acts synergistically with cetuximab to promote phagocytosis by dendritic cells, enabling better antigen presentation to cytotoxic T cells and upregulating major histocompatibility complex expression of the tumour, thus facilitating cytotoxic T-cell binding and apoptosis of the cancer cells.[26] In addition,

cetuximab inhibits the tumour's multidrug resistance mechanism in which SN-38, the active metabolite of irinotecan, is expelled from cells, which augments intracellular accumulation of SN-38 and results in improved antitumour effects.[29]

Preclinical studies have found several synergistic interactions between oxaliplatin and cetuximab. Oxaliplatin exerts antineoplastic effects by two major mechanisms: oxaliplatin-DNA adduct formation and DNA oxidative damage.[37] Because EGFR activation neutralises these oxaliplatin effects by upregulating nucleotide excision repair proteins and base excision repair proteins,[38] EGFR inhibition augments the anticancer effects of oxaliplatin. Therefore, combination of oxaliplatin with cetuximab would be expected to have a synergistic effect.[38] Cetuximab was also reported to downregulate *ERCC1*, which could further augment oxaliplatin activity.[38] The immunogenic cell death attributed to oxaliplatin[39] facilitates presentation of tumour antigens to T cells by the cetuximab-activated dendritic cells, which is another potential synergistic effect of cetuximab and oxaliplatin.

Chemotherapy and biological therapy interactions in different tumour microenvironments

In theory, the synergistic effect of cetuximab with irinotecan and oxaliplatin would be expected to be proportional to the activity of cetuximab in patients across CMS classifications (figure 1). However, although a clinical benefit of the irinotecan-cetuximab combination has been shown repeatedly,[33–36] no overall survival benefit was found with the oxaliplatin-cetuximab combination in the randomised OPUS,[40] COIN,[41] and Nordic VII[42] trials, which raises doubts regarding the effectiveness of the oxaliplatin-cetuximab combination. Huang and colleagues[43] concluded that cetuximab might differentially interact with irinotecan compared with oxaliplatin, and Grothey and Lenz[44] pointed out that "oxaliplatin and irinotecan differ greatly" in their activity as single agents. Altogether, this evidence suggests that the discrepant results of the CALGB and FIRE-3 studies could be due to the varying interaction of biological agents with either oxaliplatin or irinotecan.

The tumour microenvironment might be partly responsible for tumour resistance to chemotherapy.[45,46] CMS1 and CMS4 tumours are characterised by a fibroblast-rich microenvironment.[5] Oxaliplatin was reported to exert off-target effects on cancer-associated fibroblasts by causing them to release interleukin 17A, conferring a cell survival advantage and promoting proliferation of cancer stem cells.[47] These effects of oxaliplatin might antagonise the growth suppression and apoptosis of cancer stem cells that is induced by cetuximab.[48] Activated by oxaliplatin, cancer-associated fibroblasts have also been described to secrete TGF-β,[47] which upregulates protein kinase B,[49] antagonising the effects of cetuximab (and panitumumab) and mediating tumour resistance to biological therapies[50] by providing an intrinsic EGFR-independent survival pathway to cancer cells.[49] Furthermore, TGF-β exerts a prolonged inhibitory effect on the cetuximab-mediated ADCC by suppressing the molecular effectors of immune-cell mediated cytotoxicity that are upregulated by cetuximab.[50] Finally, all of the anti-tumour immune responses that are promoted by cetuximab, including the activation of natural killer cells,[51,52] dendritic cells,[51] macrophages,[53] and cytotoxic T cells,[53] are inhibited by TGF-β.[54–58]

