

Review

# Spontaneous Regression of Cancer: Revealing Granulocytes and Oxidative Stress as the Crucial Double-edge Sword

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Academic Editor: Graham Pawelec

Submitted: 27 January 2022 Revised: 7 March 2022 Accepted: 9 March 2022 Published: 1 April 2022

## Abstract

**Background:** It is commonly believed that cancer development is irreversible, organ-specific as well as systemic malignant disorder, often associated with harmful oxidative stress and inflammation. However, there are also well-documented cases of spontaneous cancer regression, the causative mechanisms of which are not understood. It is known that inflammation is a negative pathophysiological process that may support the development of cancer, but it is also believed that the immune system as well as oxidative stress play important roles in prevention of cancer development and defense against tumor progression. Hence, in animal models spontaneous regression of cancer could be mediated by rapid inflammatory response of granulocytes, acting against cancer mostly as innate immune response. In addition, the administration of granulocytes at the site of solid tumors can lead to tumor regression or can slow down tumor growth and extend the overall survival of animals. In both cases, similar to the radiotherapy, surgery and various chemotherapies, oxidative stress occurs generating lipid peroxidation product 4-hydroxynonenal (4-HNE). This “second messenger of free radicals” acts as growth regulating signaling molecule that exerts relatively selective cytotoxicity against cancer cells. **Conclusions:** We hypothesize that abundant inflammation and metabolic changes caused by cancer and oxidative stress producing of 4-HNE may be crucial mechanisms for spontaneous cancer regression.

**Keywords:** cancer; spontaneous regression; inflammation; polymorphonuclear cells; neutrophils; granulocytes; oxidative stress; lipid peroxidation; 4-Hydroxynonenal (4-HNE); growth control; necrosis; apoptosis; differentiation

## 1. Introduction

Cancer is often associated with chronic inflammation and oxidative stress both, in the early and in advanced stages of cancer development [1]. While it is a common opinion that cancer is irreversible organ-specific as well as systemic malignant disorder, there are also well-documented cases of spontaneous cancer regression, the causative mechanisms of which are not understood [2]. There are several reasons why spontaneous regression of cancer is still not understood. First reason is a very low incidence of spontaneous regression, i.e., relatively low number of well documented cases, which fulfil the strict criteria matching spontaneous regression: (a) pathohistological diagnosis of cancer and (b) histological verification of at least partial disappearance of cancer that was not associated with any kind of acknowledged medical treatments implemented. Nevertheless, documented cases of spontaneous regression of hypernephroma metastasis in the lungs of patients are well-known to occur after therapeutic unilateral nephrectomy removing primary cancer, while spontaneous regression of melanoma into unpigmented halo nevus was also approved for some fortunate patients, sometimes even

not aware of the initial cancer [3,4]. Actually, over decades medical records evidenced examples of spontaneous regression of cancer of almost any kind [5]. Until 90's, 741 cases of patients that met standard criteria of spontaneous regression of cancer were published [6]. Since then, the number of spontaneous regression cases reported yearly increased, and almost 90 cases were reported only in 2021 (Table 1, Ref. [7–37]). However, none of these were explained, not just because these fortunate cases are too rarely occurring and are very different, as is different also personal pathology of human cancer, but also because we still lack fundamental knowledge about biology of cancer and cancer/host relationships.

An example of this is the common consideration of inflammation as a negative pathophysiological process that may support the development of cancer, although we believe that the immune system plays a key role in the prevention of cancer and defense against tumor progression [5,38]. Indeed, shortly after tumor implantation immune cells infiltrate the site of inoculation to combat tumor, and the decay of transplanted tumor cells can be evidenced by a local decrease in the temperature [39]. However, once the de-



