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Antitumour immunity invoked by hepatic arterial infusion of first-line oxaliplatin predicts durable colorectal cancer control after liver metastasis ablation: 8–12 years of follow-up

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In colorectal cancer (CRC), hepatic arterial infusion (HAI) chemotherapy may convert primarily unresectable CRC liver metastases (CLM) into resectability, although the risk of metastatic recurrence remains high after CLM ablation. We investigated the role of antitumour immunity invoked by first-line oxaliplatin-HAI for long-term CLM outcome. In a prospective study cohort of primarily unresectable CLM, we assessed patients' fms-related tyrosine kinase 3 ligand (FLT3LG) in serum, reflecting opportune intratumoural immune activity, at baseline and following 1–3 sequences of oxaliplatin-HAI. The end points were CLM resectability and overall survival. Patients who presented an immediate twofold increment of circulating FLT3LG during the treatment and at its completion were scored as CLM resectable (16.4% with both features), were alive at final follow-up 8–12 years later. All patients experienced FLT3LG increase during the treatment course, but those who remained unresectable or had the disease converted but presented a slow and gradual FLT3LG accretion, later died of the metastatic disease. These data provide further support to our previous findings that tumour-directed immunity invoked by oxaliplatin-containing therapy predicts excellent outcome of early advanced CRC if macroscopic tumour ablation is rendered possible by the 'classic' tumour response to the cytotoxic treatment.

Introduction

In colorectal cancer (CRC), dissemination into systemic disease remains the main therapeutic challenge. Improved surgical techniques[1,2] and the introduction of perioperative oxaliplatin-based chemotherapy[3,4] have enabled an increasing number of patients with liver-confined metastatic disease to undergo treatment with potentially curative intent, although metastatic recurrence occurs in a high percentage of cases. In terms of response rates for initially unresectable CRC liver metastases (CLM), the best outcomes in randomised trials have been observed for oxaliplatin-containing triplet regimens[5,6] with favourable long-term results for both resected and nonresected patients.[7–9] Hence, primarily unresectable CLM may be converted into resectability after sufficient response to chemotherapy,[10] but deliberate patient selection is crucial for survival benefits.[11,12]

Metastatic lesions in the liver are predominantly supplied by the hepatic artery.[13,14] The technique of therapeutic hepatic arterial infusion (HAI) was developed to deliver an antineoplastic agent at high concentration directly into the liver metastases without causing systemic toxicity.[15,16] Oxaliplatin exhibits favourable

Additional Supporting Information may be found in the online version of this article.

Key words: colorectal cancer, liver metastasis, hepatic arterial infusion, oxaliplatin, immunogenic cell death

Abbreviations: CI: confidence interval; CLM: CRC liver metastases; CRC: colorectal cancer; ECOG: Eastern Cooperative Oncology Group; FLT3LG: fms-related tyrosine kinase 3 ligand; HAI: hepatic arterial infusion; ICD: immunogenic cell death; Post-1st seq: after the 1st treatment sequence (4 therapy cycles); Post-2nd seq: after the 2nd treatment sequence (8 therapy cycles); Post-3rd seq: after the 3rd treatment cycle (12 therapy cycles)

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What's new?

In colorectal cancer (CRC), hepatic arterial infusion (HAI) chemotherapy may convert primarily unresectable CRC liver metastases (CLM) into resectable CLM. The risk of metastatic recurrence remains high, however, and molecular markers for long-term therapeutic benefit are needed. In this prospective cohort study of patients who received oxaliplatin-HAI, the authors assessed serum FLT3LG to monitor intratumoural immune activity. All patients experienced FLT3LG increase during therapy, but only those who experienced a rapid and substantial tumour-directed immune response were alive 8–12 years later. Monitoring the immune response *via* serum FLT3LG may improve the selection of CLM patients for a curative-intent ablation procedure.

pharmacokinetic characteristics for a HAI regimen due to the high first-pass hepatic extraction rate.[17,18] Early-phase or retrospective studies have investigated the concept of using oxaliplatin as the HAI compound for isolated unresectable CLM with treatment toxicity, tumour response and survival as end points.[19–22] Only one prospective study has reported the intent to transform to resectable disease, in this case by HAI administration of triplet oxaliplatin-containing chemotherapy, which resulted in hepatectomy for 30% of patients.[23] The role of HAI chemotherapy in clinical practice still remains controversial, but trials that randomise between this and modern systemic therapy are ongoing.[24,25]

