Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Imatinib Synergizes with 2, 5- Dimethylcelecoxib, a Close Derivative of Celecoxib, in HT-29 Colorectal Cancer Cells: Involvement of Vascular Endothelial Growth Factor

Saba NIKANFAR [1], Somayeh ATARI-HAJIPIRLOO [2], Fatemeh KHERADMAND *[3], Amir HEYDARI [4]

1 Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2 Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
3 Department of Biochemistry, Faculty of Medicine, Solid Tumor and Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran
4 Department of Pharmacology, Faculty of Pharmacy, Urmia University of Medical Sciences, Urmia, Iran
* Corresponding Author. E-mail: fkheradmand@yahoo.com (F.KH.); Tel. +98-914-341 66 60.

Received: 05 July 2022 / Revised: 29 August 2022 / Accepted: 30 August 2022

ABSTRACT: Combining agents with molecular targets can reduce monotherapy's required dose and toxicity in cancer treatment. In this study, we investigated the cellular viability and the mRNA expression of vascular endothelial growth factor (VEGF) and nuclear factor kappa B (NF-κB) for the synergistic effects of imatinib and 2, 5- dimethylcelecoxib (DMC) combination in human colorectal cancer cells. The effects of imatinib and DMC on cell viability were assessed by MTT assay in HT-29 cells. The dose-effect relationships and drug interaction analyses were performed using the CompuSyn Software. NF-κB and VEGF mRNA expression after treating cells with imatinib (7 μM) and DMC (24 μM) separately and in combination (3.5 μM imatinib plus 12 μM DMC) were investigated using real-time RT-PCR. A strong synergy was observed in most of the combined dose pairs of imatinib- DMC in the growth inhibition of HT-29 cancer cells. Combined treatment with 3.5 μM imatinib and 12 μM DMC resulted in a significant ($p < 0.05$) decrease in VEGF and NF-κB mRNA levels as compared to the vehicle-treated control group. In addition, VEGF mRNA reduction was significant at the mentioned concentrations for the imatinib-DMC combination compared to imatinib alone ($p < 0.05$). Our results suggest VEGF as one of the cyclooxygenase2 (COX-2) independent mechanisms for the synergistic effects of imatinib-DMC. It would be beneficial to further evaluate the potential utilization of DMC for the anti-cancer application while minimizing undesired side effects related to COX-2 inhibition and reducing the side effects of imatinib therapy.

KEYWORDS: Colorectal cancer; Cyclooxygenase 2; 2,5-dimethylcelecoxib; NF-kappa B.

1. INTRODUCTION

Colorectal cancer is the third most common neoplastic disease worldwide. According to the World Health Organization, about one million people are diagnosed with this cancer annually. Colorectal cancer is characterized by a positive expression of c-kit, a type of receptor tyrosine kinase [1], and is responsive to a specific tyrosine kinase inhibitor, imatinib. Imatinib, a chemotherapeutic agent, is the first-choice drug in patients with gastrointestinal stromal tumors [2-6]. However, in most cases, imatinib elicits a partial response, and finally, drug resistance occurs. Besides, chemotherapeutic drugs in colorectal cancer treatment have adverse side effects that limit their usage, so research for alternative drug treatment and examining lower doses of these drugs in combination with others such as anti-inflammatory drugs can be beneficial [3,6,7].

Due to the anti-carcinogenic effects of non-steroidal anti-inflammatory drugs (NSAIDs) on colorectal cancer, these drugs have recently attracted extensive research [8,9].

2,5- dimethylcelecoxib (DMC) is a celecoxib analogue, which mimics all of the numerous anti-tumor effects of celecoxib *in vitro* and *in vivo* [10,11]. Besides, DMC might not lead to the cardiovascular side effects of long-term use of cyclooxygenase2 (COX-2) inhibitors that are thought to be due to the inhibition of COX-2 and subsequent imbalance of eicosanoid levels [12]

How to cite this article: Nikanfar S, Atari-Hajipirloo S, Kheradmand F, Heydari A. Imatinib Synergizes with 2, 5- Dimethylcelecoxib, a Close Derivative of Celecoxib, in HT-29 Colorectal Cancer Cells: Involvement of Vascular Endothelial Growth Factor. J Res Pharm. 2023; 27(1): 948-956.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.374

As DMC does not have COX-2 inhibitory properties, the precise mechanisms for its chemopreventive effects are not yet known. Among multiple pathways reported, nuclear factor kappa B (NF-κB) has been shown to regulate the expression of sets of genes encoding products involved in tumorigenesis [13]. The activity of NF-κB in colon cancer cell lines is abnormally high [14]. Therefore, inhibition of the NF-κB signaling pathway may improve the response of colon cancer cells to chemotherapy. In addition, the vascular endothelial growth factor (VEGF) is an important angiogenic factor that is most strongly associated with tumor growth and metastasis [15].