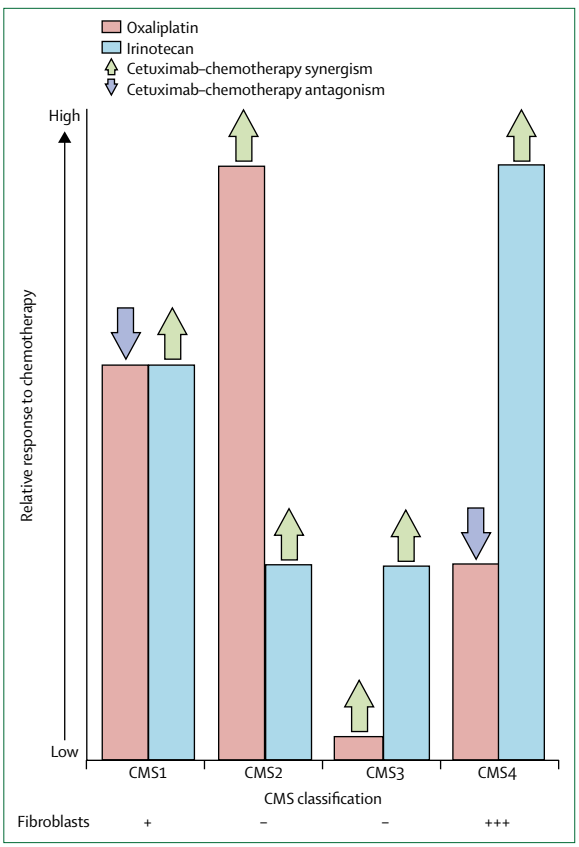

Figure 2: **Relative response by CMS tumour subtype and chemotherapy type**
The added benefit of cetuximab to oxaliplatin and irinotecan is expected to be proportional to cetuximab activity in each CMS group (green arrows). The fibroblast-rich microenvironments of the CMS1 and CMS4 subtypes are expected to have an antagonistic effect on the cetuximab-oxaliplatin synergism (purple arrows). Our hypothesis is that cetuximab activity is synergistic with irinotecan in all CMS subgroups, and is synergistic with oxaliplatin in the CMS2 and CMS3 subgroups (ie, in fibroblast-poor microenvironments). In the CMS1 and CMS4 groups, oxaliplatin activates the tumour microenvironments to release cytokines, which interfere with the anti-tumour effects of cetuximab. The relative responses to oxaliplatin in the different CMS subgroups are derived from Song and colleagues[24] and the relative responses to irinotecan are derived from Sadanandam and colleagues.[25] The amount of fibroblasts is indicated as absent (-), low (+), or high (+++). CMS=Consensus Molecular Subtypes.

Hypothesis to explain discrepancies between the FIRE-3 and CALGB studies

The previously discussed antagonistic effects of the microenvironment on the combined actions of cetuximab and oxaliplatin lead to our working hypothesis: although