As a result of the tumour cell cytotoxicity, oxaliplatin elicits immunogenic cell death (ICD), which implies that the shed tumour antigens are captured by dendritic cells for subsequent priming of tumour-targeting T-cells.[26,27] The majority of CRC cases are not inherently immunogenic[28,29] but might, if transformed to such a state by oxaliplatin, be subject to improved systemic immune surveillance with reduced risk of disease progression.[30] Our study on patients with locally advanced rectal cancer at high metastatic risk, given intensified oxaliplatin-containing therapy before radical surgery,[31] led to an ICD conceptual discovery. Patients who experienced a pronounced rise in serum levels of the fms-related tyrosine kinase 3 ligand (FLT3LG) during the neoadjuvant treatment course, interpreted as ICD induction, had significantly better progression-free survival than those without such response, implying that an advantageous systemic immune response might have been invoked by oxaliplatin.[32] The FLT3LG is a potent growth factor for antigen-presenting dendritic cells[33,34] and when administered to patients with metastatic CRC, has led to expansion of the dendritic cell population both locally in tumour and systematically.[35] In melanoma, which is the archetype immunogenic tumour entity, it was recently demonstrated that intratumoural natural killer cells are the source of the dendritic cell-stimulatory FLT3LG.[36]

Because FLT3LG in the systemic circulation may reflect an immune response generated within the tumour microenvironment, we hypothesised it may be used in the context of predicting long-term outcome of first-line oxaliplatin-HAI in patients with primarily unresectable CLM.

Materials and Methods

Ethics and approvals

The study was approved by the Committee for Research Ethics of the Capital Region of Denmark (reference number H-KA-04043-GS; the 5th of May, 2004) and the Danish Medicines Agency (reference number 2612-2538; the 16th of November, 2004), and was conducted in accordance with the Declaration of Helsinki. Besides these mandatory approvals, there was no requirement for trial registration in a public registry when our study started patient accrual in 2004, nor for preceding Institutional Review Board evaluation within the Danish specialist health services. Written informed consent by each patient was required for participation. In January 2018, anonymized biobank samples linked to de-identified selected patient data were transferred from Herlev and Gentofte Hospital, Denmark, to Akershus University Hospital, Norway, in accordance with current regulations.

Study design, treatment and end points

In this nonrandomised phase 2 study, eligible patients presented with their first recurrence of oxaliplatin-naïve isolated CLM originating from CRC adenocarcinoma and considered technically unresectable.[37] As delineated in Figure 1, patients were given a dose-intensified regimen of HAI-oxaliplatin (100 mg/m^2) on Day 1 and high-dose oral capecitabine (1,750 mg/m^2 twice daily) on Day 1–7 every 2 weeks for a maximum of 12 cycles, in accordance with a dose administration regimen described in 2003.[38] The study treatment was terminated sooner if patients experienced intolerable toxicity or disease progression, or after 8 cycles if objective radiographic response had failed to occur. Treatment response was evaluated every 4 cycles (defined as one treatment sequence). Patients who at any evaluation (after 4, 8 or 12 treatment cycles) were considered qualified for a CLM ablation procedure with either radiofrequency ablation or hepatic resection proceeded to the planned treatment option, after which the study protocol specified 6 cycles or 3 months of postoperative systemic therapy consisting of oxaliplatin (85 mg/m^2) on Day 1 and capecitabine (1,750 mg/m^2 twice daily) on Day 1–7 every 2 weeks. Patients with CLM that did not become resectable *via* the study treatment proceeded to irinotecan-based chemotherapy. CLM resectability (resectable or unresectable), irrespective of the number of HAI cycles, was the primary end point (along with the response rate[37]). The overall survival rate at censoring, recorded on the 8th of February, 2018, was a secondary end point. The present study subpopulation of 55 patients was enrolled between the 14th of December, 2004 and the 7th of October, 2009. Primary tumour *RAS* mutational status was determined retrospectively in a two-step procedure consisting of *KRAS* exon 2 analysis by the therascreen® test (Qiagen) followed by targeted DNA sequencing

