Lav. 3708

The feasibility and effect of intraperitoneal administration of regorafenib on peritoneal carcinomatosis from colorectal cancer in the rat

Ann. Ital. Chir., 2021 92: ???-???
pii: 0000000000000

Stefanos Bitsianis*, Ioannis Mantzoros*, Anna Gkiouliava**, Christos Chatzakis*, Chryssa Bekiari***, Lydia Loutzidou*, Georgios Ntampakis*, Panagiotis Christidis*, Kalliopi Domvri°, Konstantinos Porpodis°, Orestis Ioannidis*, Stamatios Aggelopoulos*

**4th Surgical Clinic, School of Medicine, Faculty of Health Science, Aristotle University of Thessaloniki, Thessaloniki, Greece*
***Department of Anaesthesiology, Georgios Papanikolaou General Hospital, Thessaloniki, Greece*
****Laboratory of Anatomy, Histology & Embryology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece.*
°Pulmonary Department-Oncology Unit, Georgios Papanikolaou General Hospital, Aristotle University of Thessaloniki, Greece.

The feasibility and effect of intraperitoneal administration of regorafenib on peritoneal carcinomatosis from colorectal cancer in the rat.

AIM: *Our goal was to investigate the potential use and efficacy of regorafenib for IPEC in an animal model of colorectal derived peritoneal metastases. Twenty four male rats were included. Carcinogenesis was induced in all rats through intraperitoneal injection of cancer.*
MATERIAL AND METHODS: *Cells at T0. At T1(Day 28) they were randomly allocated 1:1:1:1 into 4 groups and underwent median laparotomy and the corresponding intervention. Specifically, Group A: no other intervention; Group B: cytoreductive surgery; Group C: intraperitoneal chemotherapy with regorafenib; and Group D: cytoreductive surgery and intraperitoneal chemotherapy with regorafenib. At T2 (Day 56) rats were euthanized and laparotomy was performed for further investigation. The primary outcome was the experimental Peritoneal Cancer Index (ePCI) at T2. Secondary outcomes include relative change of body weight between T1 and T2, weight of the ascites, anastomotic leak/peritonitis and death.*
RESULTS: *The ePCI was significantly lower in Group D as opposed to all other groups. Comparing Group C versus Group A we found a trend towards lesser tumor progression, but no significant difference. Growth of rats in Group D was significantly least affected compared to all other groups. Animals undergoing CRS in Group B developed less ascites than Group A and C. Less ascites was found in Group D compared to Group A and C.*
CONCLUSIONS: *Intraperitoneal chemotherapy with regorafenib combined with cytoreductive surgery may impair metastases' progression.*

KEY WORDS: Regorafenib, C hemotherapy, Cytoreductive surgery, Colorectal cancer, Intraperitoneal injection

Introduction

Chemotherapy has had significant advancements the past two decades to improve not only patient survival but also patient convenience and quality of life [1-3]. Oral chemotherapy has evolved in this direction, while researchers have also been attracted by the potential of intraperitoneal chemotherapy (IPEC). IPEC was developed in an effort to achieve high concentration of chemotherapeutic regimens at the metastases' site as the blood–peritoneum barrier is considered to limit transmembrane transport of drugs and their actual bioavailability at cancer cells [4].

Regorafenib has been successfully used as salvage oral therapy for patients with metastatic colorectal cancer owing to its multiple actions towards tumor oncogenesis and expansion [5]. Its survival benefit compared to placebo, in phase III trials, has rendered it a core treatment regimen in this patient population [6]. Regorafenib

Pervenuto in Redazione Settembre 2021. Acvcettato per la pubblicazione Ottobre 2021
Correspondence to: Anna Gkiouliava, Anaesthesiology Department, George Papanikolaos "Thessaloniki", Greece (e-mail: annagkio@gmail.com)

is a multi-kinase inhibitor that alters tumor angiogenesis [VEGFR1–3 and tyrosine kinase with immunoglobulin and epidermal growth factor homology domain 2 (TIE2)], oncogenesis (KIT, RET, RAF, BRAF) and the tumor stroma [platelet-derived growth factor receptor-β and fibroblast growth factor receptor (FGFR)18] [7,8]. The multifaceted profile of regorafenib raises the hypothesis of its potential use for IPEC and has not been studied yet at preclinical or clinical level. The additive role of cytoreduction remains also to be studied.