Our primary aim in the present study was to examine the anti-proliferative effects of the imatinib-DMC combination on the colorectal cancer HT-29 cell line and define the interaction between these drugs. In addition, we conducted experiments to determine whether imatinib in combination with DMC could affect the mRNA expression of NF-κB and VEGF in HT-29 colorectal cancer cells.

2. RESULTS

2.1. Interactions between imatinib and DMC

Following the half-maximal inhibitory concentration (IC_{50}) results we obtained from our previous work [16], this study is reporting new findings for the combination of imatinib and DMC at their IC_{50} ratio (1:3.5). In the combined treatments, the concentrations of imatinib were 2.5, 3.5, 5, and 6.5 µM, while the concentrations of DMC were 8.75, 12, 17.5, and 22.75 µM, which were 3.5-fold higher than those of imatinib. The MTT assay results were analyzed by the isobologram method as described earlier. The combination of imatinib-DMC exhibited antagonistic interaction only at DMC doses of 8.75 µM and additive interaction at doses of 22.75 µM (Figure 1). However, all other combinations showed synergism in the ratios tested. Concentrations of 17 and 22 µM for DMC and 3.5 µM for imatinib were used to examine the drug interaction in some non-constant ratios. Most of the combined dose pairs at the ratios tested presented some degree of synergistic interactions.

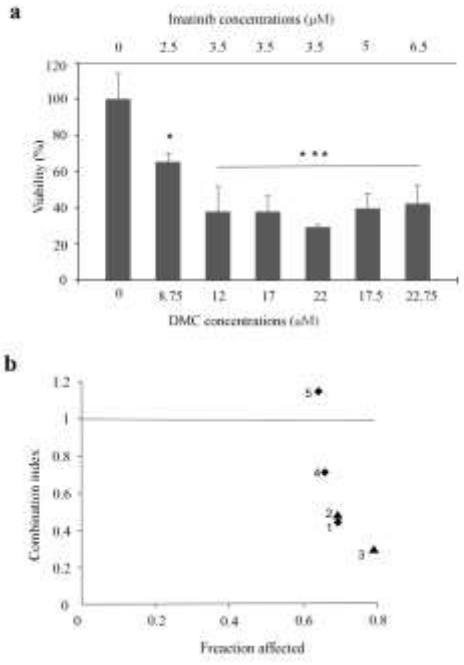

Figure 1. Growth inhibitory effects of imatinib and 2, 5- dimethylcelecoxib (DMC) at different concentrations. a) HT-29 cells were treated with different concentrations of imatinib (2.5, 3.5, 5, 6.5 µM) and DMC (8.75, 12, 17, 17.5, 22, 22.75 µM) for 24 h and analyzed by MTT assay. Columns, mean of triplicate experiments; error bars, SD. Results were analyzed by one-way ANOVA, and * represents $p < 0.05$ and *** $p < 0.0001$ versus DMSO as vehicle control. b) Combination index (CI) plot for imatinib/DMC combinations at constant (diamond) and non-constant (triangle) ratios. The antagonistic effect of DMC (8.75 µM) and imatinib (2.5 µM) was not shown. CI plot was

constructed using Chou and Talalay's method. Synergy was defined as CI lower than 1.0. The fraction affected (Fa) was calculated as [1 − (MTT signal for the experimental sample)/ (MTT signal for the untreated control)]

2.2. The effect of imatinib and DMC combination on growth inhibition of HT-29 cells *in vitro*

Based on the preliminary results of this study, a combination of 3.5 µM imatinib and 12 µM DMC was the lowest and most effective dose used for complementary experiments. Treatment with imatinib (3.5 µM) + DMC (12 µM) for 24 h caused no significant growth inhibition when compared with either drug alone (7 µM imatinib and 24 µM DMC) (Figure 2), but reduced cell growth to 38% versus control ($p < 0.05$). The combination index (CI) value for imatinib (3.5 µM) - DMC (12 µM) combination was 0.370.