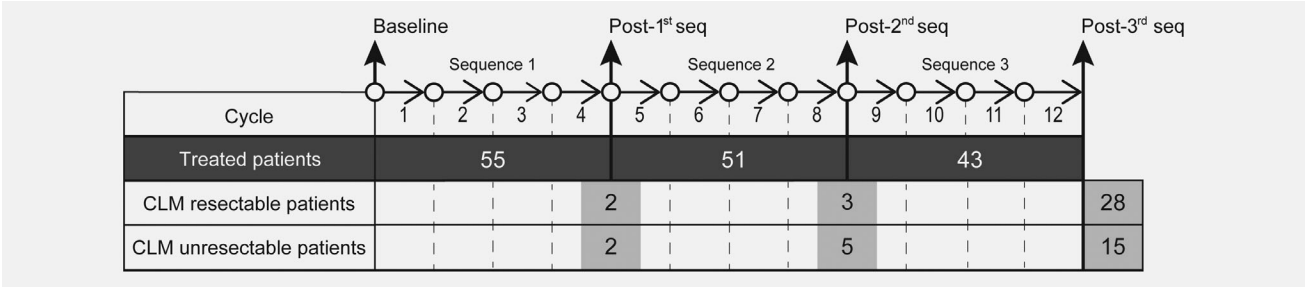

Figure 1. The study design, treatment and primary end point. Each circle indicates the start (by means of hepatic arterial infusion of oxaliplatin) of a therapy cycle (horizontal arrow), protocol-planned to every 2 weeks, with 4 cycles defining a treatment sequence. Vertical arrows indicate treatment evaluation and study-specific serum sampling [at baseline and after 4 therapy cycles (Post-1st seq), 8 cycles (Post-2nd seq) and 12 cycles (Post-3rd seq)]. The numbers of treated patients in each sequence and patients within the two groups of the primary end point (CLM, colorectal liver metastases) at each treatment evaluation are given.

(by the Ion AmpliSeq Cancer Hotspot Panel v2; Thermo Fisher Scientific) of the wild-type *KRAS* exon 2 specimens.

Assessment of serum FLT3LG

Patients had study-specific serum sampled at baseline (immediately before commencement of treatment; n = 55) and after each sequence of study treatment (at each evaluation), defined as post-1st seq (after 4 cycles; n = 43), post-2nd seq (after 8 cycles; n = 33) and post-3rd seq (after 12 cycles; n = 26). Samples were no longer collected after patients were definitely concluded as CLM resectable or unresectable, and patients could occasionally miss out from sampling of reasons unrelated to the study conduct. Blood was drawn in plain serum tubes with no additives for centrifugation to separate serum, which was left in room temperature for no more than 2 hr before storage at −80°C. Analysis of FLT3LG was undertaken in February–March 2018 (i.e. after 101–158 months of storage) using a customised Luminex Multiplex Assay (R&D Systems, Boston, MA), following the manufacturer's instructions. This is a bead-based technology for assessment of multiple serum analytes. The manufacturer advises against the use of measures outside the standard curve (below 70% of the lowest value and above 130% of the highest value of the standard curve). In the case of the FLT3LG analysis, all of the reported measurements were within these assay constraints. The mean value of duplicate measures was used in downstream analyses. One other analyte of potential relevance in the oxaliplatin-induced ICD context[39] was assayed simultaneously and will be reported separately.

Statistical analysis

The analyses were performed using the SAS software version 9.4 and STATA version 15. Differences in mean baseline serum FLT3LG values and in CLM resectability scores between various patient and tumour characteristics were analysed by independent samples t-test, χ^2-test or Fisher's exact test, as appropriate. Since our hypothesis was that serum FLT3LG during the oxaliplatin-HAI therapy might predict treatment outcome (CLM resectability and survival at censoring), we estimated linear mixed models assessing the longitudinal change of FLT3LG (as continuous variable) within the defined outcome groups. First, the overall trend in FLT3LG response over the treatment course in the entire patient cohort was estimated by a linear mixed model with fixed effects for the time of measurement. Next, the trend in FLT3LG response in the defined outcome groups was assessed by linear mixed models with fixed effects for the time of measurement, group and interaction between the time of measurement and group. All models included random effects for patients to correctly adjust the estimates for within-patient correlations due to repeated measurements. Between-group differences at each measurement were derived from the estimated linear mixed models in *post hoc* analyses. The results were presented as mean difference with 95% confidence interval (CI). Moreover, Cox proportional hazards models were estimated to assess the hazard ratio with 95% CI for overall survival for increasing values of FLT3LG measured at the single time points. An extended Cox regression model for survival was estimated with FLT3LG as time-dependent covariate. All time-dependent outcomes were calculated from the date of study enrolment. Results with p values below 0.05 were considered statistically significant. All tests were two-sided.