Drawing from these considerations, in this preclinical pilot study, we aimed to investigate the feasibility of intraperitoneal chemotherapy with regorafenib combined with cytoreductive surgery (CRS) or not and its effect on the progression of metastatic colorectal cancer in a rat model.

Materials and Methods

Protocol

This is a randomized, prospective animal study. Rats were chosen with respect to their similar biological course of colorectal cancer, previous published experimental models and facilities in our experimental lab to allow for proper conduction of the experiment. The study was designed in accordance to the European regulations for the care of animals subject to laboratory research and approved by the ethical committee of the Veterinary Medicine Department of Central Macedonia in Greece (577170/2467). The experiments and the reporting data are performed and published in accordance to the arrive (Animal Research: Reporting of *In Vivo* Experiments) guidelines 2.0(9).

Experimental Procedures

Twenty-four male Wistar rats 10-14 weeks old, weighing 200-300g were housed in a controlled temperature and humidity environment (18-22 °C and 55-60%) with interchangeable 12h intervals of light and dark. They were provided with water and food ad libitum. Male rats were chosen to prevent interference with hormone-related factors.

The HT-29 (ATCC® HTB-38™) cell line was purchased from ATCC LGC Standards (http://www.lgcstandards-atcc.org). HT-29 cell line has been isolated from a primary tumor of human colon and forms well differentiated adenocarcinoma. It was cultured in vitro and was incubated at 37 °C under atmospheric conditions of 95% humidity and 5% composition of carbon dioxide in McCoy's 5A Medium (ATCC® 30-2007™) supplemented with 10% Fetal Bovine Serum (FBS) ATCC® 30-2020™. Cell lines were cultured in Coming's tissue culture flasks (25,75 cm^2) according to the manufacturer's protocol. After cultures reached confluence, by microscope observation were then subcultured. Enzymatic detaching of HT-29 cells was achieved by 0.25% (w/v) Trypsin - 0.53 mM EDTA solution. The concentration and viability of cancer cells (cells/ml) were determined in a Neubauer chamber after trypan blue staining. Aliquots of 2 ml solutions containing 10^6/ml cancer cells were prepared for injection in each animal.

At T=0, carcinogenesis was induced in all rats. Under general anesthesia facilitated by ketamine (50mg/kg) and xylazine (5mg/kg), the rats underwent median laparotomy and cancer cells were injected at the right lower quadrant of the abdomen and the cecum mesentery. All animals were administered analgesics during the first 3 postoperative days and remained housed as aforementioned for a total of 28 days.

At T=1 (Day 28) the rats were weighed and then randomly allocated, 1:1:1:1, in 4 groups through electronic software. According to the study design, the animals were divided in Group A: control, median laparotomy without any other intervention; Group B: median laparotomy and cytoreductive surgery; Group C: median laparotomy and intraperitoneal chemotherapy with regorafenib (BAY 73-4506); and Group D: median laparotomy, cytoreductive surgery and intraperitoneal chemotherapy with regorafenib. All procedures were performed under general anesthesia. The surgeon was blinded as to the identity of the animal. Ten different sites were inspected (subcutaneous, injection site, greater omentum, liver hilum, liver, perisplenic region, mesentery, gonadal fatpad, diaphragm, parietal peritoneum). Concerning Groups B and D, right hemicolectomy combined with end-to-side anastomosis was performed in all rats, along with CRS. CRS aimed at radical removal of all macroscopic tumor deposits and involved peritonectomy, omentectomy, liver metastasectomy and removal of the mesentery nodules when feasible. In case of irresectable tumor deposits in sensitive regions such as liver hilum, spleen hilum, diaphragm or mesenterium, cauterization using an electrocoagulation device was performed in order to avoid additional animal stress and postoperative complications. In terms of IPEC with regorafenib (Group C and D), a suspension was formed with regorafenib (10 mg/kg) and 250 ml of isothermic normal saline 0.9% while in Group A and B it contained only 250 ml of isothermic normal saline 0.9%. Every 2 minutes, 10 ml of the prepared solution were infused and then removed.

After the complete administration, the abdominal cavity was rinsed with 0.9% normal saline [10]. Regorafenib was provided by Selleck Chemicals, USA. Regorafenib was administered at 10 mg/kg in a single dose for each rat which lead to exposures in the range of the respective human dose of 160 mg. [11,12].