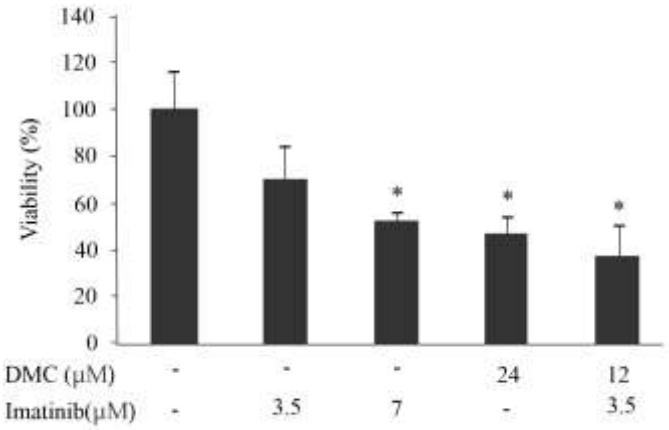

Figure 2. Anti-proliferative effects of imatinib and 2, 5- dimethylcelecoxib (DMC) treatment alone and in combination on HT-29 cells. The viable cell number was determined using the MTT colorimetric assay. The means of cell viability were compared by one-way ANOVA. * p < 0.05 as compared to the vehicle-treated control group.

2.3. Effects of imatinib and DMC treatment alone or in combination on VEGF mRNA expression

According to real-time reverse transcription polymerase chain reaction (RT-PCR) results, treatment with imatinib and DMC alone (at their IC_{50} levels) showed no significant effect on VEGF mRNA levels compared to the vehicle-treated control group in HT-29 cells. However, the combined treatment with 3.5 µM imatinib and 12 µM DMC resulted in a significant decrease in VEGF mRNA level compared to the vehicle-treated control group ($p = 0.002$) and imatinib alone ($p = 0.003$) (Figure 3).

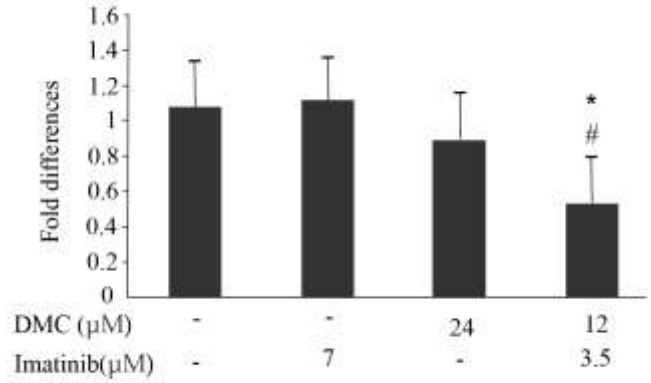

Figure 3. Effects of imatinib and 2, 5- dimethylcelecoxib (DMC) alone or in combination on VEGF mRNA levels with β-actin as the internal control by real-time RT-PCR analysis. The means of mRNA expression for three independent experiments in each treatment group were compared by one-way ANOVA.

#, $p < 0.05$ versus imatinib alone; *, $p < 0.05$ versus vehicle-control

2.4. Effects of imatinib and DMC treatment alone or in combination on NF-κB mRNA expression

In addition, qRT-PCR results showed that similar treatments with DMC but not imatinib (in their IC_{50}) had a significant (p= 0.037) effect on NF-κB mRNA expression compared to the vehicle-treated control group in HT-29 cells. However, NF-κB mRNA levels significantly (p= 0.001) decreased after combined treatment with 3.5 µM imatinib and 12 µM DMC compared to the vehicle-treated control group (Figure 4).

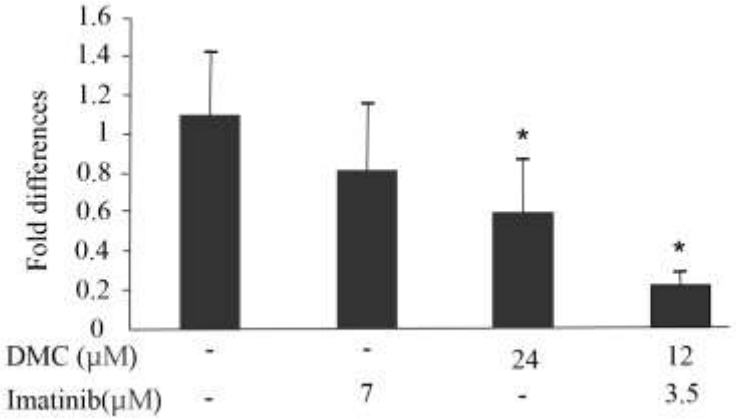

Figure 4. Effects of imatinib and 2, 5- dimethylcelecoxib (DMC) alone or in combination on NF-κB mRNA levels by real-time RT-PCR analysis. NF-κB mRNA expression was normalized to β-actin in each sample. The means of mRNA expression for three independent experiments in each treatment group were compared by one-way ANOVA.