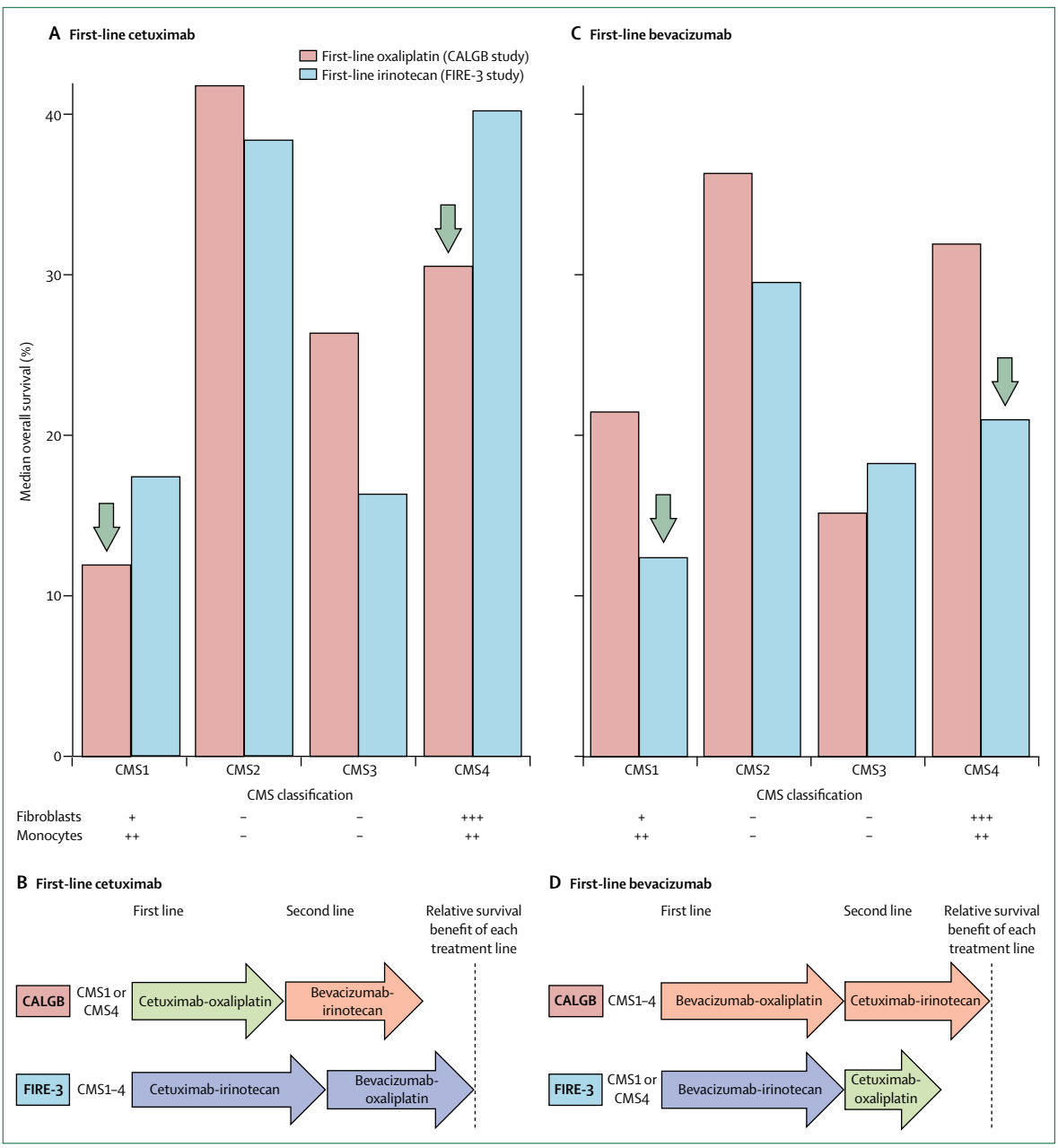

Figure 3: **Median overall survival by CMS classification and first-line biological therapy**
(A) Overall survival with first-line cetuximab. FIRE-3 used first-line cetuximab-irinotecan (blue columns). CALGB used first-line cetuximab-oxaliplatin (red columns). In the fibroblast-rich microenvironments (CMS1 and CMS4), cetuximab-oxaliplatin had a reduced survival benefit compared with cetuximab-irinotecan (green arrows). (B) Explanation of the differences in first-line cetuximab effects. The CALGB study used first-line cetuximab-oxaliplatin, which is antagonised by the CMS1 and CMS4 microenvironments (green arrow), and a synergistic second line, bevacizumab-irinotecan (orange arrow). The FIRE-3 study used two synergistic lines: first-line cetuximab-irinotecan and second-line bevacizumab-oxaliplatin (blue arrows), resulting in longer overall survival than in the CALGB study. (C) Overall survival with first-line bevacizumab. FIRE-3 used first-line bevacizumab-irinotecan (blue columns). CALGB used first-line bevacizumab-oxaliplatin (red columns). In the fibroblast-rich microenvironments (CMS1 and CMS4), first-line bevacizumab-irinotecan had a reduced survival benefit compared with first-line bevacizumab-oxaliplatin because of the antagonistic effect of second-line cetuximab-oxaliplatin (green arrows). (D) Explanation of the differences in first-line bevacizumab effects. The CALGB study used two synergistic lines (orange arrows). The FIRE-3 study used a first-line synergistic combination (blue arrow), and a second-line combination that was antagonised by the CMS1 and CMS4 microenvironments (green arrow), resulting in shorter overall survival than in the CALGB study. Data are reproduced from the FIRE-3 study[2] and the CALGB study.[3] The amounts of fibroblasts and monocytes are indicated as absent (-), low (+), moderate (++), or high (+++). CMS=Consensus Molecular Subtypes.

irinotecan probably acts synergistically when added to cetuximab in all CMS-defined microenvironments, the activity of cetuximab might be antagonised by oxaliplatin, depending on the tumour microenvironment. In a fibroblast-poor microenvironment, which is characteristic of CMS2 and CMS3 tumours, the oxaliplatin-cetuximab

combination might be synergistic. By contrast, in fibroblast-rich microenvironments, such as CMS1 or CMS4, the off-target effects of oxaliplatin induce cytokines, which might antagonise not only the antitumour effects of cetuximab, but also the cetuximab-oxaliplatin cooperation (figure 2), presumably resulting in a reduced clinical benefit of the combination for CMS1 and CMS4 tumour subtypes. It is assumed that bevacizumab acts synergistically with both irinotecan and oxaliplatin in all CMS-defined microenvironments, relative to its activity in each subtype.