All rats were followed-up for 28 days (T2, Day 56) and euthanized at T2 by means of CO_2 inhalation for further investigations. Then, median laparotomy was per-

formed. The experimental Peritoneal Cancer Index (ePCI) was measured after careful inspection of 10 regions as described by Klaver et al.1$_{10}$ Each region was classified as 0, no macroscopic tumour; 1, limited tumour growth (diameter 1-2 mm); 2, moderate tumour growth (diameter 2-4 mm); or 3, abundant (diameter more than 4 mm).The resulting sum is the ePCI, ranging from 0 to 30 and is presented in Table I. At last, tumor specimens or the diaphragm and greater omentum (in case of absence of visible tumor) were resected for pathological study to verify the presence of cancer cells. At the event of premature death of a rat, the time of death was recorded, and the aforementioned procedure was applied.

Primary and Secondary Outcomes

The primary outcome is the experimental Peritoneal Cancer Index (ePCI) at T2. Secondary outcomes include the relative change of body weight between T1 and T2, the weight of the ascites, anastomotic leak/peritonitis and death.

Statistical Analysis

This trial is a pilot study to investigate the effect of intraperitoneal chemotherapy with regorafenib combined or not with cytoreductive surgery on the progression of metastatic colorectal cancer. Therefore, no sample size calculation was performed. We assigned randomly and equally six rats in each group, and we ended up with twenty-four specimens.
Variables were classified as categorical and numerical. Numerical variables were subjected to assessment of normality though P-P, Q-Q diagrams and the Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive analysis was performed for each variable and study group. Regarding continuous variables mean ± SD and parametric tests were used, if their distribution was normal, or medians, interquartile range values and non-parametric tests if the distribution was non-normal. Categorical
to treat analysis was also performed. variables were summarized as absolute frequencies and percentages.
Data from each group were compared with repeated measure ANOVA or Friedman test according to their distribution and Levene's Test for the equality of variances assessment. In case of significant difference (p>0,05), the Bonferroni test or non- parametric Mann-Whitney was implemented. For multiple comparisons, the Bonferroni correction was used. Chi-square or Fisher's exact test were used for pairwise comparisons of proportions, as appropriate. Intention
The SPSS software (IBM Corp. Released 2016. IBM Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) was utilized for statistical analyses.

Results

Twenty four rats were included in the study and randomised into the 4 arms. Body weight at T0 did not differ between groups. Cancer cells were implanted at T0 as described. Peritoneal carcinomatosis was evident in all rats at T1 and ePCI did not differ between groups at that timepoint. Weight gain was impaired in all groups but changes among groups were similar. After inspection of the metastases, the surgeon was instructed to operate the animals depending on their assigned arm. At T2, the final weight of the rats was measured. The characteristics of the population are presented in Table I.

Primary Outcomes

The ePCI of groups A, B, C and D were 14.3±1.5, 7±2.2, 12.3±2.3 and 5.6±1.9, respectively. The ePCI was significantly lower in Group D as opposed to all other groups ($p<0.05$, Table II, Fig. I). Looking into Group C versus Group A we found a trend towards lesser tumor progression, but no statistically significant findings were recorded (p=0.2). Moreover, CRS and IPEC (Group D) significantly reduced ePCI compared to CRS alone (Group B vs Group D; 5.6±1.9 vs 12.3±2.3; p= 0.039).
The relative ePCI and weight change from T1 to T2 was computed and analyzed for each group (Table II). Growth of rats in Group A was significantly affected compared to Group B and D ($p<0.01$) but not Group C (p=0.1). Furthermore, Group D was affected the least ($p<0.05$).

Secondary Outcomes

Two rats died before T2 and thus were evaluated prematurely. One rat from Group B (0.17%) died the 4th