*, $p < 0.05$ versus untreated vehicle-control

3. DISCUSSION

Our results demonstrated that the treatment with imatinib-DMC combination for 24 h reduced cell growth significantly versus control. A strong synergy was also observed in most of the combined dose pairs of imatinib and DMC in the growth inhibition of HT-29 human colorectal cancer cells. In addition, we demonstrated that the synergistic anti-proliferative effects of imatinib-DMC were associated with a decrease in VEGF mRNA levels. Also, a reduction in NF-κB mRNA expression might be a possible mechanism for the anti-proliferative effects of this combination.

Colorectal cancer is responsive to specific tyrosine kinase inhibitor imatinib. Although imatinib is not the primary therapy of choice in this cancer, it has recently been studied successfully in phase I/II clinical trials combined with some other drugs in advanced/ metastatic colorectal cancer [17, 18]. Also, particular promising effects have been found for imatinib alone or in combination with other agents in colorectal cancer [4, 19].

Since drug resistance and side effects related to imatinib treatment may significantly limit its effectiveness, using suitable combinations of imatinib and other preventive agents can be advantageous in lowering the required dose of anti-cancer drug and minimizing undesirable side effects [20,21]. Kim et al. showed that deferasirox and imatinib combination synergistically inhibited proliferation of both imatinib-sensitive and -resistance chronic myeloid leukemia cell lines.

Due to the anti-carcinogenic effects of NSAIDs, it has been reported that using these drugs along with various chemotherapeutic agents can enhance the efficacy of chemotherapy [22, 23]. Previous studies [24,25] have shown anti-proliferative effects of COX-2-selective NSAID, celecoxib, against colorectal cancer. It has been recently shown that imatinib-celecoxib combination decreases cell viability and COX-2 expression in HT-29 cells [26]. This combination also synergistically inhibited the proliferation of K562 cells [27].

Dharmapuri et al. [21] reported that imatinib-resistant K562 cells are more sensitive to imatinib following treatment with celecoxib. In addition, celecoxib contributed to the down-regulation of genes involved in imatinib resistance [28].

However, it seems that the cardiovascular side effects related to COX-2 inhibition may limit celecoxib application. The absence of COX-2 inhibitory activity in DMC, a close structural celecoxib analog, might appear to be advantageous for studying COX-2 independent anti-tumor mechanisms of DMC [11].

VEGF has a fundamental role in the angiogenic process. In this study, treatment with sub-effective concentrations of imatinib and DMC significantly decreased VEGF mRNA levels. Therefore, the synergistic effect of this combination seems to be partly mediated by VEGF, and it can be one of the COX-2 independent targets of DMC. Schultz et al. [29} reported that imatinib and carboplatin synergistically suppressed VEGF, platelet-derived growth factor (PDGF), and PDGF-Rα/ß expression in head and neck squamous cell carcinoma [30]. However, in our study, VEGF decrease was not observed when cells were treated with the higher concentrations of either agent alone. Like our results, the VEGF level was not affected in glioma cells treated with DMC.

Consistent with Posadas et al. [31] and recent work by Atari et al. [26], imatinib exposure (7μM) lead to an insignificant increase in VEGF mRNA levels. Atari et al. demonstrated that imatinib-celecoxib combination do not increase VEGF expression [26]. In addition, celecoxib has been shown to increase VEGF expression, which seems to be involved in drug resistance [32]. Therefore, imatinib-DMC appears to be more effective than imatinib-celecoxib combination, especially in the case of VEGF inhibition. However, the safety of this combination should be evaluated in future studies.

In consistence with our findings about the effects of treatment with imatinib-DMC on NF-κB levels, it has been reported that deferasirox and imatinib combination arrested cell cycle and induced apoptosis through down-regulating the expression of NF-κB and β-catenin levels and suggested this combination as an alternative option for treatment of imatinib-resistant CML [33].

Surprisingly, imatinib-celecoxib combination did not significantly decrease NF-κB mRNA levels [26]. However, according to literature, DMC alone has also been shown to down-regulate NF-κB in multiple myeloma cells [34] and decrease the transcriptional activity of NF-κB in human cervical cancer cells [35]. In our study, NF-κB mRNA expression significantly decreased after treatment with imatinib-DMC compared to the control group. Thus NF-κB might be considered a COX-2 independent mechanism for anti-proliferative effects of imatinib-DMC combination. However, the other molecular pathways should also be considered and evaluated in future studies.

