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Immunological Effects of Remdesivir on Colon Cancer Cell Line

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ABSTRACT

Background: Colon cancer is considered the third most frequent malignant tumor in the world as well as the leading cause of mortality. Unlike other antiviral agents, remdesivir has been investigated for new therapeutic options in various cancer types, but its impact on colon cancer cell death and immune response has not been well documented.
Objectives: To assess the effects of remdesivir on the viability of SW480 colon cancer cells and the modulatory effect on immune responses in the colon cancer cell line.
Materials and methods: SW480 colon cancer cells were exposed to different doses of remdesivir (62,5, 125, 250, 500, 1000, and 2000 µg/mL). Cell viability was measured in MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay, and the immune response was probed through the expression of cytokines directly using enzyme-linked immunosorbent assay method of IL-23 (interleukin-23), TGF-β (human transforming growth factor-β), and TNF-α (tumor necrosis factor-α). The control group included cells incubated along with the same medium without the addition of any compounds, whereas the experimental group included cells exposed to different doses of remdesivir.
Results: Remdesivir exhibited the highest inhibitory effect on SW480 colon cancer cell viability at a dose of 1000 µg/µl. Remdesivir had an influence on the immune response, as evident, IL-23 and TGF-β demonstrated mild decrease, and TNF-α demonstrated a sharp increment at the highest concentration of 125 µg/µL.
Conclusion: Remdesivir has demonstrated potential for its cytotoxic effects on colon cancer cells, and it has potentially a significant modulatory effect-related cytokines.
Keywords: Antiviral agent; Colon cancer; Cytokines; Remdesivir; SW480.

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INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer globally and the second leading cause of cancer-related deaths, with an estimated 1.9 million new CRC and 935,000 CRC deaths in 2020. In the advanced stages, the prognosis remains poor, with approximately 10% five-year survival rate [1]. As such, considering the incidence and mortality of CRC, it is one of the most heavily investigated types of cancer with many continual advances in cancer research.

In the advanced stages of metastatic CRC (mCRC), therapeutic strategies for patients with mCRC remain limited due to intractable intrinsic insensitivity and acquired resistance of the tumors against all the conventional therapeutic strategies, including surgery, radiotherapy, and chemotherapy. Tumor heterogeneity further adds to the complexity of this tumor. Remarkable progress in cancer research has been seen over the recent years. Unfortunately, the use of immune checkpoint inhibitors (ICIs) in cancer therapy represents a significant unmet medical need in the treatment of colorectal cancer (CRC). This is due to multiple intrinsic resistance mechanisms within the tumor microenvironment [2].

It was perceived that with ICIs, a paradigm shift had occurred and that it was finally possible to exploit the

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body defenses to eliminate cancer cells. Pembrolizumab and nivolumab, which act against the PD-1/PD-L1 pathway, have successfully changed the treatment landscape for certain cancer types such as melanoma and some types of lung cancer. And yet, in CRC and, specifically, in microsatellite stable (MSS) CRC, these compounds are less efficient. This may be due to the notion that ICIs work optimally in an immunogenic tumor milieu with defective T-cell infiltration into the tumor microenvironment (TME) [3].

Other recent studies have focused on circumventing these constraints (and focusing on combination regimens to modulate the TME), such as a combination of botensilimab, a novel agent that blocks the immune checkpoint molecule CTLA-4, and balstilimab, an anti-PD-1 antibody, used in patients with relapsed or refractory MSS mCRC. In this case, early evidence of clinical activity with this combination yielded evidence of the hypothesis that reinvigoration of response through dual blockade of immune checkpoints could re-stimulate tumors that became refractory to immune control by the native immune system [1].

An example of its roles in CRC is lncRNAs (long noncoding ribonucleic acids), which are master gene regulators in cancer biology, positively or negatively regulating cell proliferation, apoptosis, and immune response, among other aspects of the disease. Certain lncRNAs have also been associated with poor prognosis or response to therapy. A group of lncRNAs- MYOSLID among them- that are related to cellular senescence and called CSRLs (cellular senescence-related lncRNAs) are prognostic markers and targets for the treatment of CRC [4].