**Table 1. Spontaneous regression cases reported in 2021.**

Cancer type	No of cases	Patient age and gender	Characteristics of regression	Ref.
Blastic plasmacytoid dendritic cell neoplasm	1	85 Y M	SR following sepsis by <i>Serratia marcescens</i>	[7]
Burkitt lymphoma	4	65 Y F, 16 Y M, 47 Y F, 12 Y M	SR immune-mediated	[8]
Classic Kaposi sarcoma	1	63 Y M	Partial SR	[9]
Colon cancer mismatch repair deficient	3	76 Y F, 64 Y F, 64 Y M	SR	[10]
Colorectal cancer mismatch repair deficient	3	78 Y M, 66 Y M, 73 Y M	SR	[11]
Congenital brain stem gliomas	1	Neonate F	Partial SR	[12]
Congenital brain tumor	2	2 months F, neonate M	SR	[13]
Desmoid-type fibromatosis	1	50 Y F	SR following core needle biopsy	[14]
Extragenital seminomatous germ cell tumor	1	55 Y M	SR	[15]
Gastric gastrinoma	1	37 Y M	SR following resection of metastatic gastrinoma lesion	[16]
Hepatocellular carcinoma	1	64 Y M	SR immune-mediated	[17]
Hepatocellular carcinoma	6	65 Y F, 53 Y M, 53 Y M, 48 Y M, 62 Y M, 73 Y M	SR	[18]
Hidrocystoma simplex	1	50 Y F	SR following skin biopsy	[19]
Juvenile nasopharyngeal angiofibroma	1	15 Y M	SR of residual neoplasm after partial surgical resection	[20]
Lung metastases	1	71 Y F	SR of metastases after transarterial chemoembolization of primary hepatocellular carcinoma lesion	[21]
Lung metastases	1	53 Y F	SR following resection of primary sarcoma	[22]
Lung metastases of hepatocellular carcinoma	1	78 Y M	SR suggested to be due to hemodialysis induced hypoxia with hypotension	[23]
Malignant pleural mesothelioma	1	68 Y M	SR immune-mediated	[24]
Merkel cell carcinoma	1	88 Y F	SR	[25]
Metastasis of adenocarcinoma of the gastro-esophageal junction	1	58 Y F	SR of metastatic lesions	[26]
Metastatic renal cell carcinoma	2	71 Y M, 58 Y M	Partial SR following the SARS-CoV-2 infection	[27]
Ocular surface squamous neoplasia	8	21 to 59 Y M	SR	[28]
Oropharyngeal squamous cell carcinoma	1	66 Y F	SR	[29]
Pituitary microadenoma	1	32 Y M	SR following the SARS-CoV-2 infection	[30]
Pulmonary metastasis of renal cell carcinoma	1	78 Y M	SR after metastasectomy in the contralateral lung	[31]
Small cell lung carcinoma	1	80 Y M	SR	[32]
Small cell lung carcinoma	1	83 Y F	SR	[33]
Solitary osteochondromas	4	4 Y M, 10 Y M, 10 Y M, 11 Y M	SR	[34]
Submandibular gland adenocarcinoma	1	51 Y F	SR after incisional biopsy	[35]
Uterine cervix lymphoma	1	54 Y F	Partial SR	[36]
Vestibular schwannomas	35	19 F and 16 M Age 32 to 81 Y	Spontaneous shrinkage	[37]

F, female; M, male; SR, spontaneous regression; Y, years.

fense mechanisms have ceased, the tumor progresses resulting in the raise of peripheral temperature due to neoangio-

genesis [39]. Similar paradox refers to cancer association with oxidative stress, which is believed to play a very neg-

ative carcinogenic role in the development of cancer, while many types of major anticancer therapies rely on targeted local cytotoxic anti-cancer oxidative stress (radiotherapy, chemotherapy with some cytostatic drugs like doxorubicin, cis-platinum and cyclophosphamide, etc.), which is often associated with undesirable systemic consequences [40].

The first well-documented cases of at least partial spontaneous regression of human cancer were recorded by Everson and Cole about sixty years ago [41] and were further supported by numerous other cases recognized by the National Cancer Institute in Bethesda, which dedicated in 70's the conference on spontaneous regression of cancer [42]. This conference revealed that palliative surgery/trauma was the most frequent event associated with spontaneous regression in humans. The same finding seems to be valid also nowadays as the search of relevant scientific literature using Scopus reveals that out of more than four thousand publications dealing with spontaneous regression of cancer, almost every second (1.8 thousand) has surgery listed among key words.

One of major obstacles for understanding so complex interactions between cancer growth/regression with oxidative stress and inflammation is the lack of convenient translational models either *in vivo* or *in vitro*. In spite of that, there were different animal studies published over decades using salamander or even murine models, which lead to conclusion that systemic stress response to surgical trauma, including inflammation, altered metabolism and oxidative stress associated with trauma and/or with consequential wound healing/regeneration could be crucial for the onset of spontaneous regression of cancer [43–47].

When considering these possibilities, one should also consider the fact that lipids are necessary course of energy and structural elements of biomembranes, therefore any kind of intensively proliferating cells, malignant as well as non-malignant cells involved in tissue regeneration/wound healing requires more lipids than their counterpart [48]. Even more, it is known for sixty years already that malignant cells express higher request for lipids, also changing their composition to maintain stable growth, thus resembling more each other as cancer cells than their nonmalignant counterpart cells [49]. Altered metabolism, marked by hypoxic glycolysis or the Warburg Effect that enables cancer cells to survive an insufficient cellular respiration caused by altered mitochondria [49] is causing oxidative stress acting as another key element of carcinogenesis [50].