The limitations of this work include 1) small sample size, 2) using only one cell line, and 3) not measuring the effects of gene expression in other dose pairs. To the best of our knowledge, there is no available work reporting the combinational effect of imatinib and DMC on cell viability. However, decreased cell viability and increased apoptosis have been reported after treatment with imatinib and celecoxib [26, 28] and OSU-03012, a celecoxib-derived phosphoinositide-dependent kinase-1 (PDK-1) inhibitor [36].

4. CONCLUSION

In conclusion, the present *in vitro* study with colon cancer cell lines demonstrated that imatinib-DMC combination significantly decreased cell viability compared to the control group. In this regard, our study presents VEGF as an important COX-2–independent molecular target involved in mediating the synergistic anti-proliferative effects of imatinib-DMC combination. NF-κB might be considered another COX-2 independent mechanism for the anti-proliferative effects of this combination, and its role should be more extensively investigated in future studies. Therefore, these findings provide further insights into the possible use of DMC alone or in combination with other drugs without the undesired cardiovascular risks related to COX-2 inhibition. However, DMC's clinical usefulness and safety should be considered in future research.

5. MATERIALS AND METHODS

5.1. Reagents and chemicals

Human colorectal cancer cell line HT-29 was obtained from the Iranian Biological Resource Center (IBRC, Tehran, Iran). DMC was ordered from Sigma-Aldrich (St. Louis, MO, USA). Imatinib mesylate, available under the trade name Glivec® or Gleevec, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA).

5.2. Cell culture and drug treatment

HT-29 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with stable glutamine (PAA), supplemented with 10% fetal bovine serum (PAA) and 100 units/mL of penicillin at 37˚C with 5% CO_2. All drugs were dissolved in dimethyl sulfoxide (DMSO), which was used as the vehicle to deliver drugs. The final concentrations of DMSO in vehicle-control wells were equal to that of the test wells and did not exceed 0.1% throughout the study [37]. Before use, the stock solutions of drugs (20 mM) were prepared and diluted in DMEM. The drug concentrations used are listed in the table.

Table. Drug concentrations used in this study

	Vehicle control group	Imatinib alone (IC_{50})	Dimethylcelecoxib alone (IC_{50})	Combination (1:3.5 ratio)				Combination (non-constant ratio)	
Dimethylcelecoxib (μM)	0	0	24	8.75	12	17.5	22.75	17	22
Imatinib (μM)	0	7	0	2.5	3.5	5	6.5	3.5	3.5

5.3. Cell viability

Cell proliferation was analyzed by the MTT method in triplicate. Briefly, HT-29 cells (5,000 cells/well) were seeded in 96-well plates. At 24 h after seeding, the cells were treated with distinct concentrations of imatinib and DMC or their combination at a ratio of 1:3.5. One day after treatment, 10 μl of MTT reagent was added per well and the plates were incubated at 37˚C for another 3 h. The reaction was stopped by the removal of MTT-containing media. After that, 100 μl of Crystal Dissolving Solution was added to solubilize formazan crystals. Absorbance at 570 nm was recorded using a microplate reader (Stat Fax 2100, Awareness Technology Inc.). Experiments were repeated at least three times in triplicate. The cell viability was calculated as the absorbance ratio of treated samples to the untreated vehicle control.

5.4. Analyses of drug effects and interactions

Analyses of the dose-effect relationship and drug interaction for imatinib in combination with DMC were performed according to the median-effect method of Chou and Talalay [38]. Using the CompuSyn Software. The combined effect of imatinib and DMC was analyzed using the same method by calculating the CI. CI <1 indicates synergism, CI =1 indicates additive effect, and CI > 1 indicates antagonism [39].

5.5. RNA extraction and cDNA synthesis

Total RNA of about 5×10^5 HT-29 treated cells was extracted using the Total RNX-Plus Solution Kit (CinnaGen Co., Iran) according to the manufacturer's protocol. The purity of the extracted RNA was confirmed by measuring the ratio of optical density at 260 nm to that at 280 nm, and the integrity was examined by electrophoresis on the agarose gel. Complementary DNA (cDNA) synthesis was performed using 2 μg of total RNA by 2-steps RT-PCR Kit (Vivantis, Malaysia) according to the manufacturer's instructions. The synthesized cDNA was directly used as a template for quantitative RT-PCR using Bio-Rad iQ5 cycler Sequence detection system (Bio-Rad Laboratories Inc.).