Discrepancies in overall survival

Our hypothesis was first tested by referring to the differences in overall survival observed in patients classified in the same CMS group who received the same biological therapy, as reported by the two studies[2,3] (figure 3). We examined the discrepancy in overall survival found by the FIRE-3 and CALGB studies for first-line cetuximab in the CMS1 and CMS4 tumour subtypes (figure 3A). In the CALGB study, according to our working hypothesis, first-line cetuximab coupled with oxaliplatin probably had an antagonistic effect in patients with CMS1 and CMS4 microenvironments, which would explain the reduced overall survival of 11·7 months (95% CI 10·9–18·0) in the CMS1 subgroup and 30·8 months (24·4–43·5) in the CMS4 subgroup (figure 3A). By contrast, in the FIRE-3 study, the synergistic combination of cetuximab with irinotecan would provide a greater overall survival benefit, which would explain the improved overall survival of 17·9 months (95% CI 7·1–28·7) in the CMS1 subgroup and 40·1 months (20·3–59·9) in the CMS4 subgroup (figure 3A). Thus, in patients with the same tumour microenvironments who received the same first-line biological therapy (cetuximab), a different overall survival was observed depending on the first-line chemotherapy backbone.

In a clinical setting, the overall survival associated with first-line cetuximab or first-line bevacizumab is actually representative of cumulative overall survival obtained after first-line treatment, second-line treatment, and other treatment lines for each group (figure 3B). Our hypothesis can also explain the discrepant results of the studies with regards to first-line bevacizumab in patients with tumours classified as CMS1 and CMS4.

Compared with the FIRE-3 study, the CALGB study found an improved overall survival with first-line bevacizumab in the CMS1 and CMS4 subgroups (figure 3C). The CALGB study used a sequence of two synergistic combinations for patients with CMS1 and CMS4-defined tumour microenvironments: first-line bevacizumab-oxaliplatin and second-line cetuximab-irinotecan (administered to some of the patients; figure 3D), which resulted in a cumulative overall survival of 22·5 months (95% CI 15·9–32·6) for the CMS1 subgroup and 32·7 months (26·3–37·5) for the CMS4 subgroup (figure 3C). By contrast, in the FIRE-3 study, after the overall survival benefit obtained following the synergistic first-line bevacizumab-irinotecan, 41% of the patients crossed over to second-line cetuximab-oxaliplatin,[2] which probably had an antagonistic effect in patients classified as CMS1 and CMS4 (figure 3D). This effect would reduce the contribution of the second-line cetuximab-oxaliplatin to the cumulative overall survival of patients who received first-line bevacizumab in the FIRE-3 study, and could explain the worse median overall survival of 13·1 months (95% CI 8·5–17·6) in the CMS1 subgroup and 21·1 months (14·8–27·3) in the CMS4 subgroup, compared with 22·5 months (15·9–32·6) and 32·7 months (26·3–37·5), respectively, in the CALGB study (figure 3C).

A second mechanism explaining the worse overall survival in patients who received first-line bevacizumab might be related to the antagonistic effect between bevacizumab and cetuximab (or panitumumab) when administered concurrently, in combination with chemotherapy.[59,60] Because of its long half-life, bevacizumab might be detectable in serum for more than 3 months after its last first-line administration,[61] and might overlap with the second-line cetuximab for several months. Sequential administration of cetuximab after bevacizumab was shown to reduce blood vessel permeability, interfering with diffusion and tumour cell binding of cetuximab.[62] This might explain the possibility that the effect of both EGFR inhibitors, panitumumab and cetuximab, is reduced if they are administered after bevacizumab,[63–67] with a substantially better residual activity for panitumumab compared with cetuximab.[68] Because some patients classified as having CMS2 tumours also have fibroblast and TGF-β signatures,[69,70] the two inhibitory mechanisms (ie, the antagonism of oxaliplatin-treated fibroblast-rich microenvironments, and the overlap of first-line bevacizumab with second-line cetuximab), might have cooperated to substantially reduce the contribution of the second-line cetuximab-oxaliplatin to the cumulative overall survival in patients treated with first-line bevacizumab in the FIRE-3 study in three of the four CMS tumour subtypes (CMS1, CMS2, and CMS4; figure 3C).

Analysis of the median overall survival obtained by the two studies according to CMS classification (figure 4) unexpectedly answers a pertinent clinical question: what is the best combination of first-line chemotherapy and biological therapy for each tumour subtype? The CALGB study reported the overall survival of patients who were given predominantly first-line oxaliplatin and biological therapy, whereas FIRE-3 reported the overall survival of patients who were exclusively given biological therapy with an irinotecan backbone. Because each study contributed half of the information regarding the interaction of different chemotherapy agents with the two biological therapies (figure 4), we would argue that the studies are complementary, rather than discrepant.