This shift towards understanding these molecules can potentially be transformed into a clinical benefit by enabling an approach tailored to the patient (precision medicine) as well as allowing the researchers to identify responders to immunotherapy or targeted therapies via novel biomarkers. For instance, lncRNAs might function as biomarkers for stratifying patients. Eryilmaz et al. explored the cytotoxic and antitumor action of remdesivir [5]. This drug displayed an interesting means of action through the suppression of the NF-kappa B signalling pathway and this resulted in the cure of cancer since it could show inhibition of proliferation rate and induction of apoptosis in tumor cells [5].

An alternative approach to optimize cancer immunotherapy is to block some molecules that regulate the T-cell immune response, an example is the transmembrane protein CD43, a key regulator of T-cell activation and antitumour immune responses, is widely and abundantly expressed in many different immune cells, such as lymphocytes, neutrophils, and others. Liu and colleagues demonstrated that CD43 can be specifically targeted in CRC for immunotherapy. The targeting of CD43 can optimize the interaction between T cells and tumor cells, which can promote tumor immune response by overcoming the immune escape mechanism of CRC cells, thereby increasing the efficiency of existing immunotherapy [6].

Resistance to immunotherapy remains a major issue in CRCs, and several investigations have described strong connections between the immune suppressive cells and cytokines comprising TME and mechanisms of resistance. Marquedant et al. demonstrated these mechanisms and outlined possible pathways for modulating the TME to enhance ICI efficacy and the role of dendritic cells (DCs) in reducing resistance: Increasing DC numbers might potentially sensitize CRC tumors to ICI therapy to a higher level of efficacy, including objective response rate and duration of survival. Overall, increasing the numbers of DCs would lead to increased antigen presentation, which in turn would stimulate T-cell activation and proliferation [7–9].

Remdesivir is an antiviral drug, and drug repurposing is an important method for detecting its activity against colon cancer cell lines. The current study was conducted to evaluate the remdesivir effects on the SW480 colon cancer cells as well as the subsequent immune effects in these cells.

MATERIALS AND METHODS

The study was performed in laboratory of the College of Medicine, University of Babylon, Iraq, during August 2023 to March 2024. The Ethical approval reference No. was 236 issued on July 18, 2023, by the College of Medicine, University of Babylon, Iraq. The study contained the use of cancer cell line with the use of remdesivir. The research utilized a cell line commonly used in chemotherapy applications. Cells were cultured in Dulbeccos Modified Eagle Medium (DMEM), which is used for reducing the chances of microbial contamination in cell culture. Between 0.5 and 1 mL of penicillin-streptomycin solution are added to 100 mL of cell culture media for a final concentration of 50 to 100 I.U./mL penicillin and 50 to 100 µg/mL streptomycin. It was enriched with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C, 5% CO_2, and 95% humidity. Before treatments, cells were cultivated in 75 cm^2 culture flasks until achieving 70–80 percent confluence. The experimental groups comprised untreated control cells and cells subjected to remdesivir treatment. Remdesivir concentrations were determined based on initial cytotoxicity experiments.

Cytotoxicity assay

The proliferation of cells was evaluated after 24 hours of 5×10^3 cells per 0.1 mL seeding in 96-well microplates utilizing the 3(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay. The resulted MTT powder solution in phosphate-buffered saline was introduced into the wells of a 96-well microplate. The initial group of cells is designated as the control. The second group of cells was administered with varying concentrations (62,5, 125, 250, 500, 1000, and 2000 µg/ml) of remdesivir. Dimethyl sulfoxide (DMSO) was introduced to the wells following 4 hours of incubation at 37°C, and the absorbance was quantified at 570 nm. Cisplatin is a specific anti-CRC drug was used as a positive control. Cell viability was calculated using the following equation:
%Cell viability (%CV) = optical density (OD) of test – OD of blank)/(OD of control – OD of blank) × 100%