5.6. Real-time RT-PCR

Primers were designed for real-time RT-PCR and their sequences for NF-κB were 5'-GGAGATCGGGAAAAAGAGC-3' (sense) and 5'- GACTCCACCATTTTCTTCCTC-3' (anti- sense). The primers for VEGF were 5'- AGGAGGAGGGCAGAATCATC-3' (sense) and 5'-GGCACACAGGATGGCTTGAA-3' (anti-sense). The β-actin gene was used as the reference gene for normalization by the following primers: forward, 5'-CTGGAACGGTGAAGGTGACA-3' and reverse, 5'-TGGGGTGGCTTTTAGGATGG-3'. Real-time RT-PCR was performed by using the AccuPower® 2X Greenstar qPCR master mix (Bioneer, South Korea) in a total volume of 25 μl according to the manufacturer's instructions. The reactions were prepared in a 96-well optical plate for 10 min at 95˚C, followed by 40 cycles of 20 sec at 95˚C and 45 sec at 59˚C. In addition, a no- template control was used to test the potential contamination and primer dimer formation. The relative expression of each mRNA was calculated using the 2-ΔΔCt method, where Ct is the threshold cycle [40], and relative expression levels of mRNA were normalized to β-actin.

5.7. Statistical analysis

Parametric tests were used because distributions of values were normal. The results are presented as mean ± standard deviation (SD) of triplicate experiments repeated at least three times. Statistical significance of differences between mean values was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test using SPSS 16 statistical analysis software (SPSS Inc. Chicago, IL). The level of significant difference was set at $p < 0.05$. The fold differences of gene expression normalized to control were presented graphically in the form of histograms using the Microsoft Excel computer program.

Acknowledgements: This study was conducted with financial support that was provided by the Urmia University of Medical Sciences. The authors would like to thank members of the Cellular and Molecular Research Center of Urmia University of Medical Sciences, Urmia, Iran.

Author contributions: Concept – S.N.; Design – S.N. , F.KH; Supervision – F.KH; Resources – S.N.; materials -S.AH; Data Collection and/or Processing – S.AH; Analysis and/or Interpretation – A.H; Literature Search – A.H.; Writing – S.N., S.AH.; Critical Reviews – F.KH, A.H.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

REFERENCES

1. Pratschke KM, Atherton MJ, Sillito JA, Lamm CG. Evaluation of a modified proportional margins approach for surgical resection of mast cell tumors in dogs: 40 cases (2008–2012). J Am Vet Med Assoc. 2013; 243(10): 1436–1441. [CrossRef]
2. Weitz J, Koch M, Debus J, Hohler T, Galle PR, Buchler MW. Colorectal cancer. Lancet. 2005; 365(9454): 153–165. [CrossRef]
3. Kumari S, Sherriff JM, Spooner D, Beckett R. Peripheral neuropathy induced by red yeast rice in a patient with a known small bowel gastrointestinal tumour. BMJ Case Rep. 2013; 2013:bcr2013009060. [CrossRef]
4. Attoub S, Rivat C, Rodrigues S, Van Bocxlaer S, Bedin M, Bruyneel E, et al. The c-kit tyrosine kinase inhibitor STI571 for colorectal cancer therapy. Cancer Res. 2002; 62: 4879–4883.
5. Stahtea XN, Roussidis AE, Kanakis I, Tzanakakis GN, Chalkiadakis G, Mavroudis D, Kletsas D, Karamanos NK. Imatinib inhibits colorectal cancer cell growth and suppresses stromal-induced growth stimulation, MT1-MMP expression and pro-MMP2 activation. Int J Cancer. 2007; 121(12): 2808–2814. [CrossRef]
6. Farag S, Verschoor AJ, Bosma JW, Gelderblom H, Kerst JM, Sleijfer S, Steeghs N. J Clin Pharmacol. 2015; 55:920–925. [CrossRef]
7. Rossi F, Yozgat Y, de Stanchina E, Veach D, Clarkson B, Manova K, Giancotti FG, Antonescu CR, Besmer P. Imatinib upregulates compensatory integrin signaling in a mouse model of gastrointestinal stromal tumor and is more effective when combined with dasatinib. Mol Cancer Res. 2010; 8(9): 1271–1283. [CrossRef]
8. Hamoya T, Fujii G, Miyamoto S, Takahashi M, Totsuka Y, Wakabayashi K, Toshima J, Mutoh M. Effects of NSAIDs on the risk factors of colorectal cancer: a mini review. Genes Environ. 2016; 38(1): 1-7. [CrossRef]
9. Grancher A, Michel P, Di Fiore F, Sefrioui D. Aspirin and colorectal cancer. Bull Cancer. 2017; 105(2): 171-80. [CrossRef]
10. Schönthal AH. Anti-tumor properties of dimethyl-celecoxib, a derivative of celecoxib that does not inhibit cyclooxygenase-2: implications for glioma therapy. Neurosurg Focus. 2006; 20(4): E21. [CrossRef]
11. Backhus LM, Petasis NA, Uddin J, Schönthal AH, Bart RD, Lin Y, Starnes VA, Bremner RM. Dimethyl celecoxib as a novel non-cyclooxygenase 2 therapy in the treatment of non-small cell lung cancer. J Thorac Cardiovasc Surg. 2005; 130(5): 1406–1412. [CrossRef]
12. Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. Bmj. 2006; 332(7553): 1302-8. [CrossRef]
13. Garg A, Aggarwal BB. Nuclear transcription factor- kappaB as a target for cancer drug development. Leukemia. 2002; 16(6): 1053–1068. [CrossRef]
14. Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-kB collaboration and crosstalk in cancer. Cytokine Growth Factor Rev. 2010; 21(1): 11–19. [CrossRef]