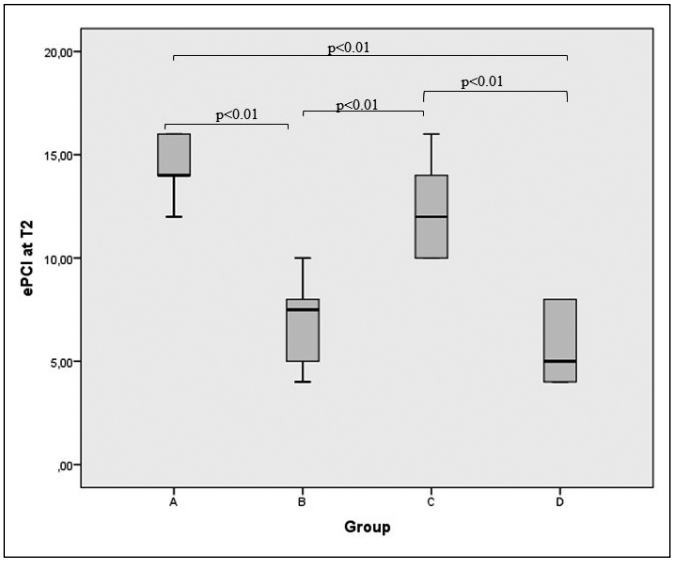

Fig. 1: ePCI at T2 between groups.

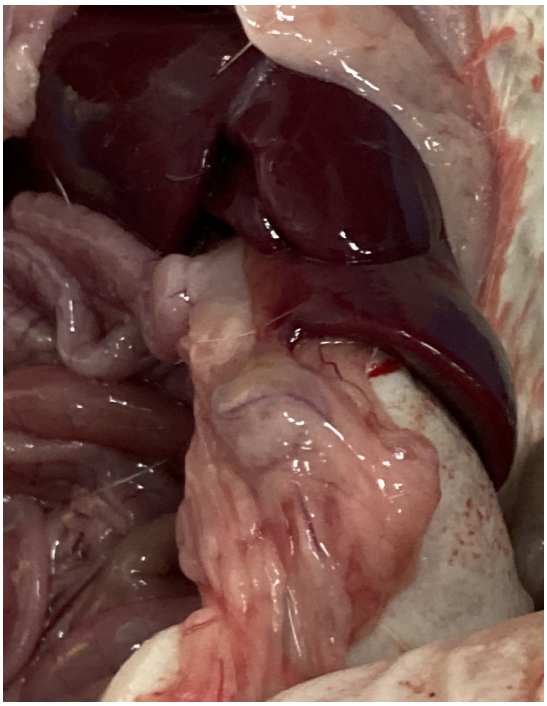

Fig. 2: Metastasis at the greater omentum found upon laparotomy.

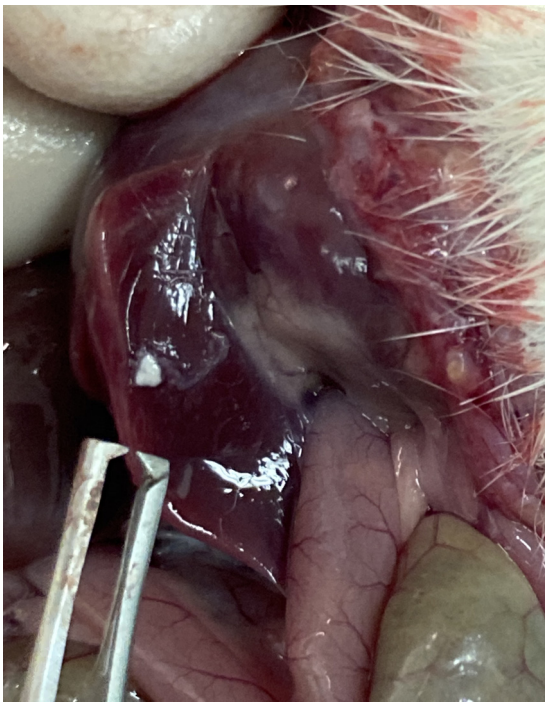

Fig. 3: Metastasis at the liver found upon laparotomy.

TABLE I - *Characteristics of animals at T2.*

	Group A (n=6)	Group B (n=6)	Group C (n=6)	Group D (n=6)
Bodyweight (g)	262 ± 11.9	274 ± 11.5	276 ± 8.4	289 ± 5.8
Tumor score per site				
Subcutaneous	0	0	0	0
Injection site	3	2(1-2)	3(2-3)	2(1-2)
Greater omentum	3(2-3)	2(1-2)	2(2-3)	1.5(0-2)
Liver hilum	0(0-1)	0(0-1)	0(0-1)	0
Liver	3(2-3)	0.5(0-1)	2(1-3)	0.5(0-1)
Perisplenic	0	0	0	0
Mesentery	3(2-3)	1(1-2)	2.5(2-3)	1(1-2)
Gonadal fatpads	0	0	0	0
Diaphragm	0	0	0	0
Parietal peritoneum	3(2-3)	1.5(0-2)	2.5(2-3)	1(0-2)
Overall ePCI	14.3 ± 1.5	7 ± 2.2	12.3 ± 2.3	5.6 ± 1.9

Values are expressed in mean and standard deviation or median and range with respect to the distribution of the data.

postoperative day and one from Group D (0.17%) the 5th postoperative day, while their death was attributed to peritonitis following anastomotic leak.