Over the years, different research groups identified altered lipid metabolism to play fundamental role in cancer development, thus allowing malignant transformation of affected cells that in turn become resistant to toxicity of lipid peroxidation [51,52]. Namely, lipid composition of cancer cells often favors saturated fatty acids, which are less susceptible to lipid peroxidation than are (poly)unsaturated fatty acids. Thus by altering membrane fluidity and protein dynamics cancer cells are exposed to less lipid peroxida-

tion [53]. These metabolic changes also alter signal transduction, protect cancer cells from toxicity of lipid peroxidation and even decrease uptake of anticancer drugs. Complementary to that, lipids also serve as a source of bioactive lipid mediators, which regulate various important processes of cancer development, including cell proliferation and the onset of metastasis. Parallel metabolic alteration of antioxidants, in particular of glutathione, the crucial scavenger of lipid peroxidation products [50], and differences on the level of cell differentiation and immunogenicity between different types of transformed cells could offer explanation why some types of cancer tend to regress spontaneously more than do other cancer types. However, these aspects of carcinogenesis and cancer/host relationships were not well studied so far, and we hope that by this review paper we will inspire some research in this direction.

## 2. Oxidative Stress, Oxidative Burst of Granulocytes and Lipid Peroxidation

A basic feature of cell physiology, notably respiration and cell signaling, is oxidative respiration associated with production of reactive oxygen and nitrogen species (ROS/RNS). Cells are in a stable state when the rate of ROS/RNS generation is balanced by the scavenging capacity of various antioxidant compounds. Interactions between the ROS/RNS and these antioxidants in the redox homeostatic balance produce metabolic responses to endogenous as well as exogenous signals [54]. These signals modulate the appropriate induction of adaptation processes or alternatively, the activation of cell death mechanisms [55]. Therefore, cellular redox homeostasis plays a key role in physiological as well as in many pathophysiological processes. Elevated ROS levels that cannot be counteracted by the cellular antioxidant abilities induce redox imbalance that results in uncontrolled oxidative stress, which causes oxidative modifications of structure and function of cellular components and consequently leads to oxidative injuries [55]. Thus, redox imbalance plays a significant role in development of numerous disease conditions, including cancer development [56].

Indeed, one of the hallmarks of cancer cells is deregulated redox homeostasis resulting in excess of ROS that promote malignant transformation of the stressed cells and support cancer growth and dissemination [57]. In order to prevent ROS-induced toxicity, cancer cells alter metabolic pathways and increase their antioxidant defense ability, induce expression of proto-oncogenes and activate transcription factors. It is commonly believed that ROS gain their carcinogenic effects causing DNA mutations, which lead to genomic instability and facilitate tumor invasion and metastasis.

Irrespective of the fact that ROS play an important role in tumor promotion, it is believed that they also show tumor suppressing effects. For anti-cancer effects of ROS important role is attributed to the oxidative burst of inflammatory

cells, especially macrophages and granulocytes which can act against tumor cells *in vitro* and *in vivo* [58–62]. It has been revealed also that spontaneous regression or complete resistance of the organism to cancer cells is mediated by rapid infiltration of granulocytes, mostly as a consequence of innate immune response [63]. In addition, administration of granulocytes at the site of solid tumors can lead to tumor regression or can slow down tumor growth and extend the overall survival of animals [60]. As the size of tumor cells is often bigger than that of leukocytes, the ingestion of tumor cells by granulocytes is not likely to occur. However, it seems that the mechanisms essential for killing microorganisms are also important in granulocyte mediated tumor cell cytotoxicity, although it is more likely that granulocytes can cause regression of tumor cells by different mechanisms, production of ROS being the most prominent one denoted as oxidative burst.

Thus, it was demonstrated that activated granulocytes cause unspecific lysis of tumor cells mediated by ROS, acting as effector molecules of the oxygen-dependent killing of cancer cells by granulocytes [64]. However, oxidative burst of granulocytes has also important role in tumor progression [59] as well as in regression [60] in respect to the type of tumor cells. Oxidative burst of granulocytes is already pronounced in tumor-bearing animals in the earliest stage of tumor development, while further tumor progression is associated with a constant increase in the oxidative burst of granulocytes. In case of tumor regression, the oxidative burst of granulocytes eventually decreases to normal. Therefore, one may assume that an elevated functional activity of granulocytes may result in tumor regression while continuous growth of tumor is associated with gradual increment in granulocyte oxidative burst that might even promote the tumor progression.