Data availability

Request to inspect and analyse the data that underlie the results reported in this article should be directed to the corresponding author, and access will be provided in accordance with the General Data Protection Regulation of the European Union.

Results

Patient characteristics and circulating FLT3LG

As shown in Table 1, the 55 patients (64% males) had a mean age of 60 (minimum, maximum: 41, 74) years, of whom 48 reported normal performance status [Eastern Cooperative Oncology Group (ECOG) score[40] 0]. Eighty percent of primary tumours were left-sided or rectal, and half of the cases had mutant *RAS* status (7 of 11 right-sided and 20 of 44 left-sided/rectal cases). With the exception of significantly lower measures for the seven patients with reduced performance status (ECOG score 1–2),

Table 1. Baseline characteristics of the cohort

	n (%)	FLT3LG, pg/ml Mean (SD)	*p*[1]
Age, years Mean (minimum, maximum)	60 (41, 74)		
Sex			
Male	35 (63.6)	77.6 (25.1)	
Female	20 (36.4)	72.8 (20.7)	0.47
ECOG performance status			
0	48 (87.3)	78.9 (21.6)	
1–2	7 (12.7)	55.4 (27.6)	0.01
Primary tumour location			
Right-sided	11 (20.0)	71.7 (18.5)	
Left-sided or rectal	44 (80.0)	76.9 (24.7)	0.52
Tumour RAS status			
Wild-type	28 (50.9)	81.7 (24.3)	
Mutant	27 (49.1)	69.9 (21.6)	0.06

[1]By independent samples *t*-test.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; FLT3LG, fms-related tyrosine kinase 3 ligand; SD, standard deviation.

baseline FLT3LG values did not differ between the various patient and tumour strata (listed in Table 1). For the whole patient group (Fig. 2), FLT3LG showed an initial increase before a less marked continuous rise and a final slight decline, with measures (mean ± standard deviation; in pg/ml) of 75.9 ± 23.5 at baseline, 133.5 ± 53.8 at post-1st seq, 147.3 ± 53.4 at post-2nd seq and 128.7 ± 37.0 at post-3rd seq.

CLM resectability and patient survival

As indicated in Figure 1, two patients had disease progression at post-1st seq and five others had progression or no objective response at post-2nd seq; hence, seven patients were scored as CLM unresectable based on the criterion of failed treatment response over the initial 4–8 cycles. In addition, 15 patients were scored as technically unresectable at post-3rd seq despite a radiographic response. In contrast, two patients had the CLM converted to resectable disease at post-1st seq, another three at post-2nd seq and yet 28 at post-3rd seq, altogether of whom 30 proceeded to CLM ablation because three patients with complete treatment response remained radiologically free of disease.

Among the 33 patients who were scored as CLM resectable (60.0% of enrolled individuals) and thus, might be cured of their metastatic disease, 9 (27.3%) were alive at censoring, resulting in an overall survival rate of 16.4% for the total study population. Median time to death was 71.0 (minimum, maximum: 22.0, 147.0) months for patients in the resectable category as opposed to 23.5 (minimum, maximum: 5.0, 63.0) months for patients who remained with unresectable CLM. The median follow-up time for the nine patients alive was 122.0 (minimum, maximum: 100.0, 144.7) months—the oldest study patient was 87 years old at censoring.