The ascites weight at T2 did not differ between Group A and C (p=0.07) and between Group B and D (p=0.07). Animal undergoing CRS in Group B developed less ascitic fluid than Group A (p<0.01) and C (p=0.02). Also, in Group A and C greater volume of ascites was found compared to Group D (p<0.01).

Discussion

This study focused on survival and remission of peritoneal metastases to investigate the effect of a novel treatment strategy to tackle these cases. Regorafenib was administered intraperitoneally in a colorectal cancer derived peritoneal carcinomatosis animal model. Rats treated with regorafenib showed a trend of deceleration of the metastases' progression compared to rats (p=0.2). According to the literature, no study has investigated regorafenib administration intraperitoneally and it is only used as a component of oral chemotherapy. The CORRECT and the CONCUR trials were the first phase III human studies, and both reported prolonged survival for patients receiving oral regorafenib compared to placebo [13,14]. Regorafenib has been shown to manipulate the ATP-binding cassette (ABC) transporter, ABCB1 and the breast cancer resistance protein (BCRP) which are associated with multidrug resistance [15,16]. Noteworthy, rego-

TABLE II - *Relative % changes of ePCI and weight from T1 to T2.*

	Group A (n=6)	Group B (n=6)	Group C (n=6)	Group D (n=6)
ΔePCI, %	49 ± 15	-19 ± 13	32 ± 11	-42 ± 14
	$p<0.01^a$, $p< 0.01^b$, $p=0.2^c$, $p<0.01^d$, $p<0.01^e$, $p<0.05^f$, $p<0.01^g$			
Δweight, %	0.5 ± 0.6	4 ± 0.7	2 ± 0.4	6 ± 1.8
	$p<0.01^a$, $p< 0.01^b$, $p=0.1^c$, $p<0.01^d$, $p<0.05^e$, $p<0.05^f$, $p<0.01^g$			

[a] Denotes Anova's test result. [b] Indicates difference between group A and B, [c] between group A and C, [d] between group A and D, [e] between group B and C, [f] between group B and D and [g] between group C and D.

rafenib is effective regardless of the type of RAS and BRAF mutation [17].

Many substances have been tested in vitro including oxiplatin, mitomycin C and cisplatin to determine the Thermal Enhancement Ratio which is crucial to justify the benefit for hyperthermic intraperitoneal chemotherapy [18,19]. In a rat model, gemcitabine- and taurolin-based hyperthermic IPEC failed to reduce ePCI scores compared to surgery alone or surgery and mitomycin C (p=0.03) [20]. Among chemotherapeutic agents, oxaliplatin and mitomycin C are incorporated in the ESMO guidelines (class III, B). Despite this fact, several studies provided evidence that did not support the use of oxaliplatin-based hyperthermic IPEC. The COLOPEC trial aimed to determine the efficacy of adjuvant hyperthermic IPEC in patients with locally advanced colon cancer. Their results raised questions regarding the efficacy of oxaliplatin as there was no difference in peritoneal-free survival at 18-months (80.9%; CI: 73.3-88.5) [21].

The PRODIGE 7 trial investigated the effects of oxaliplatin-based hyperthermic IPEC added to cytoreductive surgery for patients with colorectal peritoneal metastases. Its addition did not improve median overall survival (HR 1.00; CI: 0.63-1.58) for those patients and caused more adverse events compared to cytoreductive surgery only (26% vs 15%; p=0.035) at 60 days [22]. Spielberg et al. also demonstrated an unfavorable profile for oxaliplatin-based hyperthermic IPEC. In his retrospective study, where hyperthermic IPEC was introduced with either oxaliplatin or mitomycin C, the former group presented with more complications (66.2% *vs* 35.3%; p=0.003) and no improvement in median overall survival [23].