Although this seems paradoxical, it is not. Namely, several findings suggest that, depending on their polarization, tumor associated inflammatory cells, whether these are macrophages (TAM) or neutrophils (TAN), have distinct ROS profiles. Activated TAM1 and TAN1 have anti-tumor phenotype and produce higher amount of ROS compared to TAM2 and TAN2 with pro-tumor phenotype, respectively [65–67]. This is particularly relevant for the “cold” tumor environment, as for example in most brain and breast cancers, that has weak response to immunotherapy due to low infiltration of effector T cells. Namely, reprogramming of TAM2 to increase the co-expression of C-C motif chemokine ligand 5 and C-X-C motif chemokine ligand (CXCL) 9 promotes recruitment of effector CD8<sup>+</sup> T cells and reinforces their anti-tumor function [68–70]. CXCL1 is another intrinsic factor, produced by both tumor cells and TAN2, and was recognized as one of the major factors responsible for neutrophil infiltration and non-T-cell-inflamed tumor microenvironment [71,72]. Excessive arginase produced by TAN2 impairs T cell function, while TAN1 promote recruitment and activation of CD8<sup>+</sup> T cells

[73,74]. In addition, excessive ROS generated by TAN1 is mediated by the action of myeloperoxidase (MPO) [66]. Indeed, in a tumor regression model we have reported a massive infiltration of granulocytes [63] and strong presence of MPO and its product acrolein already 5 hours at the site of tumor implantation [61]. As acrolein can induce ROS formation, this reactive aldehyde was suggested to be further responsible for induction of self-catalysed chain reaction of lipid peroxidation [61,62]. This is also supported by the finding that initial formation of acrolein in tumor regression was accompanied by lipid peroxidation and modification of proteins by other lipid peroxidation derived aldehydes [61]. Beside immune cells, there are other extracellular regulators of redox state in tumor microenvironment [75]. Intracellularly, majority of ROS are in any type of cells produced by mitochondria and membrane bound NADPH oxidase, although organelles like peroxisomes and endoplasmic reticulum are other sources of ROS generation, as recently reviewed in particular for cancer cells [76]. As mentioned above, excessive ROS can induce lipid peroxidation that acts as a double-edged sword in carcinogenesis exhibiting either a pro- or anti-tumor effect [77]. Moderate increase in ROS promotes tumorigenesis, while further increase in ROS reaches the cytotoxic threshold inducing cancer cell death. To keep the ROS level below the cytotoxic threshold, tumor cells have altered antioxidant defenses including those of glutathione and thioredoxin antioxidant defense systems as well as the function of the NRF2 “master” regulator of the antioxidant responses [78]. In addition, presence of high levels of transition metals can contribute to redox imbalance and oncogene expression [79], and by the generation of hydroxyl radical via Fenton reaction directly promote lipid peroxidation [80]. It has been suggested that one of the mechanisms by which oxidative burst of granulocytes may lead to tumor destruction is by influencing the NRF2 signaling pathway [81] that may be regulated by ROS as well as by NF- $\kappa$ B [82]. Today it is well recognized that NRF2 has one of the key roles in tumor progression, including TAN and TAM polarization [83]. Moreover, ROS can function in multiple points (i.e., upstream or downstream) within a given pathway, like in the NF- $\kappa$ B pathway. ROS can stimulate the NF- $\kappa$ B pathway in the cytoplasm but inhibit NF- $\kappa$ B pathway in the nucleus [84]. Thus, ROS produced by oxidative burst of granulocytes may influence the NF- $\kappa$ B signaling pathway by repressing the NRF2-ARE pathway and thus lead to malignant destruction [81].

Furthermore, granulocytes may interact with tumor cells through four intercellular redox signaling pathways: NO/peroxynitrite, nitryl chloride, metal catalyzed and HOCl signaling pathway [67,85]. Intercellular induction of apoptosis is mainly achieved through the HOCl signaling pathway. This pathway depends on the superoxide anions generated both by transformed cells and by granulocytes. Granulocytes employ NADPH oxidase to generate the superoxide anions that is further reduced to hydrogen perox-



ide. By the action of MPO, hydrogen peroxide is further converted into reactive HOCl. At low levels HOCl does not affect cells directly; however, at high concentrations it exhibits cytotoxic effects either directly or indirectly by modifying biomolecules. As described above, the interaction of HOCl with the superoxide anions further yields highly reactive hydroxyl radicals that can trigger apoptosis through induction of lipid peroxidation.