Examination of the 16 possible combinations of first-line chemotherapy, first-line biological therapy, and

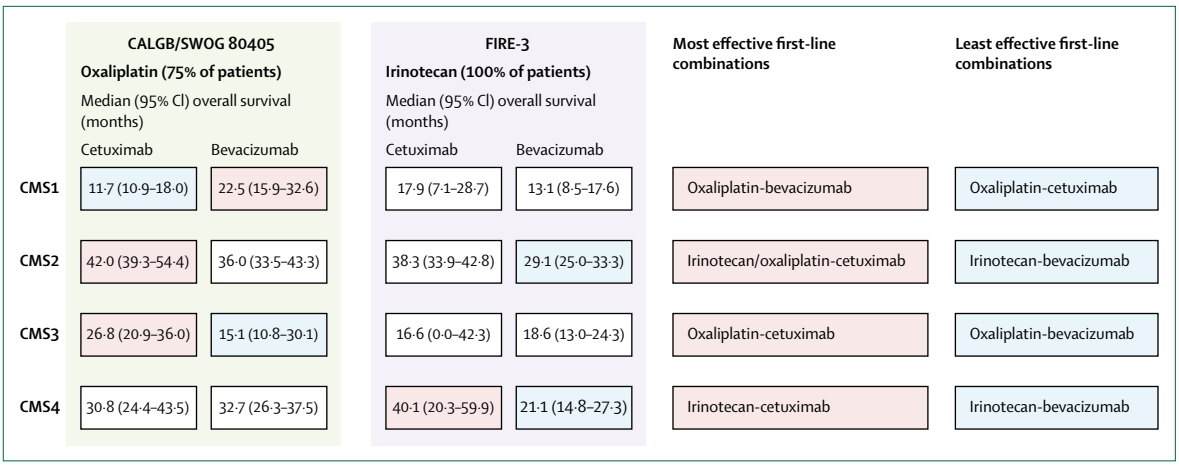

Figure 4: **Overall survival by first-line biological therapy and chemotherapy combinations and CMS classification**
Most effective combinations for each CMS subtype are highlighted in red. Least effective combinations for each CMS subtype are highlighted in blue. 80% of the left-sided tumours (CMS2 and CMS4) could benefit from irinotecan with cetuximab. Cetuximab is part of the most effective combination for 86% of tumours (CMS2, CMS3, and CMS4). Data are reproduced from the FIRE-3 study[2] and the CALGB study.[3] CMS=Consensus Molecular Subtypes.

tumour subtype indicates that for each tumour subtype, there is a different optimal first-line combination (figure 4, highlighted in red). The other combinations are less effective, and the least effective combinations at increasing overall survival for each subtype are highlighted in blue (figure 4). Because the FIRE-3 and CALGB study discussions addressed only the biological therapy being used, and not the complex interactions between biological therapy, chemotherapy, and tumour subtype, the authors reached discrepant conclusions regarding the choice of the optimal first-line biological therapy. The discrepancy seems to be closely related to the overall survival outcome of patients who received first-line bevacizumab, who had considerably worse overall survival in the FIRE-3 compared with the CALGB (25·0 months *vs* 29·0 months). By contrast, the overall survival of the first-line cetuximab groups was almost the same in the two studies (28·7 months *vs* 29·9 months).[1–3] The worse overall survival obtained in the FIRE-3 first-line bevacizumab group can also be explained by the fact that the FIRE-3 study used the least effective first-line combinations for CMS2 and CMS4 patients, representing about 75% of the cohort (figure 4). By contrast, in the CALGB study, the overall survival of the first-line bevacizumab group was increased, because only a minor subgroup (CMS3), representing less than 15% of the patients, was given the least effective first-line combination (figure 4).