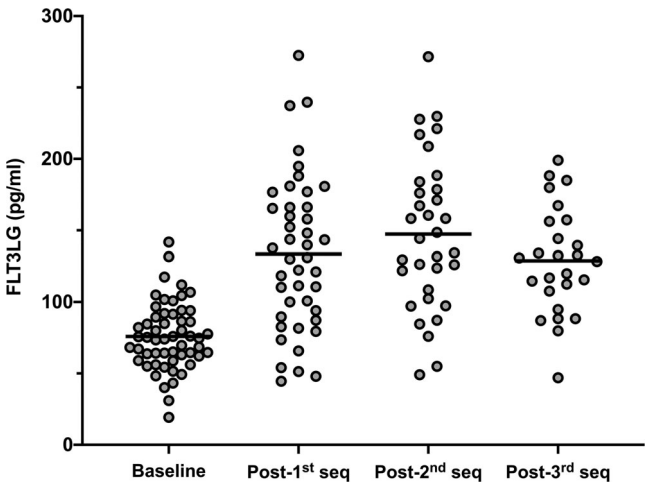

Figure 2. Serum levels of fms-related tyrosine kinase 3 ligand (FLT3LG) during the study treatment [at baseline (n = 55) and after 4 therapy cycles (Post-1st seq; n = 43), 8 cycles (Post-2nd seq; n = 33) and 12 cycles (Post-3rd seq; n = 26)]. The horizontal line in each data cluster represents the mean value.

FLT3LG and treatment outcome

In order to explore the serum FLT3LG response over the treatment course (Fig. 2), patients were stratified according to treatment outcome (CLM resectability and survival at censoring) and linear mixed models were estimated, with the results displayed in Figure 3. The significant overall trend in the FLT3LG response ($p < 0.001$; upper panel) was essentially reproduced within the short-term outcome groups, with no trend difference between resectable and unresectable cases (middle panel). The distribution of age, sex and primary tumour sidedness was also equal in resectable and unresectable cases, while tumour wild-type *RAS* status was significantly more common in patients who had the disease converted to resectability (Supporting Information Table S1).

As illustrated by the lower panel of Figure 3, the overall trend of the serum FLT3LG response differed (p = 0.005) for patients in the resectable category who were alive (n = 9) or dead (n = 24) at censoring. The patients in this category who later died, all but three of metastatic recurrence, presented an almost constant and altogether less than twofold increment of FLT3LG over the entire treatment course. In contrast, the surviving individuals (i.e. those cured of the metastatic disease by the multimodal study treatment) had an immediate twofold FLT3LG increase from mean 76.5 (95% CI: 50.8–102.3) pg/ml at baseline to mean 158.9 (95% CI: 136.3–181.6) pg/ml at post-1st seq before a continued rise to mean 179.6 (95% CI: 156.7–202.5) pg/ml at post-2nd seq. The relative incline over the second treatment sequence, however, was essentially similar to that in the patient group that later died. At completion of the maximum number of therapy cycles (post-3rd seq), the two patient groups again had identical circulating FLT3LG values, which for the surviving individuals were a consequence