Quantitative enzyme-linked immunosorbent assay (ELISA) protocols

The proteins IL-23, TGF-β, and TNF-α were quantified utilizing specific ELISA kits (Human Interleukin 23, IL-23 ELISA Kit (Cat. No: E0074Hu-Korain-China), Human Transforming Growth factor β (TGF-β) ELISA Kit (Cat. No: E3051Hu-Korain-China), and human tumor necrosis factor alpha (TNF-α), TNF-A ELISA Kit (Cat. No: E0082Hu-Korain-China). The protocols for ELISA kits stipulate the utilization of pre-coated antibody plates, biotinylated detection antibodies, and suitable wash and substrate solutions. Absorbance was quantified at 450 nm within 30 minutes post-application of the stop solution, in accordance with kit protocols, to ascertain the concentration of proteins in the su-

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pernatants of cell cultures. Quantitative ELISA technique includes fine-tuning procedure for accurate measurement of target protein or biomarker. Data are separated by centrifugation, leaving a fresh supernatant. Standards are reconstituted and serially dilute to make a standard curve for the calibration of concentration. Pre-coated ELISA plate (which is already incubated with the antibodies) recognizes the sample's antigen. After incubation, biotinylated antibodies and enzyme-bound streptavidin were added one by one, and then thoroughly washed to get rid of bound substances. The solution was added to the substrate to produce a reproducible color shift in relation to antigen concentration. Aqueous solution halts the reaction, and the absorbance of this solution at 450 nm was measured 10 minutes later. Washes must be done well, as stated in protocols to eliminate noise and improve specificity, and the kit's instructions include streamlined instructions for cross-contamination and repeatability.

The biomarker and colon-specific antigen were calculated using quantitative measurement by a standard curve of known concentrations of the biomarker, which gives the concentrations of the experimental samples.

Quality control

As part of the control for reliability and reproducibility, we conducted several quality controls during the study. They incubated SW480 colon cancer cells at 37°C, 5% CO_2, and 95% humidity and monitored for contamination regularly with a sterile incubator and administered penicillin-streptomycin. Every reagent (MTT, ELISA kit etc.) was purchased from a trusted vendor and used as per the instructions of the manufacturer. Each experiment was replicated three times to limit error, and appropriate controls were used: Cells were used as negative controls and cisplatin as a positive control. All the instrument spectrophotometers and incubators were set up in advance. Also, ELISA experiments were confirmed by drawing standard curves for each cytokine measured.

Statistical analysis

The analysis was conducted using the statistical package for the social sciences (SPSS) version 23 (IBM, Chicago, USA). Mean ± SEM was used to present the data all through the paper unless stated otherwise. The ANOVA tests was used to assess the differences between the mean values of various treatment groups. Tukey's test was utilized to identify the specific groups exhibiting statistically significant differences. A statistically significant difference was established with a P-value of less than 0.05.

RESULTS

Effect of remdesivir on cell viability

The cells (which are considered as negative control group, using the carrier, DMSO) without any treatment) are very much viable. The cell viability is almost at 100%, however the viability decreased with the incremental doses of remdesivir. The cell viability of colon cancer cells is significantly (P-value < 0.05) affected in the treated group in comparison with controls. In the lowest dosage 62.5 µg/Ml, the viability was approximately 60%, which means there is a moderate cytotoxic effect. The cell viability reduces further to 30% and below as the dosage increases to 125 µg/mL and 250 µg/mL. The descending trend line continues as the concentration increases 500 µg/mL and above. At this point, the viability of cells reduced to almost zero percent when treated with concentrations more than 750 µg/mL of remdesivir, which is significant (P-value < 0.05) when compared to the control group (Figure 1).