Discrepancy in the efficacy of bevacizumab versus cetuximab

We also examined whether our working hypothesis could explain the opposing conclusions of the FIRE-3 and CALGB studies regarding the optimal first-line biological therapy (figure 5). The CALGB study, in which the main first-line chemotherapy was oxaliplatin, found that bevacizumab and cetuximab were equally effective. In the first-line bevacizumab arm of the CALGB study, a sequence of two synergistic regimens was used, which both contributed to an optimal cumulative survival (figure 5B). By contrast, the first-line cetuximab group, which was also coupled with oxaliplatin, was probably antagonised in the CMS1 and CMS4 microenvironments (according to the current hypothesis). Despite a synergistic second line (figure 5B), the cumulative overall survival benefit for those patients classified as CMS1 or CMS4 who received first-line cetuximab was reduced compared with that of patients who received first-line bevacizumab (figure 5A). As a result, the overall survival curve of the first-line line cetuximab group in the CALGB study (CMS1–4) was lowered, closing the gap between the survival curves of the first-line cetuximab and first-line bevacizumab groups (figure 5B). The gap between the overall survival curves was not statistically significant, which led to the conclusion of the CALGB study that the two biological therapies have an equal effect on overall survival.[3]

A similar argument can explain the opposing conclusion of the FIRE-3 study—namely first-line cetuximab is superior to first-line bevacizumab. In the first-line cetuximab arm of the FIRE-3 study, patients were given the first-line combination of cetuximab-irinotecan (figure 5C), followed by second-line bevacizumab-oxaliplatin (given to 95 [47%] of 204 patients).[2] These two synergistic lines provided an optimal first-line and second-line survival gain for the first-line cetuximab group (figure 5D). By contrast, the first-line bevacizumab group in the FIRE-3 study was given the synergistic first-line bevacizumab-irinotecan combination, but at progression, 79 (41%) of 191 patients were crossed over to the default second-line cetuximab-oxaliplatin combination.[2] Cetuximab-oxaliplatin was probably antagonised in patients with CMS1 and CMS4 tumours (figure 5D), thus reducing the overall survival benefit