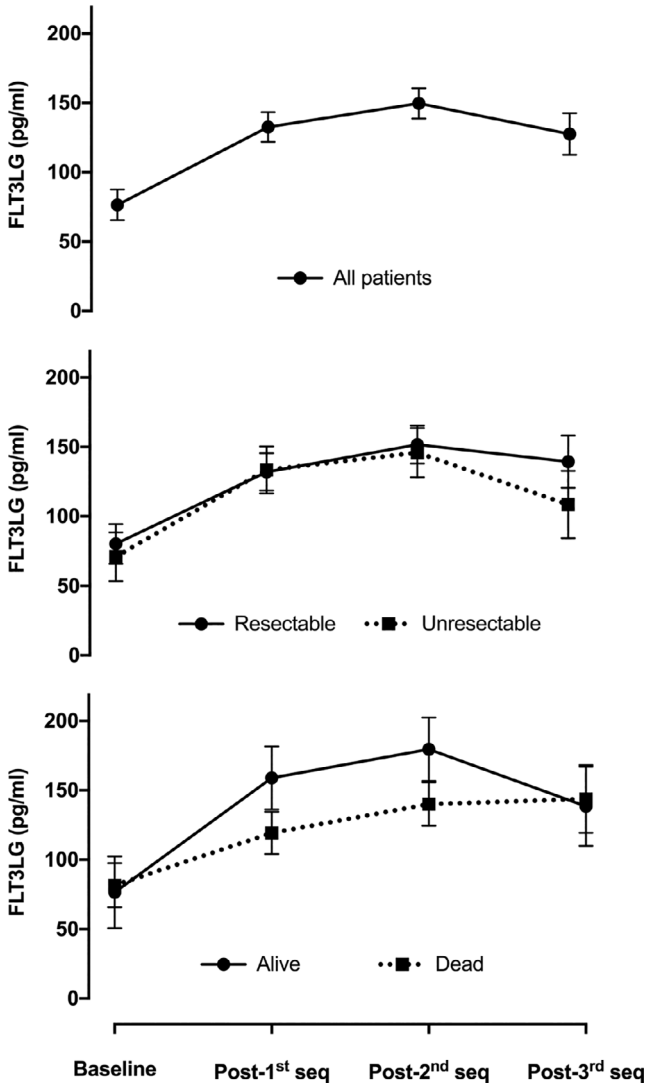

Figure 3. Serum levels of fms-related tyrosine kinase 3 ligand (FLT3LG) during the study treatment [at baseline and after 4 therapy cycles (Post-1st seq), 8 cycles (Post-2nd seq) and 12 cycles (Post-3rd seq)] in all study patients (upper panel) and patients stratified to treatment outcome groups [colorectal liver metastases resectability (resectable, unresectable; middle panel) and survival at censoring (alive, dead) for the resectable cases (lower panel)]. Results of linear mixed models; means and 95% confidence intervals.

of a decline over the third treatment sequence. However, among the resectable cases, none of FLT3LG values at any point of measurement over the treatment course was predictive of overall survival, nor were the longitudinal FLT3LG measures entered into the model simultaneously for all individual patients within the total study population (Supporting Information Table S2).

Post hoc analyses (Table 2) showed that in the group of resectable cases, the early rise in circulating FLT3LG in patients who were alive at censoring translated into mean differences relative to those who later died of 39.6 (95% CI: 12.3–66.9) pg/ml ($p = 0.004$) at post-1st seq and 39.5 (95% CI: 11.8–67.3) pg/ml ($p = 0.005$) at post-2nd seq. The differences at completion of the first and second treatment sequences were essentially also seen within the total study population when comparing the nine surviving individuals with the 46 patients who died. Additionally, patients scored with unresectable CLM after three therapy sequences had serum FLT3LG values that were lower than the measures in resectable cases by a small margin, with a mean difference of 31.0 (95% CI: 0.10–61.8) pg/ml ($p = 0.049$).

Table 2. Differences in serum FLT3LG during the study treatment between patients with various outcomes

Outcome	Point of measurement	FLT3LG, pg/ml Mean difference (95% CI)[1]	p^1
Resectable *versus* unresectable	Post-3rd seq	31.0 (0.10–61.8)	0.049
Alive *versus* dead (within the resectable patient category)	Post-1st seq	39.6 (12.3–66.9)	0.004
	Post-2nd seq	39.5 (11.8–67.3)	0.005
Alive *versus* dead (within the total study population)	Post-1st seq	32.3 (5.70–58.9)	0.017
	Post-2nd seq	36.6 (9.60–63.6)	0.008

[1]Results of *post hoc* analyses.
Abbreviations: CI, confidence interval; FLT3LG, fms-related tyrosine kinase 3 ligand; Post-1st seq, after 4 therapy cycles; Post-2nd seq, after 8 therapy cycles; Post-3rd seq, after 12 therapy cycles.

Discussion

In order to improve the selection of patients with isolated, primarily unresectable CLM for curative-intent ablation after chemotherapy, a better understanding of the underlying biology and the development of molecular markers for long-term therapeutic benefit are needed.[41] We noted that patients who during first-line oxaliplatin-HAI presented a rapid and substantial rise in circulating FLT3LG over the initial treatment, regarded as a marker of invoked tumour-defeating immunity,[32–34] and at treatment completion could proceed to CLM ablation, were alive at final follow-up 8–12 years later. Essentially, all study patients experienced serum FLT3LG increase after start of the therapy. However, 40% of cases did not have the CLM converted to resectable disease, and more than 70% of those who were finally scored as technically resectable presented a slow and gradual FLT3LG accretion. The patients with either of these features died in the study follow-up. Consequently, complete tumour eradication appeared to be contingent on conversion to resectable disease (for macroscopic tumour clearance) along with a manifest antitumour immune response (for elimination of disseminated microscopic tumour cells).