Increased lipid peroxidation leads to a decrease in polyunsaturated fatty acids (PUFAs), including eicosapentaenoic, gamma-linolenic, arachidonic and docosahexaenoic acid, which promote cancer cells death via apoptosis [86]. PUFAs promote overexpression of cytochrome P450 leading to the depletion of glutathione and suppression of activity of carnitine palmitoyl transferase I [87]. On the other hand, it was suggested that *Bcl-2* induced by these PUFAs may prevent apoptosis, while n-3 PUFAs downregulate the expression of the *RAS* gene leading to the suppression of cancer development [88]. However, once *Bcl-2* is phosphorylated its ability to interfere with apoptosis is diminished and lipid peroxidation enhanced leading to apoptosis. Tumor cells treated with long-chain fatty acids show an increase in lipid peroxidation, depletion of antioxidants and phosphorylation of proteins [87]. These results suggest that these PUFAs induce apoptosis by enhancing lipid peroxidation, suppressing *Bcl-2* expression possibly through phosphorylation and increasing P450 activity. Thus, these PUFAs could act at the level of gene/oncogene expression and exert a cytotoxic effect on cancer cells.

The end products of lipid peroxidation are reactive electrophilic aldehydes that have much longer half-life than ROS and can diffuse from their sites of formation and share a lot of bioactivities with ROS. For these reasons, they are also considered as “second messengers of ROS”. Due to their high reactivity, the aldehydes derived from lipid peroxidation, such as malonaldehyde (MDA), hexanal, 4-hydroxynonenal (4-HNE) and acrolein have received much attention [89]. The most abundant aldehyde is MDA, acrolein is the most reactive [90], while 4-HNE has displayed the highest biological activities and has therefore been the most intensively studied. While ROS are short living and react on short efficiency distance, 4-HNE has strong binding affinity for proteins, forming relatively stable adducts. These adducts can diffuse from the site of origin changing structure and function of targeted proteins. Consequently, 4-HNE can influence proliferation, differentiation and apoptosis of cancer cells on one hand, while on the other it can affect genome functionality, too. These processes are crucial in regulation of the normal behavior of cells, hence their modulation by 4-HNE can interfere with the control of normal or malignant cell growth and metabolism thereby supporting and/or inhibiting development of tumors. The relevance of 4-HNE in modulation of cancer growth can be easily perceived by its interactions with signaling pathways modulating the Hanahan and

Weinberg’s hallmarks of cancer [91].

MDA is another reactive end product of lipid peroxidation, which shows primarily high affinity for DNA, but its elevated levels in the cytoplasm also result in formation of MDA-proteins adducts through the formation of N- $\epsilon$ -(2-propenal)lysine or 1-amino-3-iminopropene-type and pyridyl-dihydropyridine-type lysine-lysine cross-links [90]. The formation of MDA-protein and MDA-acetaldehyde-protein adducts is indispensably associated with a pro-inflammatory reaction within the whole organism because intense production of MDA-protein adducts leads to the activation of Th17 lymphocytes and triggers autoimmune reactions [92].

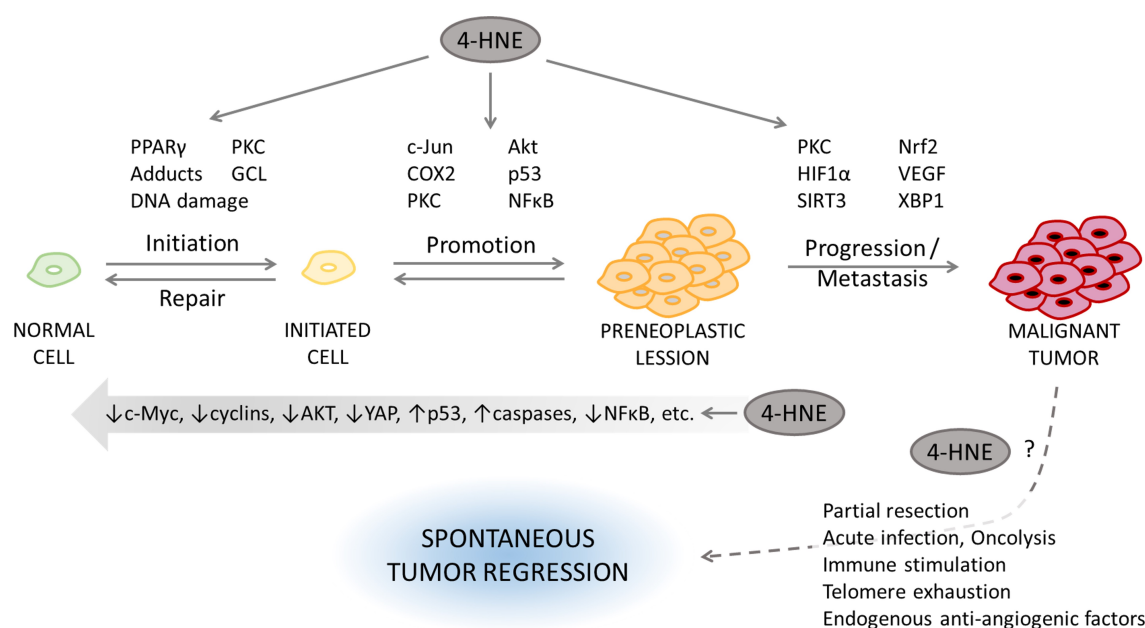
Cytotoxicity of acrolein is associated with the formation of Michael adducts with thiol groups of cysteines, which affects activities of proteins. The acrolein-protein adducts modify proteins even at the level of their biosynthesis and can also influence their degradation. It was shown that acrolein-protein adducts cause the activation of p53, p21 and p38 proteins, directly leading to the activation of ligases, suggesting the role of acrolein in protein degradation [93]. Acrolein-protein adducts are also often effective cell signal for apoptosis, as acrolein may attach to and activate ERK or JNK kinases, thus indirectly initiating apoptosis [94].