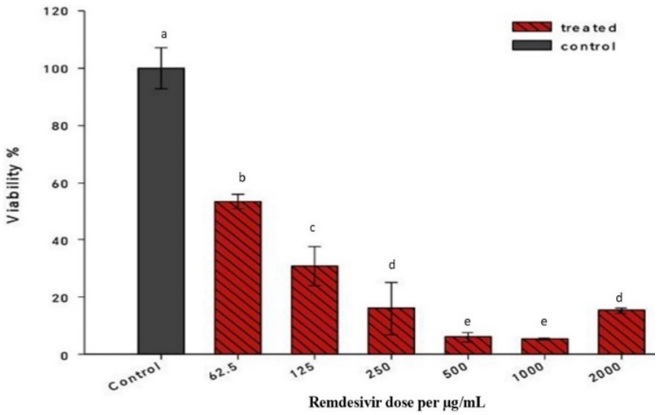

Figure 1. Effects of remdesivir on the viability of SW480 colon cancer cells. Different small letters above bars: Statistically significant difference (P-value < 0.05). Similar small letters above bars: Not significant difference (P-value > 0.05).

Modulation of IL-23 production by remdesivir

Overall, it is evident that at concentrations 62.5 µg/mL and 125 µg/mL of remdesivir, IL-23 production is higher. However, this is not significant difference when compared to the untreated control. Additionally, at 500 µg/mL, the level of IL-23 exhibited drastic decline, this demonstrates that remdesivir can effectively (P-value < 0.05) repress immunoregulatory. This pattern has showed that although remdesivir can modulation on IL-23 production, the pattern of concentration exerts influence on remdesivir potency in downregulation of IL-23 (Figure 2).

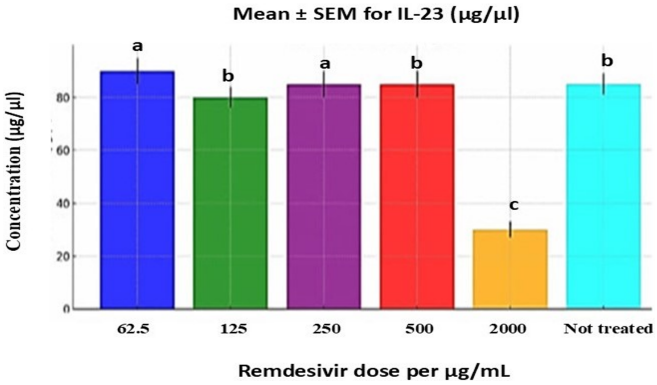

Figure 2. Effects of remdesivir on the immunoregulatory cytokine, IL-23 production in the SW480 colon cancer cells. Different small letters above bars: Statistically significant difference (P-value < 0.05). Similar small letters above bars: Not significant difference (P- value > 0.05).

Effects of remdesivir on TGF-β Levels

TGF-β production was constant over the use of remdesivir, with no significant difference for the lower concentrations of 62.5, 125, and 250 µg/mL (P-value> 0.5). At the highest concentration, 2000 µg/mL, the TGF-β production from the tumor cells went down significantly (P-value $<$ 0.05) compared with these in the control cells (Figure 3).

Effect of remdesivir on TNF-α production

At 125 µg/mL of remdesivir, the production of TNF-α (inflammation-linked cytokine) increased significantly (P-value $<$ 0.05). This surge of TNF-α followed by an increased cell death may indicate an initial immune-stimulating effect of remdesivir at this concentration. However, the number of cells producing TNF-α decreases sharply with each subsequent increase in remdesivir, and approaching almost zero at 500 µg/mL (P-value $<$ 0.05) as shown in Figure 4.

DISCUSSION

The current observations revealed that remdesivir has a dose-dependent cytotoxic effect on SW480 colon cancer cells, as reflected by a decrease in the cell viability as the concentration increases. Cell viability reduced to ∼60% implying a moderate cytotoxic effect at lower concentration (62.5 µg/mL). While the cell viability was reduced to almost zero at higher concentrations (500 µg/mL and up). This outcome overlaps with the results from Eryilmaz et al. [5], who reported a cytotoxic effect of remdesivir on several cancer cell lines: Flow cytometric analysis revealed a significant inhibition of cell proliferation and increase in apoptosis, in a dose-dependent manner after 48 hours of treatment in all cancer cell lines, due to inhibited activity of RNA-dependent RNA polymerase (RdRp) [10]. Shayan et al. [11] reported on oncolytic HSV-1 virotherapy on colon cancer cell lines, including SW480, reporting that certain viral therapies could exert a similar cytotoxic effect on cancer cells under hypoxic conditions [12].

The current study revealed that there is a concentration-dependent effect of remdesivir on IL-23 production; at lower concentrations, it has no remarkable effect on IL-23 levels, and at 500 µg/mL it significantly decreased IL-23 levels. This suppression of IL-23 production can modulate immune response and downregulate tumor-promoting inflammation. These observations are also in keeping with the findings of Popēna et al. [10], who showed that extracellular vesicles (EVs) from CRC cells, such as those originating from the cell line SW480, can impact immune cell (macrophage and monocyte) cytokine secretion profile, altering cytokine production in favor of an immunosuppressive environment. Lower concentrations suppress IL-23 activity, which, by suppressing IL-23 activity, can counteract the immune-suppressive effects promoted by tumor microenvironment-remodeling EVs. This is another mechanism by which remdesivir can be a modifier of the tumor microenvironment.

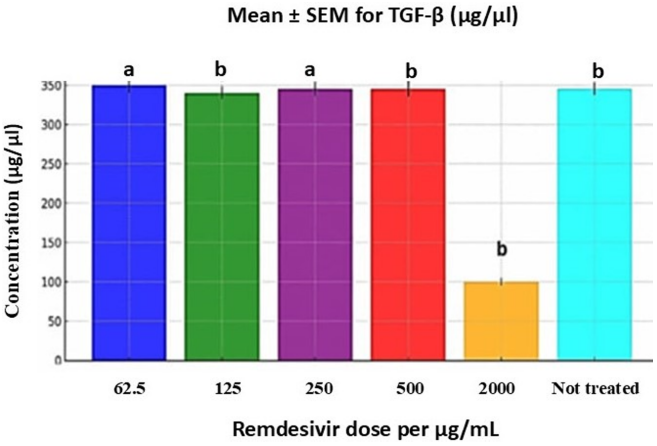

Figure 3. Effects of remdesivir on the production of a tumor-promoting cytokine, TGF-β, in the SW480 colon cancer cells. Different small letters above bars: statistically significant difference (P-value $<$ 0.05). Similar small letters above bars: Not significant difference (P-value $>$ 0.05).

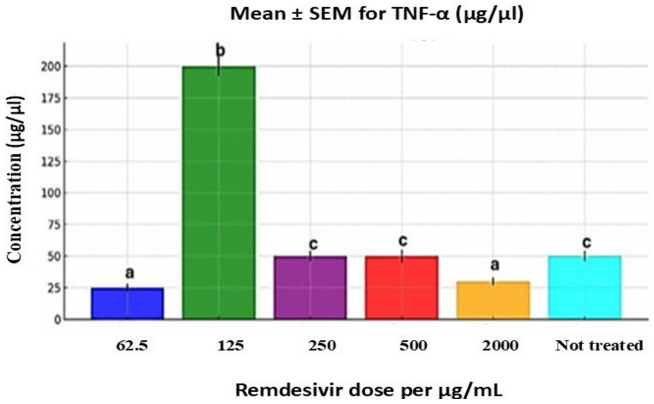

Figure 4. Effects of remdesivir on the production of the TNF-α in the SW480 colon cancer cells. Different small letters above bars: Statistically significant difference (P-value $<$ 0.05). Similar small letters above bars: Not significant difference (P-vale $>$ 0.05).

It was also clear that the effect on TGF-β production was related to the concentration of remdesivir, with no effect observed at lower concentrations of the drug, but an appreciable fall at 500 µg/mL. TGF-β has been implicated in tumor growth and immune suppression. Inhibition of TGF-β would be expected to be anti-tumor and antimetastatic by disrupting the immunosuppression that favors tumor growth. This result is in line with the findings of Hartman et al. (2009), who have demonstrated the role of Estrogen receptor β (Erβ) in SW480 colon cancer cells by showing that ERβ expression attenuates TGF-β signaling and subsequently reduces cell proliferation and migration [13]. Although remdesivir does not directly target ERβ, it might achieve similar antitumoral effects by disrupting TGF-β (like the current study finding) signaling pathways that are required for tumor growth and metastasis.

At a low concentration of (125 µg/mL), there was a strong increase in TNF-α, which is indicative of a pro-inflammatory response. As the drug concentration increased, TNF-α dramatically decreased, showing suppression in the cell viability at higher doses. This biphasic effect on TNF-α aligns with a recent preclinical study [14], which demonstrated that targeting the epidermal growth factor receptor (EGFR) can initially stimulate immune responses. However, on the long term, it can diminish the production of pro-inflammatory cytokines [15]. In this way, the remdesivir effect on TNF-α might be

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part of its broader immunomodulatory activity and could be a factor facilitating tumor-associated inflammation control.

Remdesivir was explored during the COVID-19 pandemic for its antiviral properties. Hagman et al. [16] found that a few groups reported that remdesivir lowered viral dynamics with ultimately little impact on mortality. If the effects of remdesivir on cancer cells are like those observed during COVID-19, it seems that remdesivir cytotoxicity might be more pronounced in the context of cancer particularly for cancer cell lines like SW480 that could simply suffer direct inhibition of the cellular replication machinery. These results align with a systematic review and meta-analysis on remdesivir combined with other drugs for COVID-19 therapy [13], indicating in-vivo efficacy and safety with mixed results in different patient populations. Because remdesivir exerts distinct effects on different biological contexts, the current data shows dose-dependent effects on cytokine production and cell viability. These differential effects appear to be distinct from its known antiviral effect since both the anti-cytokine and anti-apoptotic effects were observed at concentrations hundreds of times below those needed for antiviral activity. The remdesivir immunosuppressive effects could be due to interactions with enzymes or molecules other than the viral base-proofreading complex [5, 17].

It was observed that the inhibition of SW480 colon cancer cell survival by remdesivir at 1000 g/L was in line with recent data suggesting it may be anti-tumor. Remdesivir significantly reduced cell viability in several cancer cell lines such as prostate cancer, hepatocellular carcinoma, and malignant melanoma at 10, 25, and 50 µM [5].

The moderate decrease in IL-23 and TGF-β and a dramatic uptick in TNF-α at 125 g/L point to a detailed modulation of cytokine production by remdesivir [18]. IL-23 and TGF-β help to differentiate and maintain Th17 cells involved in inflammatory responses and some autoimmune diseases [19]. Such a decrease in these cytokines can indicate a slowing down of Th17-driven processes. The strong rise in TNF-α, a pro-inflammatory cytokine, on the other hand, may indicate inflammatory reactions. This two-pronged modulation reveals the subtle immunosuppression effect of remdesivir [20].

Other antivirals, by contrast, have been more variable in their effect on cancer cell proliferation and immunity. In one study of remdesivir and cyclosporine, for example, it was shown that both drugs on their own blocked human coronavirus OC43 in cells of CRC, but the combination of the two had a synchronized effect, increasing antiviral activity [20].

The MTT assay is one of the most widely used tests for cell viability; it reveals how much living cells metabolically function by breaking down yellow tetrazolium salt into purple crystals of formazan using enzymes in the mitochondrial dehydrogenase system. However, interference responses can distort its resolution. Any general decrease of MTT by reduction of reducing agent or metabolite in the medium may overestimate cell viability, and colored compounds like excessive amounts of remdesivir can perturb absorbance at 570 nm. Also, MTT can be in part diminished by enzymes or metabolites of dead or destroyed cells that distort the results. Though less sensitive and non-toxic than other cytotoxicity tests like the Resazurin (Alamar Blue) test, for example, or the lactate dehydrogenase (LDH) release test, which directly measures cell death, the MTT assay is still attractive because it is so easy and affordable. However, it does need to be handled carefully with control sets and the same experimental setting so that interference is minimized. Blank absorbance was subtracted to correct for background signals and statistical interpretation confirmed the results. For several purposes, the MTT assay is still a viable cell test that can be performed on its own because it is easy, cheap, and reproducible in controlled conditions. Because it is based on the metabolic activity of mitochondria, it is a direct, quantifiable measure of cell function, and is therefore an ideal test of cytotoxicity. Research has indicated that with the right controls and optimized protocol, the MTT assay can be highly reliable. It can also give quantitative data, making it useful for high-throughput screening, especially in pre-clinical drug efficacy studies [21].

The current research has a few limitations that should be recognized. For one thing, the doses used (62.5–2000 g/mL) were far larger than the peak plasma concentration in humans (3.027 g/mL), and the results might not have direct clinical utility. These are normal levels that would be used for in vitro studies to quantify dose dependence, but not physiological ones that would have to be confirmed in vivo experiments. Second, remdesivir's half-maximal inhibitory concentration (IC50) was not determined in the study, which is an important indicator of drug effectiveness. Last, though we can find dose-dependent modulation of cytokine regulation by remdesivir in the study, the molecular causes of these immunologic shifts are not known. Further studies are highly recommended to resolve these limitations to make the current results more translational.

CONCLUSION

Remdesivir is a dose-dependent cytotoxic to SW480 colon cancer cells, destroying viability as low as 62.5 g/mL and almost annihilating cell viability as high as 500 g/mL. Crucially, the research also finds that remdesivir tampers with immune responses by altering cytokine release. At high concentrations, it slashes IL-23 and TGF-β levels which are cancer-promoting and immune-suppressive cytokines. On the other hand, when administered at low levels (125 g/mL), remdesivir stimulates an overproduction of TNF-α, an anti-inflammatory cytokine that can promote anti-tumor immune responses.

ETHICAL DECLARATIONS

Acknowledgments

None.

Ethics Approval and Consent to Participate

The study was approved by the Ethical Approval Committee (Reference number 236 on July 18, 2023) of the College of Medicine, University of Babylon, Iraq. Informed consent was not required for cell line study.

Consent for Publication

No personal data is included.

Availability of Data and Material

The datasets produced and/or analyzed during the present study can be obtained from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Authors' Contributions

Al-Shibly IKA and Ghaleb RA contributed to the design and implementation of the research. Hameed AK was contributed to all the laboratory work, the data analysis, and the writing of the manuscript. Al-Shibly IKA was contributed to reviewing and proofreading of the manuscript. All authors read and approved the final version of the manuscript.

REFERENCES

[1] L. Cao *et al.* Prognostic cellular senescence-related lncrnas patterns to predict clinical outcome and immune response in colon cancer. *Frontiers in Immunology*, 15:1450135, 2024.

[2] Z. Wu, F. Zhang, Y. Wang, Z. Lu, and C. Lin. Identification and validation of the lncrna myoslid as a regulating factor of necroptosis and immune cell infiltration in colorectal cancer following necroptosis-related lncrna model establishment. *Cancers*, 14(18):4364, 2022.

[3] K. Ding *et al.* The next bastion to be conquered in immunotherapy: microsatellite stable colorectal cancer. *Frontiers in Immunology*, 14:1298524, 2023.

[4] Y.-y. Li *et al.* Targeting cd43 optimizes cancer immunotherapy through reinvigorating antitumor immune response in colorectal cancer. *Cellular Oncology*, 46(3):777–791, 2023.

[5] I. E. Eryilmaz, C. C. Bergel, U. Egeli, and G. Cecener. The cytotoxic and antitumoral effects of remdesivir, an antiviral rdrp inhibitor, on different cancer cells in vitro. *Molecular & Cellular Toxicology*, 20(3):649–660, 2024.

[6] P. Hong *et al.* Cd24 promotes metastasis and chemoresistance by directly targeting arf6-erk pathway in esophageal squamous cell carcinoma. *Cancer Letters*, 594:216994, 2024.

[7] Y. Rosenstein, J. K. Park, W. C. Hahn, F. S. Rosen, B. E. Bierer, and S. J. Burakoff. Cd43, a molecule defective in wiskott-aldrich syndrome, binds icam-1. *Nature*, 354(6350):233–235, 1991.

[8] G. Guney Eskiler, I. E. Eryilmaz, B. Yurdacan, U. Egeli, G. Cecener, and B. Tunca. Synergistic effects of hormone therapy drugs and usnic acid on hormone receptor-positive breast and prostate cancer cells. *Journal of Biochemical and Molecular Toxicology*, 33(8):e22338, 2019.

[9] A. J. Bullock *et al.* Botensilimab plus balstilimab in relapsed/refractory microsatellite stable metastatic colorectal cancer: a phase 1 trial. *Nature Medicine*, 30(9):2558–2567, 2024.

[10] I. Popēna *et al.* Effect of colorectal cancer-derived extracellular vesicles on the immunophenotype and cytokine secretion profile of monocytes and macrophages. *Cell Communication and Signaling*, 16:1–12, 2018.

[11] S. Shayan, A. Arashkia, G. Bahramali, A. Abdoli, M. S. S. Nosrati, and K. Azadmanesh. Cell type-specific response of colon cancer tumor cell lines to oncolytic hsv-1 virotherapy in hypoxia. *Cancer cell international*, 22(1):164, 2022.

[12] M. E. Ohl *et al.* Association of remdesivir treatment with survival and length of hospital stay among us veterans hospitalized with covid-19. *JAMA network open*, 4(7):e2114741–e2114741, 2021.

[13] C. Chen *et al.* The efficacy and safety of remdesivir alone and in combination with other drugs for the treatment of covid-19: A systematic review and meta-analysis. *BMC Infectious Diseases*, 23(1):672, 2023.

[14] T. Lamtha *et al.* A novel nanobody as therapeutics target for egfr-positive colorectal cancer therapy: exploring the effects of the nanobody on sw480 cells using proteomics approach. *Proteome Science*, 20(1):9, 2022.

[15] F. Alowaidi *et al.* Assessing stemness and proliferation properties of the newly established colon cancer 'stem'cell line, csc480 and novel approaches to identify dormant cancer cells. *Oncology reports*, 39(6):2881–2891, 2018.

[16] K. Hagman *et al.* Effects of remdesivir on sars-cov-2 viral dynamics and mortality in viraemic patients hospitalized for covid-19. *Journal of Antimicrobial Chemotherapy*, 78(11):2735–2742, 2023.

[17] J. Hartman *et al.* Tumor repressive functions of estrogen receptor β in sw480 colon cancer cells. *Cancer research*, 69(15):6100–6106, 2009.

[18] J. G. Krueger *et al.* Il-23 past, present, and future: a roadmap to advancing il-23 science and therapy. *Frontiers in immunology*, 15:1331217, 2024.

[19] H.-Y. Hsu *et al.* Remdesivir and cyclosporine synergistically inhibit the human coronaviruses oc43 and sars-cov-2. *Frontiers in Pharmacology*, 12:706901, 2021.

[20] M. Ghasemi, T. Turnbull, S. Sebastian, and I. Kempson. The mtt assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *International journal of molecular sciences*, 22(23):12827, 2021.

[21] V. Blay, B. Tolani, S. P. Ho, and M. R. Arkin. High-throughput screening: today's biochemical and cell-based approaches. *Drug Discovery Today*, 25(10):1807–1821, 2020.

http://doi.org/10.33091/amj.2025.154591.1964