15. Carmeliet P. Blood vessels and nerves: Common signals, pathways and diseases. Vol. 4, Nature Reviews Genetics. 2003; 4(9): 710–720. [CrossRef]

16. Atari-Hajipirloo S, Nikanfar S, Heydari A, Kheradmand F. Imatinib and its combination with 2,5-dimethyl-celecoxibinduces apoptosis of human HT-29 colorectal cancer cells. Res Pharm Sci. 2017; 12(1): 67–73. [CrossRef]

17. Kelley RK, Hwang J, Magbanua MJM, Watt L, Beumer JH, Christner SM, Baruchel S, Wu B, Fong L, Yeh BM, Moore AP, Ko AH, Korn WM, Rajpal S, Park JW, Tempero MA, Venook AP, Bergsland EK. A phase 1 trial of imatinib, bevacizumab, and metronomic cyclophosphamide in advanced colorectal cancer. Br J Cancer. 2013; 109(7): 1725–1734. [CrossRef]

18. Hoehler T, Von Wichert G, Schimanski C, Kanzler S, Moehler MH, Hinke A, Seufferlein T, Siebler J, Hochhaus A, Arnold D, Hallek M, Hofheinz R, Hacker UT. Phase I/II trial of capecitabine and oxaliplatin in combination with bevacizumab and imatinib in patients with metastatic colorectal cancer: AIO KRK 0205. Br J Cancer. 2013; 109(6): 1408–1413. [CrossRef]

19. Abdel-Aziz AK, Azab SSE, Youssef SS, El-Sayed AM, El-Demerdash E, Shouman S. Modulation of imatinib cytotoxicity by selenite in HCT116 colorectal cancer cells. Basic Clin Pharmacol Toxicol. 2015; 116(1): 37–46. [CrossRef]

20. Radujkovic A, Topaly J, Fruehauf S, Zeller WJ. Combination treatment of imatinib-sensitive and -resistant BCR-ABL-positive CML cells with imatinib and farnesyltransferase inhibitors. Anticancer Res. 2006; 26(3 A): 2169–2177.

21. Dharmapuri G, Doneti R, Philip GH, Kalle AM. Celecoxib sensitizes imatinib-resistant K562 cells to imatinib by inhibiting MRP1-5, ABCA2 and ABCG2 transporters via Wnt and Ras signaling pathways. Leuk Res. 2015; 39(7): 696–701. [CrossRef]

22. Xiao H, Zhang Q, Lin Y, Reddy BS, Yang CS. Combination of atorvastatin and celecoxib synergistically induces cell cycle arrest and apoptosis in colon cancer cells. Int J Cancer. 2008; 122(9): 2115–2124. [CrossRef]

23. El-Awady RA, Saleh EM, Ezz M, Elsayed AM. Interaction of celecoxib with different anti-cancer drugs is antagonistic in breast but not in other cancer cells. Toxicol Appl Pharmacol. 2011; 255(3): 271–86. [CrossRef]

24. Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, Tang J, Rosenstein RB, Wittes J, Corle D, Hess TM, Woloj GM, Boisserie F, Anderson WF, Viner JL, Bagheri D, Burn J, Chung DC, Dewar T, Foley TR, Hoffman N, Macrae F, Pruitt RE, Saltzman JR, Salzberg B, Sylwestrowicz T, Gordon GB, Hawk ET. Celecoxib for the Prevention of Sporadic Colorectal Adenomas. N Engl J Med. 2006; 355(9): 873–884. [CrossRef]

25. Arber N, Eagle CJ, Spicak J, Rácz I, Dite P, Hajer J, Zavoral M, Lechuga MJ, Gerletti P, Tang J, Rosenstein RB, Macdonald K, Bhadra P, Fowler R, Wittes J, Zauber AG, Solomon SD, Levin B. Celecoxib for the Prevention of Colorectal Adenomatous Polyps. N Engl J Med. 2006; 355(9): 885–895. [CrossRef]

26. Atari-Hajipirloo S, Nikanfar S, Heydari A, Noori F, Kheradmand F. The effect of celecoxib and its combination with imatinib on human HT-29 colorectal cancer cells: Involvement of COX-2, Caspase-3, VEGF and NF-κB genes expression. Cell Mol Biol. 2016; 62(2): 68–74.