Research over the use of mitomycin C has also produced some inconclusive results. The introduction of Mitomycin-based IPEC did not improve survival for patients undergoing systematic chemotherapy with cytoreductive surgery, albeit did not increase their morbidity, as stated by Baratti et al. at his retrospective case series [24]. Therefore, the additional role of IPEC paired with cytoreductive surgery is a field for further studies [25]. As might be expected, cytoreduction to remove macroscopic metastases and lysis of adhesions are crucial for the effective action of the drug administered intraperitoneally [26]. In our model, we may presume an additive effect of regorafenib combined with CRS which is expressed by the ePCI change between group B and D (p<0.05). Yet, these results are primitive and further extensive research should be carried out to determine the exact role of regorafenib-based IPEC. In a multicentric retrospective study, survival was significantly related to the completeness of cytoreduction but not IPEC with mitomycin or fluorouracil. Of note, IPEC techniques varied among centers such that conclusive comments over the efficacy of pharmacological interventions is prohibited [27]. In an editorial, Sugarbaker et al. points out the challenging drug resistance and proper methodology that will take into account not only practical details but also pharmacological properties of substances used [28].

This pilot study was the first to examine the effect of intraperitoneal administration of regorafenib in a rat model of peritoneal carcinomatosis. This is not devoid of limitations as there is lack of previous data regarding such use and appropriate dosage of regorafenib. More preclinical trials should be designed to delineate the technique of regorafenib-based IPEC and perform further investigations.

Conclusions

Should efficacy and safety combined be advocated by additional and detailed evidence regarding regorafenib-based IPEC; it may emerge as a powerful tool against peritoneal carcinomatosis

conflict of interest.

Riassunto

In questo studio, ci proponiamo di indagare l'uso potenziale e l'efficacia di regorafenib per IPEC in un modello animale di metastasi peritoneali derivate dal colon-retto.

METODI: Sono stati utilizzati 24 ratti maschi. La cancerogenesi è stata indotta in tutti i ratti mediante l'iniezione intraperitoneale di cellule tumorali a T0. Al T1 (giorno 28) sono stati assegnati in modo casuale

1:1:1:1 in 4 gruppi e sono stati sottoposti a laparotomia mediana e all'intervento corrispondente. Nello specifico, Gruppo A: nessun altro intervento; Gruppo B: chirurgia citoriduttiva; Gruppo C: chemioterapia intraperitoneale con regorafenib (BAY 73-4506); e Gruppo D: chirurgia citoriduttiva e chemioterapia intraperitoneale con regorafenib. Al T2 (giorno 56) i ratti sono stati soppressi ed è stata eseguita una laparotomia mediana per ulteriori indagini. L'esito primario era l'indice sperimentale di cancro peritoneale (ePCI) a T2. Gli esiti secondari includono la variazione relativa del peso corporeo tra T1 e T2, il peso dell'ascite, la perdita anastomotica/peritonite e la morte.
RISULTATI: L'ePCI era significativamente più basso nel Gruppo D rispetto a tutti gli altri gruppi. Confrontando il Gruppo C con il Gruppo A abbiamo trovato una tendenza verso una minore progressione del tumore, ma nessuna differenza significativa. È stata calcolata la variazione di peso relativa da T1 a T2. La crescita dei ratti nel gruppo D è stata significativamente la meno colpita rispetto a tutti gli altri gruppi. L'animale sottoposto a CRS nel gruppo B ha sviluppato meno liquido ascitico rispetto al gruppo A e C. Meno ascite è stata trovata nel gruppo D rispetto al gruppo A e C. La chemioterapia intraperitoneale con regorafenib ha agito in sinergia con la chirurgia citoriduttiva nel gruppo D che ha ottenuto risultati migliori in termini di ePCI rispetto al gruppo B.
CONCLUSIONI: La chemioterapia intraperitoneale con regorafenib è fattibile e combinata con la chirurgia citoriduttiva può compromettere la progressione delle metastasi.

References

1. Jacobs JM, Ream ME, Pensak N, Nisotel LE, Fishbein JN, Macdonald JJ, et al: *Patient experiences with oral chemotherapy: Adherence, symptoms, and quality of life*. J Natl Compr Canc Netw, 2019; 17(3):221. doi: 10.6004/jnccn.2018.7098.