The above-mentioned end products of lipid peroxidation may contribute to the progression of granulocyte mediated oxidative damage of tumor cells. Namely, induction of apoptosis in transformed cells depends exclusively on extracellular ROS during the first 20 hours of malignant transformation, indicating a crucial role of ROS signaling in early carcinogenesis [95], while ROS signaling was also suggested to be used by natural host antitumor systems during the induction of selective apoptosis in transformed cells [85].

### 3. 4-Hydroxynonenal

Advance of understanding biology and biochemistry of 4-HNE and related products of lipid metabolism indicates that products of lipid peroxidation can regulate physiological signaling for the non-malignant cells and can gain selective cytotoxic effects against cancer cells [96]. These findings were strongly supported by several clinical trials that have shown abundant presence of 4-HNE and other lipid peroxidation products in viable normal tissue surrounding cancer as well as in necrotic cancer tissues [77,82,97–100]. Accordingly, herewith we propose that 4-HNE might also have the role in defense of normal cells against invading cancer, acting as a kind of natural anticancer substance.

In favor of our hypothesis are findings revealed by studies on spontaneous regression of melanoma B16 in mice upon partial hepatectomy and after treatment with 4-HNE, as well as regression of W256 cancer in rats due to the inflammatory response to cancer based on the oxida-



**Fig. 1. Dual roles of 4-HNE in tumorigenesis.**

tive burst of granulocytes generating cytotoxic products of lipid peroxidation, especially 4-hydroxynonenal (4-HNE) [44,47,61,63,101]. This particular aldehyde is known to act as “second messenger” of free radicals generated after various types of stress, including toxic chemicals, irradiation, mechanical trauma/surgery and inappropriate nutrition (overload of lipids and prooxidants) [102].

Early data on 4-HNE as growth modulating factor were provided by *in vitro* experiments using cancer cells [103], which lead to conclusion that growth controlling effects of 4-HNE are due to its interaction with cytokines and related humoral growth factors and/or due to its influence on cellular autocrine growth (dis)regulation. Accordingly, it was of interest to define synthesis and accumulation of 4-HNE within normal and malignant cells and its interference with growth regulating cytokines *in vivo*, especially in case of cancer. Early studies using monoclonal antibodies specific for the 4-HNE-protein conjugates showed differences of the 4-HNE appearance between normal kidney tissue and renal tumors, with variations in intensity depending on tumor type and the type of kidney cells analyzed [104]. Moreover, a decrease of the 4-HNE content in colon carcinoma tissue was revealed by Biasi *et al.* [105] in comparison to normal colon tissue. In correlation with reduced 4-HNE levels in human colon cancer, the expression of TGF- $\beta$ 1 was also decreased, indicating possible regulatory role of 4-HNE in colon carcinogenesis. Noteworthy, both 4-HNE and the TGF- $\beta$ 1 suppressed *in vitro* growth of colon cancer cells acting in synergic way through upregulation of JNK [105].