Liver-confined disease represents about one-third of metastatic CRC cases, with complete macroscopic ablation considered an option for potential cure.[42] However, recurrent disease occurs in the majority of patients, and even with

contemporary multimodal treatment only one in five patients is cured.[42,43] Primary tumour right-sidedness and mutant *RAS* status have been reported as unfavourable prognostic factors.[44,45] The current study population displayed distributions of age, sex and tumour sidedness and mutational status in agreement with other reports on isolated CLM.[20,21,23,46] We observed that wild-type *RAS* status was strongly associated with CLM conversion to resectability by oxaliplatin-HAI. The significantly longer overall survival for those patients has been reported previously.[37]

Oxaliplatin-based chemotherapy administered systemically is considered the standard-of-care for the majority of CLM patients, particularly if conversion of technically unresectable disease to resectability is the primary goal.[10] Prospective studies of oxaliplatin-HAI in combination with a systemically administered fluoropyrimidine, given either as early-line therapy or after failure of systemic palliative therapy, have reported various response and survival rates.[22] Modern oncological approaches have yielded median overall survival of unselected patients with metastatic CRC of approximately 30 months.[10] The randomised FIRE-3 trial on chemotherapy combined with biologically targeted agents in the first line for metastatic CRC showed median overall survival of 36 months for patients with wild-type *RAS* liver-confined disease.[47] In the present study, 60% of cases were converted to resectability and had median overall survival of close to 6 years. More than one quarter of the resected patients were cured. Altogether, these are remarkable results for patients with disseminated CRC, providing additional evidence to the importance of optimal patient selection and treatment approaches in organ-limited disease. We acknowledge that eligible study patients, a high percentage of whom had normal performance status, had their first metastatic event and thus were selected for good prognosis. Yet, for both the resected and nonresected patient groups, disease recurrence or progression seemed to be dominant, warranting a deeper understanding of the involved biological mechanisms in order to further improve the cure rate.

From experimental models it has long been known that FLT3LG is central in the development of distinct subsets of dendritic cell lineages[48] and that this factor, in combination with tumour irradiation, causes long-term protective antitumour immunity.[49] This knowledge has fostered the recent enthusiasm for administering FLT3LG together with radiotherapy as an *in situ* vaccination strategy in combination with immune checkpoint-blocking therapy, in order to obtain systemic therapeutic effects.[34] In the present analysis, we found that intratumoural delivery of oxaliplatin caused FLT3LG release into the circulation, supporting the notion that oxaliplatin may promote an intratumoural immune response that specifically recruits dendritic cells to present antigens released by the dying tumour cells to cytotoxic lymphocytes, ultimately providing systemic immunity. The likely freedom from recurrent metastasis in CLM-ablated patients who had experienced a rapid initial rise in circulating FLT3LG during the oxaliplatin-HAI therapy appears to agree with the theory that FLT3LG production reflects an innate immune response, specifically comprising natural killer cells, within the tumour microenvironment.[36]

However, genuine cure of the metastatic disease was additionally contingent on therapeutic conversion to resectability as a result of the 'classic' cytotoxic effect of chemotherapy. We made a corresponding observation in study patients with locally advanced rectal cancer at high metastatic risk, treated with neoadjuvant oxaliplatin and radiotherapy before radical surgery.[31] Those who had an immediate rise in circulating FLT3LG during the neoadjuvant course were cured after pelvic tumour clearance enabled by sufficient tumour regression after the cytotoxic therapy.[32] Within this frame of reference, it is tempting to speculate whether patients in the present study who presented an insufficient FLT3LG accretion, yet achieved CLM resectability, might have obtained long-term survival from the addition of immune checkpoint-blocking therapy.

There are obvious limitations of this spin-off study. First, the cohort is small, without a control group. However, this is our second study reporting on oxaliplatin effects on circulating FLT3LG and the advantageous outcome of early advanced CRC. Next, a number of serum samples were missing and FLT3LG could not be consecutively followed for all of the patients. Moreover, the analyses were undertaken without information about dose adjustments or accounting for common predictors of CLM outcome such as the location, number and size of liver lesions[42] but comprised the study's main primary and secondary end points.

In conclusion, our study provides indications that systemic tumour-directed immunity invoked under chemotherapy predicts excellent outcome of isolated CLM if macroscopic tumour ablation is rendered possible by the 'classic' tumour response to the cytotoxic therapy. Information about the circulating FLT3LG profile over the course of treatment may be useful for the selection of patients with primarily unresectable CLM most likely to benefit from a curative-intent ablation procedure.

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Conflict of interest

The authors declare no conflict of interest.

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