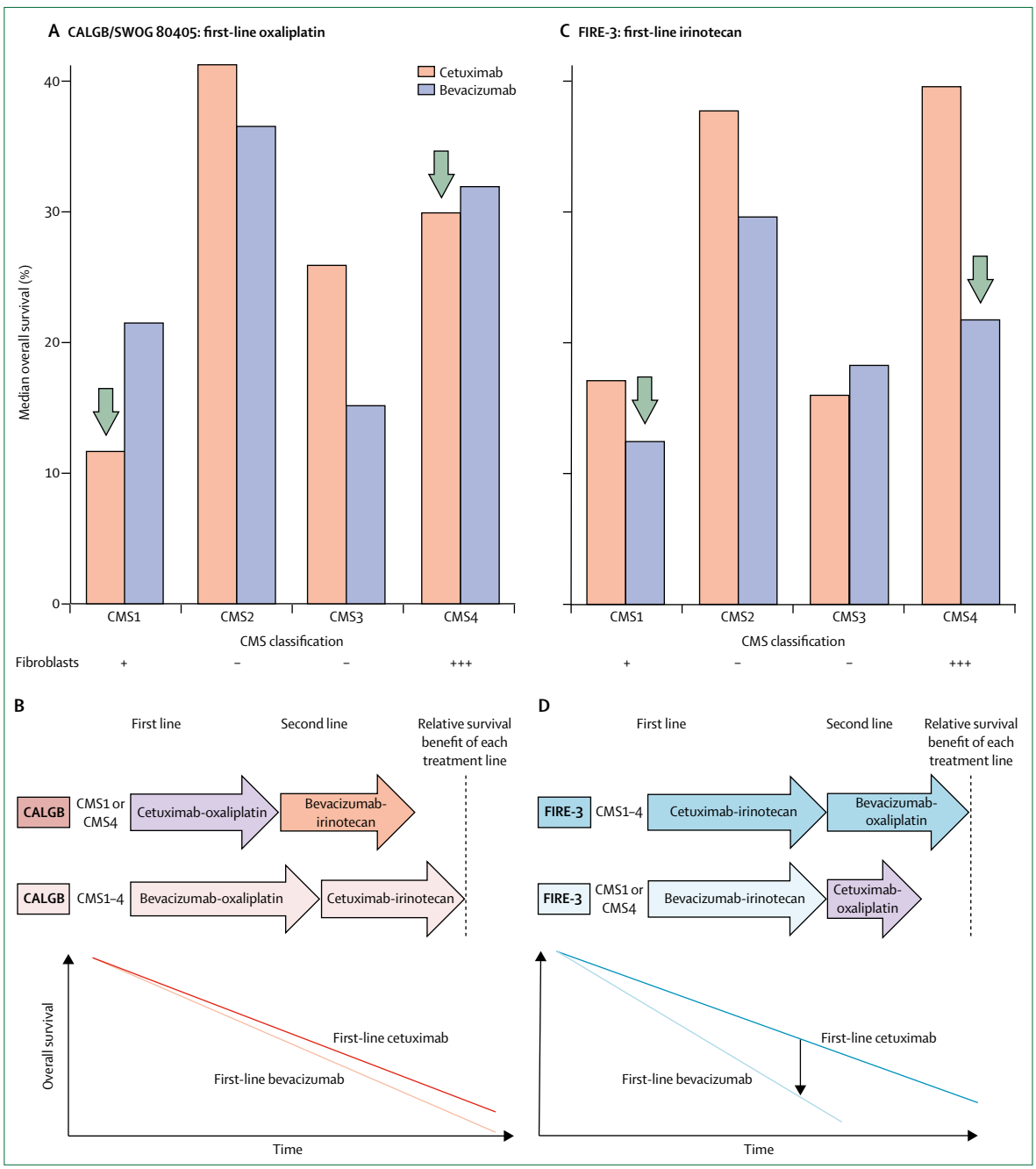

Figure 5: **Median overall survival by CMS classification and backbone chemotherapy**
(A) Overall survival with first-line oxaliplatin, which was used in the CALGB study. First-line cetuximab-oxaliplatin has a reduced survival benefit compared with the synergistic first-line bevacizumab-oxaliplatin in the CMS1 and CMS4 subtypes, caused by antagonism in the fibroblast-rich microenvironments (green arrows). (B) Explanation of the CALGB findings. The reduced survival benefit of first-line cetuximab-oxaliplatin is explained by the sequence of an antagonistic first-line treatment (purple arrow) and a synergistic second-line treatment (orange arrow) in CMS1 and CMS4. The first-line bevacizumab-oxaliplatin arm was given a sequence of two synergistic lines (pink arrows). The overall survival of all subtypes (CMS1–4) given first-line cetuximab is reduced by the shortened survival of CMS1 and CMS4 patients, resulting in a lowered survival curve for first-line cetuximab (red line) that is close to the survival curve for first-line bevacizumab (pink line). This explains the CALGB conclusion: cetuximab and bevacizumab have the same clinical value (when coupled with first-line oxaliplatin). (C) Overall survival with first-line irinotecan, which was used in the FIRE-3 study. First-line cetuximab-irinotecan resulted in longer median overall survival than first-line bevacizumab-irinotecan in the CMS1 and CMS4 subtypes (green arrows). (D) Explanation of the FIRE-3 findings. The FIRE-3 first-line cetuximab treatment arm sequenced two synergistic lines (blue arrows). The first-line bevacizumab arm sequenced a synergistic first line (light blue arrow) and an antagonistic (in CMS1 and CMS4) second line (purple arrow). As a result, the survival of all first-line bevacizumab patients was reduced by the lower survival gain contributed by the CMS1 and CMS4 patients, resulting in a lowered survival curve for the first-line bevacizumab patients (light blue line) and a larger gap from the first-line cetuximab survival curve (blue line). This explains the FIRE-3 conclusion: cetuximab is more effective than bevacizumab (when coupled with first-line irinotecan). Data are reproduced from the FIRE-3 study[2] and the CALGB study.[3] The amount of fibroblasts is indicated as absent (-), low (+), or high (+++). CMS=Consensus Molecular Subtypes.

Search strategy and selection criteria

We searched PubMed for articles in English published between Jan 1, 2005 and Dec 31, 2018 with the terms: "CALGB", "FIRE3", "microenvironment", "cetuximab", "bevacizumab", "irinotecan mechanism of action", "oxaliplatin mechanism of action", "CMS classification", "bevacizumab cetuximab interaction", "irinotecan cetuximab interaction", "oxaliplatin cetuximab interaction", "WT-Ras", "sidedness and cetuximab", "molecular signatures and cetuximab", "molecular signatures and oxaliplatin", and "molecular signatures and irinotecan".

contributed by the second-line treatment to the cumulative overall survival of the first-line bevacizumab group. As a result, the overall survival curve of the first-line bevacizumab group was lowered, increasing the gap between the survival curves of first-line cetuximab and first-line bevacizumab (figure 5D). The difference between the two survival curves was statistically significant, which led the FIRE-3 study to conclude that cetuximab is superior to bevacizumab as first-line treatment.[4]

We suggest that the opposing conclusions of the two studies were reached by assuming that only the first-line biological therapy contributed to overall survival, and not considering the contribution of the complex interplay between first-line biological therapy, chemotherapy, and tumour type, or the effects of the subsequent second-line biological therapy and chemotherapy combination acting on a tumour and microenvironment that have probably been changed by the first-line treatment.