The pattern of 4-HNE histological appearance is not universal but is dependent on the histological origin of cancer [106]. Thus, in brain tumors the amounts of 4-HNE-

protein adducts were found to increase with increasing malignancy of these tumors [107–109]. It was also observed that the presence and localization of 4-HNE in normal tissues may reflect its physiologic roles as well as its causative involvement in the early onset of pathological processes [110]. In favor to this is the change of subcellular location of 4-HNE observed in patients with duodenal ulcer, where 4-HNE was found not only in the cytoplasm of the glandular cells in gastric mucosa, but also in the nuclei of these cells [111], persisting even after eradication of *H. Pylori* [112], unless patients received also amaranth oil [113]. Similar findings were observed for the Long-Evans Cinnamon (LEC) rat model of hepatitis and liver carcinogenesis based on the hepatic accumulation of copper [114]. These animals spontaneously develop jaundice and acute hepatitis that progressively worsens to liver carcinoma. The presence of 4-HNE in the nuclear region of liver cells in the early stages of such carcinogenesis suggests its involvement in the development of liver cancer [114]. To gain better insight into the pathways by which 4-HNE may regulate carcinogenesis, we present here the critical signaling pathways involved in carcinogenesis that are sensitive to 4-HNE.

The 4-HNE is important signaling factor regulating cell growth [96] and is involved in all hallmarks of cancer, from initiation and promotion to tumor progression and metastasis (Fig. 1). However, as 4-HNE has a dose- and cell type-dependent dual effects it also triggers effects essential for cancer regression, such as inhibition of cell growth, induction of apoptosis and reduction of metastatic capacity. 4-HNE contains 3 functional groups that can readily modify nucleophilic amino acid side chain of proteins via Michael addition or the Schiff base formation [115] altering protein structure and function. It can also readily form

adducts with DNA, eliciting mutagenic effects by inducing transversions and transitions [116]. In cells where base excision repair and nucleotide excision repair, main mechanisms involved in removal of HNE-adducts, are not efficient, it can cause mutations that may promote tumorigenesis. Indeed, 4-HNE induces G:C to T:A transversions at *p53* codon 249, which is a mutational hotspot in human cancers [117], while 4-HNE also inhibits nucleotide excision repair itself, thus contributing to tumorigenesis.

Formation of protein adducts with different proteins may affect normal cell function resulting in either cell adaptation or death. In order to understand its role, the presence of 4-HNE-protein adducts, as a biomarker of oxidative stress and lipid peroxidation, has been assessed in various tissues in a number of different pathologies, including cancer. Mass spectrometry is currently the most sensitive and advanced technique used for the detection of 4-HNE-protein adducts [115], however due to the high costs and limited instrument availability, together with the fact that 4-HNE is usually bound to proteins, immunochemical methods like immunohistochemistry, western blots and ELISA are the most frequently used [110,118–121].

4-HNE adduction to enzymes may result in the enzyme inhibition/inactivation or enzyme activation. As example, 4-HNE inhibits activity of thioredoxin reductase but it activates matrix metalloproteinase-13 [122]. Both events are associated with aggressive tumor phenotype [123,124]. It was observed that 4-HNE can induce normal and cancer cells differentiation (K562 and HL-60 leukemic cells) [125]. Differentiation of HL-60 cells by 4-HNE is initiated by a huge increase in peroxisome proliferation activating receptor  $\gamma$  (PPAR $\gamma$ ), but not of PPAR $\alpha$  expression, indicating targeted effects of 4-HNE on PPAR $\gamma$  ligands [126]. Further research implicated PPAR $\gamma$  in differentiation of various types of cancer cells [90]. Fatty acids, prostaglandin 15d-PGJ2 as well as products of linoleic acid oxidative metabolism are known endogenous ligands for PPAR $\gamma$  [127], thereby indicating that 4-HNE, also originating from the oxidized linoleic acid, may act in similar manner. Protein kinase C (PKC) is another target of 4-HNE, and depending on the PKC isoform affected it may promote tumorigenesis at different stages, from differentiation and promotion of tumor growth to increasing metastatic capacity and promoting neoangiogenesis [96].

Moreover, 4-HNE is a well-known inducer of the NRF2/Keap/ARE pathway, main pathway involved in the cellular antioxidant defenses. Although initially this was perceived as desirable in the context of ROS detoxification, today it is clear that NRF2 pathway upregulation is also beneficial to neoplastic cells contributing to tumor progression [78]. Intracellular 4-HNE formation in tumor infiltrating dendritic cells leads to overactivation of IRE1 $\alpha$ -XBP1, ultimately blunting anti-tumor immune response [128]. Study in breast cancer cells found that 4-HNE induces cell growth, vascular endothelial growth factor (VEGF) and hypoxia in-

ducible factor 1 alpha (HIF-1 $\alpha$ ) in a SIRT3-dependent manner suggesting its role in growth, invasion and angiogenesis of breast cancer cells [129].