27. Li RJ, F.J.Gong GSZ. Cytotoxic activities of Celecoxib on leukemic cells and the synergistic effects of Celecoxib with Imatinib thereupon. Zhonghua Yi Xue Za Zhi. 2006; 86: 1417–1420

28. Arunasree KM, Roy KR, Anilkumar K, Aparna A, Reddy GV, Reddanna P. Imatinib-resistant K562 cells are more sensitive to celecoxib, a selective COX-2 inhibitor: Role of COX-2 and MDR-1. Leuk Res. 2008; 32(6): 855–864. [CrossRef]

29. Schultz JD, Rotunno S, Riedel F, Anders C, Erben P, Hofheinz RD, Faber A, Thorn C, Sommer JU, Hörmann K, Sauter A. Synergistic effects of imatinib and carboplatin on VEGF, PDGF and PDGF-Rα/β expression in squamous cell carcinoma of the head and neck in vitro. Int J Oncol. 2011; 38(4): 1001–1012. [CrossRef]

30. Virrey JJ, Liu Z, Cho H-Y, Kardosh A, Golden EB, Louie SG, Gaffney KJ, Petasis NA, Schönthal AH, Chen TC, Hofman FM. Antiangiogenic Activities of 2,5-Dimethyl-Celecoxib on the Tumor Vasculature. Mol Cancer Ther. 2010; 9(3): 631–641. [CrossRef]

31. Posadas EM, Kwitkowski V, Kotz HL, Espina V, Minasian L, Tchabo N, Premkumar A, Hussain MM, Chang R, Steinberg SM, Kohn EC. A prospective analysis of imatinib-induced c-KIT modulation in ovarian cancer: a phase II clinical study with proteomic profiling. Cancer. 2007; 110(2): 309–317. [CrossRef]

32. Xu B, Wang Y, Yang J, Zhang Z, Zhang Y, Du H. Celecoxib induces apoptosis but up-regulates VEGF via endoplasmic reticulum stress in human colorectal cancer in vitro and in vivo. Cancer Chemother Pharmacol. 2016; 77(4): 797–806. [CrossRef]

33. Kim DS, Na YJ, Kang MH, Yoon SY, Choi CW. Use of deferasirox, an iron chelator, to overcome imatinib resistance of chronic myeloid leukemia cells. Korean J Intern Med. 2016; 31(2): 357–366. [CrossRef]

34. Kardosh A, Soriano N, Liu YT, Uddin J, Petasis NA, Hofman FM, Chen TC, Schönthal AH. Multitarget inhibition of drug-resistant multiple myeloma cell lines by dimethyl-celecoxib (DMC), a non-COX-2 inhibitory analog of celecoxib. Blood. 2005; 106(13): 4330–4338. [CrossRef]

35. Deckmann K, Rörsch F, Geisslinger G, Grösch S. Dimethylcelecoxib induces an inhibitory complex consisting of HDAC1/NF-κB(p65)RelA leading to transcriptional downregulation of mPGES-1 and EGR1. Cell Signal. 2012; 24(2): 460–467. [CrossRef]

36. Tseng PH, Lin HP, Zhu J, Chen KF, Hade EM, Young DC, Byrd JC, Grever M, Johnson K, Druker BJ, Chen CS. Synergistic interactions between imatinib mesylate and the novel phosphoinositide-dependent kinase-1 inhibitor OSU-03012 in overcoming imatinib mesylate resistance. Blood. 2005; 105(10): 4021–4027. [CrossRef]

37. Yang Z, Xiao H, Jin H, Koo PT, Tsang DJ, Yang CS. Synergistic actions of atorvastatin with gamma-tocotrienol and celecoxib against human colon cancer HT29 and HCT116 cells. Int J Cancer. 2010; 126(4): 852-63. [CrossRef]

38. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul. 1984; 22: 27–55. [CrossRef]

39. Chou TC. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev. 2006; 58(3): 621-81. [CrossRef]

40. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25(4): 402–408. [CrossRef]