2. Jacobs JM, Pensak NA, Sporn NJ, MacDonald JJ, Lennes IT, Safren SA, et al.: *Treatment satisfaction and adherence to oral chemotherapy in patients with cancer.* J Oncol Pract. 2017; 13(5):e474-e85. doi: 10.1200/JOP.2016.019729.

3. Seretis C, Youssef H: *Quality of life after cytoreductive surgery and intraoperative hyperthermic intraperitoneal chemotherapy for peritoneal surface malignancies: A systematic review.* Eur J Surg Oncol. 2014; 40(12):1605-13. Epub 2014/09/23. doi: 10.1016/j.ejso.2014.08.477. PubMed PMID: 25242382.

4. Elias D, Goere D, Dumont F, Honore C, Dartigues P, Stoclin A, et al.: *Role of hyperthermic intraoperative peritoneal chemotherapy in the management of peritoneal metastases.* Eur J Cancer, 2014; 50(2):332-40.

5. Abou-Elkacem L, Arns S, Brix G, Gremse F, Zopf D, Kiessling F, et al.: *Regorafenib inhibits growth, angiogenesis, and metastasis in a highly aggressive, orthotopic colon cancer model.* Mol Cancer Ther, 2013; 12(7):1322-31.

6. Li J, Qin S, Xu R, Yau TCC, Ma B, Pan H, et al.: *Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): A randomised, double-blind, placebo-controlled, phase 3 trial.* Lancet Oncol. 2015; 16(6):619-29. doi: 10.1016/S1470-2045(15)70156-7.

7. Roed Skarderud M, Polk A, Kjeldgaard Vistisen K, Larsen FO, Nielsen DL: *Efficacy and safety of regorafenib in the treatment of metastatic colorectal cancer: A systematic review.* Cancer Treat Rev, 2018; 62:61-73. doi: https://doi.org/10.1016/j.ctrv.2017.10.011.

8. Schmieder R, Hoffmann J, Becker M, Bhargava A, Muller T, Kahmann N, et al.: Regorafenib (BAY 73-4506): *Antitumor and antimetastatic activities in preclinical models of colorectal cancer.* Int J Cancer. 2014; 135(6):1487-96. doi: https://doi.org/10.1002/ijc.28669.

9. Percie DU Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al.: *The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research.* Br J Pharmacol, 2020; 177(16):3617-24. Epub 2020/07/15. doi: 10.1111/bph.15193.

10. Klaver YL, Hendriks T, Lomme RM, Rutten HJ, Bleichrodt RP, De Hingh IH: *Intraoperative hyperthermic intraperitoneal chemotherapy after cytoreductive surgery for peritoneal carcinomatosis in an experimental model.* Br J Surg, 2010; 97(12):1874-80. Epub 2010/09/02. doi: 10.1002/bjs.7249. PubMed PMID: 20806291.

11. Mross K, Frost A, Steinbild S, Hedhom S, Buchert M, Fasol U, et al.: *A phase I dose-escalation study of regorafenib (BAY 73-4506), an inhibitor of oncogenic, angiogenic, and stromal kinases, in patients with advanced solid tumors.* Clin Cancer Res, 2012; 18(9):2658-67. Epub 2012/03/17. doi: 10.1158/1078-0432.ccr-11-1900. PubMed PMID: 22421192.

12. Kissel M, Berndt S, Fiebig L, Kling S, JI Q, Gu Q, et al.: *Antitumor effects of regorafenib and sorafenib in preclinical models of hepatocellular carcinoma.* Oncotarget, 2017; 8(63):107096-108. Epub 2018/01/02. doi: 10.18632/oncotarget.22334.

13. Li J, Qin S, Xu R, Yau TC, Ma B, Pan H, et al.: *Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): A randomised, double-blind, placebo-controlled, phase 3 trial.* Lancet Oncol, 2015; 16(6):619-29. Epub 2015/05/20. doi: 10.1016/s1470-2045(15)70156-7.

14. Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, et al.: *Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): An international, multicentre, randomised, placebo-controlled, phase 3 trial.* Lancet. 2013;381(9863):303-12. Epub 2012/11/28. doi: 10.1016/s0140-6736(12)61900-x.

15. Wang Y-J, Zhang Y-K, Zhang G-N, Al Rihani Sb, Wei M-N, Gupta P, et al.: *Regorafenib overcomes chemotherapeutic multidrug resistance mediated by ABCB1 transporter in colorectal cancer: In vitro and in vivo study.* Cancer Lett, 2017; 396:145-54.