Conclusion

By examining the results of the CALGB and FIRE-3 studies from a different perspective, we have found that their results could be complementary, rather than discrepant. Our analysis of the CALGB and FIRE-3 studies has several important clinical implications. Firstly, on the basis of the findings of the two studies, we should perhaps redirect our attention from trying to determine the best first-line biological therapy, and instead focus on the concept that overall survival depends on the best first-line biological therapy-chemotherapy combination, which predetermines the crossover to the best second-line therapies, thus ensuring the optimal overall survival of a patient with a specific tumour subtype (figure 4).

Secondly, CMS signatures are not yet validated as a predictive test, because their consistency in the temporal evolution of colorectal cancer is not known and their concordance with sequential metastatic lesions remains to be determined. Accordingly, the approach of using these tumour signatures needs to be refined to support clinical decision making that aims to improve patient outcomes.[71] Although CMS classification is not predictive, our analyses suggest that first-line cetuximab combinations can offer improved overall survival for almost 86% of patients with *KRAS* wild-type tumours (CMS2–4), especially for those with left-sided tumours (figure 4).

The possibility that irinotecan could be the preferred cetuximab partner over oxaliplatin as the optimal first-line combination for the majority of left-sided tumours (figure 4; CMS2 or CMS4, around 80% of patients), and possibly also for right-sided tumours, should be further explored. The APEC trial results[72] are consistent with this concept; patients with right-sided tumours who were given the cetuximab-oxaliplatin combination (probably antagonised by the fibroblast-rich microenvironment) had an overall survival of 21·8 months compared with 31·2 months for patients who were given the synergistic cetuximab-irinotecan combination.

The challenge of validating the present hypothesis remains—namely that the efficacy of cetuximab is partially antagonised by the oxaliplatin-activated fibroblast-rich microenvironments in patients with colorectal tumours classified as CMS1 and CMS4. Further examination of the COIN[41] or NORDIC VII[42] trials according to CMS classification could help to clarify this issue. The present hypothesis generates provocative questions related to the optimal treatment of CMS subgroups according to sidedness, because CMS2 or CMS4 tumours, which are predominant on the left side of the colon and which generally respond to cetuximab, might respond differently if they are on the right side, where bevacizumab might be a more effective treatment. We do not know whether cetuximab and panitumumab have similar interactions with oxaliplatin in the CMS1 and CMS4 microenvironments, because no such analysis has yet been done for the major panitumumab trials. In theory, a previous bevacizumab treatment, or TGF-β release by CMS1 and CMS4 microenvironments in response to oxaliplatin, would be expected to interfere equally with both cetuximab and panitumumab at the tumour-cell level, although the effect of cetuximab might be more disrupted at the microenvironment level because of inhibition of its unique effects on ADCC, natural killer cells, macrophages, and T-cell activation.

Addressing the questions raised by our working hypothesis could provide new opportunities to optimise personalised treatment (and possibly adjuvant treatment) of metastatic colorectal cancer subtypes. This could be by anticipating the optimal treatment sequence or, preferably, by predetermining the best first-line chemotherapy-biological therapy combination for individual *KRAS* wild-type colorectal cancer tumours.

Contributors

DA did the literature search, collected data, and wrote the manuscript. SS and VH (authors of the FIRE-3 study) helped to interpret the data with discussions and comments.

Declaration of interests

DA received honoraria for participation in advisory boards from Merck, Bayer, and Teva, speaker fees from Merck and MSD, and travel expenses to meetings from Merck. SS received honoraria for participation in advisory boards from Merck, Roche, Amgen, and Samsung, speaker fees from Merck, Roche, Amgen, Sanofi, Lilly, Sirtex, Takeda, Taiho, and

Servier, and travel expenses to meetings from Merck, Roche, Lilly, and Sanofi. VH received honoraria for participation in advisory boards from Merck, Roche, Amgen, Sanofi, Lilly, Sirtex, Boehringer Ingelheim, Taiho, and Servier, speaker fees from Merck, Roche, Amgen, Sanofi, Sirtex, Servier, and MSD, travel expenses to meetings from Merck, Roche, Amgen, Sirtex, Servier, MSD, and Bristol-Myers Squibb, and funding of research activity from Merck, Pfizer, Amgen, Roche, Servier, and Sirtex.

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