As mentioned earlier, 4-HNE is a two-faced bioactive aldehyde that may also exhibit effects that may reverse tumorigenesis. 4-HNE promotes G0/G1 cell cycle blockade [130] most likely by downregulation of cyclins [131] and inhibits expression of *c-Myc* oncogenes in HL-60 cells [132] displaying anti-proliferative effects. Moreover, proapoptotic effects of 4-HNE have been evidenced by its upregulation of *p53* and the expression of proapoptotic Bax in SK-N-BE human neuroblastoma cells [133]. The 4-HNE mediated activation of c-Jun N-terminal kinase (JNK) results in enhanced apoptosis of colon adenocarcinoma cells [134]. Moreover, in T24 bladder cancer cells 4-HNE was found to mediate proteasomal degradation of YAP oncogene, impairing cancer cell proliferation, invasion and angiogenesis, and finally inducing apoptosis [135]. Furthermore, although at low concentrations 4-HNE activates NF- $\kappa$ B pathway promoting cell differentiation, proliferation and expression of pro-inflammatory cytokines contributing to pro-tumorigenic microenvironment, at high concentrations 4-HNE inhibits NF- $\kappa$ B pathway promoting apoptosis [136]. 4-HNE also has dual regulatory role on AKT that seems to be cell type dependent.

Multiple exposures of the human osteosarcoma cells (HOS) to sub-lethal doses of 4-HNE reduced both, their osteoblast differentiation markers and mitotic index, increasing apoptosis of HOS cells [137]. It is of high importance that such effects of 4-HNE are selectively cytotoxic for osteosarcoma, but not for the normal bone cells that could even be stimulated to growth *in vitro* by the same concentrations of 4-HNE, which are cytotoxic for their malignant counterpart HOS cells [138]. Such beneficial effects of 4-HNE were recently revealed to be associated with the level of differentiation of HOS cells, most likely due to the change of lipid metabolism of differentiated cancer cells favoring PUFAs that generate 4-HNE upon oxidative stress [139]. Therefore, 4-HNE could act as a pluripotent growth regulator of cancer cell growth, even if present at non-toxic concentrations, at least *in vitro*. Namely, already from the early discovery of the abilities of 4-HNE to modulate cell growth, 4-HNE was described as a biphasic growth factor, stimulating cell growth at low doses, while having suppressive/cytotoxic at high doses [103]. It seems likely that 4-HNE might exhibit differential effects for cancer and for normal cells, as mentioned above for the bone cells and HOS cells. In favor of these were also findings on the interference of 4-HNE with angiotensin II, that made 4-HNE nowadays to be recognized also as pathogenic co-factor of otosclerosis [140,141]. It is also worth mentioning that due to these growth regulatory effects of 4-HNE enhancing proliferation and differentiation of normal, nonmalignant, at least mesenchymal cells, the recently proposed concept of beneficial effects of oxidative stress for enhancement of tis-

sue regeneration after injury is becoming popular, which is also supported by the findings of 4-HNE as crucial growth enhancing factor of bioglass used to enhance healing of the fractured bones [142,143].

Selective, anti-cancer effect of 4-HNE was first observed for human leukemic cells, which were sensitive to cytotoxic 4-HNE treatment that otherwise enhanced the *in vitro* growth of normal, healthy human lymphocytes [144]. That might also reflect differential pattern of lipid metabolism and protein synthesis for normal and for malignant cells, especially in case of inflammation, since the overall effects of 4-HNE depend on the aldehyde's concentration, on the type of cells and on the patho/physiological state. Namely, the effects of 4-HNE depend on its binding to the cellular proteins, while the pattern of the protein synthesis reflects the character of the cells (normal or altered by disease), which are accordingly differently modified by 4-HNE [145,146]. It should be mentioned that 4-HNE was also proposed to be growth regulating, protein-modifying signaling molecule acting on similar principles even in yeast cells upon their modification causing PUFA synthesis that led to initially increased sensitivity of oxidative stress, followed by adaptation based on enhanced catalase activities [120,147–149].

It should also be mentioned that beside its regulatory and cytotoxic roles, 4-HNE might be likely candidate for explanation of spontaneous regression of cancer mechanisms involving lipid peroxidation also because 4-HNE is important for therapeutic effects and side effects of numerous drugs, in particular of anticancer drugs doxorubicin, cyclophosphamide and cisplatin [77,150]. For different cancer types it was found that overexpression of Bcl-2, an anti-apoptotic protein, is associated with therapeutic resistance. