Point-by-point response to the reviewers' comments

Any differences between the point-by-point response to reviewers and the final article text result from minor corrections made during proofreading.

Manuscript Number: 049701

Title: Exploring the potential link between mRNA COVID-19 vaccinations and cancer: A case report with a review of haematopoietic malignancies with insights into pathogenic mechanisms

Dear Editor Prof. Wafik El-Deiry,

Please find below our detailed, point-by-point responses to the reviewers' comments. We express our profound gratitude to the reviewers for their rigorous and comprehensive scientific evaluation of the manuscript. Their valuable insights and essential recommendations have greatly enhanced the article's quality and structural robustness.

Note: In the following text, each response is coloured in red, while additions or modifications to the manuscript in response to the reviewers' comments will be written in *italic* (with line numbers indicated).

Furthermore, the following paragraph has been added to the manuscript (lines 74-80) in order to cite 2 important papers that have been recently published:

"*Another study suggesting a possible link between spike protein expression and cancer progression after modRNA vaccination was recently published by Kuperwasser and El-Deiry, describing their review of 300 cases of cancers reported in the peer-reviewed literature following receipt of the COVID genetic vaccinations, and exploring possible mechanisms of oncogenesis [29]. Zhang and El-Diery had previously published on the ability of spike protein to suppress P53 activity in cancer cells potentially driving oncogenesis [30].*"

Reviewer #1:

Dear Editor

I have already been asked to review this manuscript by another journal
I have completed three rounds of review and the authors have responded to all my suggestions for modifications
The manuscript is now very balanced, and I would be grateful if you would accept it for publication. It is indeed very important to publish case reports of post-Covid mRNA vaccine cancer accompanied by biological mechanisms consistent with a possible causal relationship.

We thank the reviewer sincerely for the valuable suggestions, and strong endorsement of the manuscript.

Reviewer #2:

The study by Patrizia Gentilini et al discusses the link between cancer and COVID-19 mRNA vaccination starting from a case report and reviewing the literature afterword.

The manuscript is easy to read and informative. It presents convincing arguments and can be interesting to many readers and clinicians.

I have the following observations:

1) This sentence (see below, lanes 48-51) should be changed to include a statement of why the paper was rejected and what is therefore the gap present in literature, which does not allow to estimate the real increase in cancer after COVID-19 administration, and what are the type of studies needed for clarifying this issue.
"According to a recent (retracted) study performed in Japan, the age-adjusted death rates for leukaemia, breast, pancreatic, and lip/oral/pharyngeal cancers increased significantly in 2022 after a large portion of the Japanese population had received the third dose of the modRNA vaccine, as compared to 2020, the first year of the pandemic, when no mass global genetic vaccinations were given [22]."

In response to the reviewer's comment, the article text has been modified as follows:

Revised text (lines 47-56):
"*According to a recent study performed in Japan, the age-adjusted death rates for leukaemia, breast, pancreatic, and lip/oral/pharyngeal cancers increased significantly in 2022 after a large portion of the Japanese population had received the third dose of the modRNA vaccine, as compared to 2020, the first year of the pandemic, when no mass global genetic vaccinations were given [22]. Such study was subsequently retracted with a summary notice by the journal stating that: "the correlation between mortality rates and vaccination status cannot be proven with the data presented" [23], though without comprehensive evidence substantiating this claim. This underscores a critical literature gap: the absence of population studies verifying cancer incidence by vaccination status in order to estimate the true cancer incidence or mortality increases following COVID-19 vaccination.*"

2) IgG4 discussion (up to lanes 471).

Although the development of IgG4 responses to the spike protein is a well-established phenomenon reported by several studies, it is unclear to me how these IgG4 induction may affect the development of cancer. At least, the authors report a series of observations but failed to give a rational.
One could reason that anti-Spike antibodies of the IgG4 subtypes may be in close contact with the tumor in a way that they block the immune responses needed to eradicate the tumor. To have this mechanism operative such IgG4 need to reach the tumor environment. What is recruiting these suppressive IgG4 into the tumor? This is not explained by the authors, however, given the expression of spike protein (after vaccination) in any body district, it could be that expression of spike in a tissue precedes the tumor growth and may recall anti-spike antibodies of the IgG4 subtypes by two main reasons:
a) spike itself can be expressed in tumor cells and in the tumor environment (reference number 88 is indeed cited in the previous sentences).
b) Tumor cells can express antigens that are specific of the tumors, which cross-react with anti-spike antibodies of IgG4 subtypes by "molecular mimicry".
Both hypotheses can be valid.
Regarding the second hypothesis there is a very recent paper showing cross-reactivity of T-cells (not antibodies in this case) specific for Spike protein with a cardiac protein called Kv2.1 (the voltage-dependent K+ channel), which can explain the occurrence of myocarditis after COVID-19 vaccination (Circulation. 2025 Oct 30;152(21):1485-1500. doi: 10.1161/CIRCULATIONAHA.125.074644), attributed to molecular mimicry.
The expression level of Kv2.1 was observed to be higher in the highly metastatic prostate cancer cells (BMB Rep. 2021 Feb 28;54(2):130-135. doi: 10.5483/BMBRep.2021.54.2.210), which can be

an example on how a cross reactive immune-response can be induced against tumor antigens. If this happens with IgG4 antibodies anti-Spike, which recognize a tumor antigen and accumulate in the tumors to block effective immune responses is unknown, however the authors could discuss this possible mechanism occurrence.

We thank the reviewer for these insightful comments and constructive suggestions regarding the mechanistic rationale linking IgG4 responses to cancer progression. We have substantially revised and expanded this section (lines 478-512) to directly address these concerns:

"***(vi)*** *The role of the immunoglobulin subtype IgG4 in immunomodulation contributing to cancer endpoints including immunosuppression and immune evasion. Wang et al. found that IgG4-containing B lymphocytes and IgG4 concentration were significantly increased in cancer tissues, as well as in the serum of patients with cancer [96]. Both were positively correlated with worse prognoses and increased cancer malignancy. Previous studies have reported that IgG4 is locally produced in melanoma, playing an important role in evading immune system control and promoting tumour progression [53,97]. The increased production of IgG4 occurs with prolonged and repeated exposures to singular antigens and their interaction with antibodies of the IgG and IgE classes through their Fc domains [98]. IgG4 is in fact endowed with a dual role, as it can suppress or stop inflammation by competing with inflammatory IgE for binding to the antigen, in the case of allergies and infections from helminths and filarial parasites or, on the contrary, IgG4 can lead to serious autoimmune diseases [99] and cancer, playing an essential role in the "immune evasion" of cancer cells [100]. Recent studies indicate that repeated modRNA vaccinations against COVID-19 shifts the antibody response towards the IgG4 subclass with a decrease in FcγR-dependent effector activity and an increased COVID-19 infection fatality rate [101–103]. In cohorts of healthy healthcare workers, it was demonstrated that several months after the second dose, the SARS-CoV-2-specific antibodies were increasingly composed of immunosuppressive IgG4, which were further increased by a third modRNA vaccination and/or by subsequent infections of SARS-CoV-2 viral variants [102]. IgG4 antibodies, among all spike-specific IgG antibodies, increased on average from 0.04% shortly after the second vaccination to almost 20% after the third vaccination [103]. Spike protein/galectin-3 molecular mimicry facilitates recruitment of vaccine-induced IgG4 to the tumour microenvironment [104]. Once localized there, IgG4 promotes cancer progression through specific immunosuppressive mechanisms: binding anti-tumour IgG1 antibodies to block effector cell function, engaging inhibitory FcγRIIB receptors on innate immune cells, and creating oncogenic microenvironments through epitope targeting [104]. Moreover, a review of 10 studies on patients with IgG4-related disease (IgG4-RD), which features excess IgG4, revealed elevated rates of several cancers, particularly pancreatic cancer and lymphoma [105]. These patients also showed increased galectin-3 levels, a protein linked to IgG4 class switching [106]. Galectin-3 shares near-identical homology with the spike protein's N-terminal domain, potentially driving IgG4 switching via molecular mimicry [104,106,107]. Mechanistically, tumour cells expressing vaccine-derived spike protein recruit spike-specific IgG4 (induced by repeated mRNA vaccination/galectin-3 mimicry) to the tumour microenvironment, promoting cancer progression by: a) binding anti-tumour IgG1 to block effectors, b) engaging inhibitory FcγRIIB receptors, and c) creating oncogenic microenvironments [104]. Thus, repeated mRNA vaccines may drive cancer progression via spike-specific IgG4 recruitment and immunosuppression*"

3) Regarding the pro-tumorigenic potential of vaccines there is this recent paper that can be discussed by the authors (Medicina (Kaunas). 2025 Sep 17;61(9):1687. doi: 10.3390/medicina61091687).

We thank the reviewer for highlighting this recent paper, which had escaped our attention. Following their suggestion, we have added it as an eighth point among the potential mechanisms discussed in the literature (lines 519-524), as follows:

"***(viii)*** *A recent hypothesis paper proposes that mRNA vaccines' LPNs, via hepatic tropism, may transiently dysregulate liver metabolism in susceptible individuals, potentially promoting leukemogenesis through five mechanisms: folate sequestration starving bone marrow precursors; LNP-induced phospholipid dysregulation; indoleamine 2,3-dioxygenase-mediated tryptophan catabolism creating immunosuppression; hepcidin-driven iron sequestration with compensatory overload; and heightened hepatic NADPH demand diverting stromal support [103].*"

Reviewer #3:

In may opinion the manuscript , entitled: Exploring the potential link between mRNA COVID-19 vaccinations and cancer ... , Authors Patrizia Gentilini et. Al, could be accepted for publication, pending major revision, 1) the patient shoved neutropenia before the vaccination, so it is possible that a leukemic process was smoldering, this point should be discussed

We appreciate the reviewer's suggestion that pre-vaccination neutropenia could indicate a smouldering leukaemic process and are happy to address this point. In response to this valuable comment, we have added the following statement to the Discussion section (lines 337-348):

"Regarding the pre-vaccination neutropenia observed this case report in April 2021, although rare pancytopenic prodromes preceding overt ALL have been reported in ~1.3–2.2% of paediatric cases, characterized by severe pancytopenia and abnormal lymphoid cells in bone marrow aspirates [71], such features are not typical of ALL, which is a rapidly progressive malignancy characterized by the sudden accumulation of lymphoblasts in the marrow and blood [72]. The patient's isolated mild leukopenia (WBC 2,450/µL, neutrophils 650/µL , normal Hb/platelets, no atypical forms), detected during routine wellness screening for sports practice, more likely reflects a benign transient neutropenia from viral or other non-malignant causes rather than smouldering leukemia. Notably, the complete blood count one month post-second vaccine dose showed absolute neutrophils more than doubled to 1400/µL, with normal WBC, Hb, and platelets. A precursor ALL process would be unlikely to show post-vaccination neutrophil increase alongside no evidence of anaemia or thrombocytopenia."

2) the discussion section should be shortened.

We acknowledge the reviewer's concern regarding the length of the Discussion section. The sections significantly extending the text are the direct quotations from regulatory documents (FDA, WHO, EMA). These were included at the explicit request of another reviewer, as they correctly highlight safety concerns previously recognized by regulatory agencies. Although they increase length, these excerpts provide consolidated primary documentation that readers can reference directly within a single article, avoiding dispersion across multiple sources. Therefore, we propose retaining these sections for their important documentary value.