16. Zhang Y-K, Wang Y-J, Lei Z-N, Zhang G-N, Zhang X-Y, Wang D-S, et al.: *Regorafenib antagonizes BCRP-mediated multidrug resistance in colon cancer.* Cancer Lett, 2019; 442:104-12.

17. Arai H, Battaglin F, Wang J, Lo Jh, Soni S, Zhang W, et al.: *Molecular insight of regorafenib treatment for colorectal cancer.* Cancer Treat Rev, 2019; 81:101912.

18. Helderman Rfcpa, Loke DR, Kok HP, Oei AL, Tanis PJ, Franken Napk, et al.: *Variation in clinical application of hyperthermic intraperitoneal chemotherapy: A review.* Cancers (Basel). 2019; 11(1):78.

19. DE Bree E, Tsiftsis DD: E*xperimental and pharmacokinetic studies in intraperitoneal chemotherapy: From laboratory be;nch to bedside.* Recent Results Cancer Res: Springer; 2007; 53-73.

20. Raue W, Kilian M, Braumann C, Atanassow V, Makareinis A, Caldenas S, et al.: *Multimodal approach for treatment of peritoneal surface malignancies in a tumour-bearing* rat model. Int J Colorectal Dis, 2010; 25(2):245-50. doi: 10.1007/s00384-009-0819-7.

21. Klaver CE, Wisselink DD, Puntcj, Snaebjornsson P, Crezee J, Aalbers AG, et al.: *Adjuvant hyperthermic intraperitoneal chemotherapy in patients with locally advanced colon cancer (COLOPEC): a multicentre, open-label, randomised trial.* Lancet Gastroenterol Hepatol, 2019; 4(10):761-70.

22. Quenet F, Elias D, Roca L, Goere D, Ghouti L, Pocard M, et al.: *Cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy versus cytoreductive surgery alone for colorectal peritoneal metastases (PRODIGE 7): A multicentre, randomised, open-label, phase 3 trial.* Lancet Oncol, 2021; 22(2):256-66. Epub 2021/01/22. doi: 10.1016/s1470-2045(20)30599-4. PubMed PMID: 33476595.

23. Spiegelberg J, NEeff H, HolznerP, Runkel M, Fichtner Feigl S, Glatz T: *Comparison of hyperthermic intraperitoneal chemotherapy regimens for treatment of peritoneal-metastasized colorectal cancer.* World J Gastrointest Oncol, 2020; 12(8):903-17. doi: 10.4251/wjgo.v12.i8.903. PubMed PMID: 32879667.

24. BaratTI D, Kusamura S, Azmi N, Guaglio M, Montenovo M, Deraco M: C*olorectal peritoneal metastases treated by perioperative systemic chemotherapy and cytoreductive surgery with or without mitomycin c-based hipec: A comparative study using the peritoneal surface disease severity score (PSDSS*). Ann Surg Oncol, 2020; 27(1):98-106. doi: 10.1245/s10434-019-07935-2.

25. Glehen O, Kwiatkowski F, Sugarbaker PH, ELIAS D, Levine EA, De Simone M, et al.: *Cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for the management of peritoneal carcinomatosis from colorectal cancer: A multi-institutional study*. J Clin Oncol, 2004; 22(16):3284-92. Epub 2004/08/18. doi: 10.1200/jco.2004.10.012. PubMed PMID: 15310771.

26. De Bree E, Witkamp A, ZoetmuldeR FA: *Intraperitoneal chemotherapy for colorectal cancer*. J Clin Oncol, 2002; 79(1):46-61.

27. Elias D, Gilly F, Boutitie F, Quenet F, Bereder J-M, Mansvelt B, et al.: *Peritoneal colorectal carcinomatosis treated with surgery and perioperative intraperitoneal chemotherapy: Retrospective analysis of 523 patients from a multicentric french study.* J Clin Oncol, 2010; 28(1):63-8. doi: 10.1200/jco.2009.23.9285. PubMed PMID: 19917863.

28. Sugarbaker PH: *Intraperitoneal delivery of chemotherapeutic agents for the treatment of peritoneal metastases: Current challenges and how to overcome them.* Expert Opin Drug Deliv, 2019; 16(12):1393-401. doi: 10.1080/17425247.2019.1693997.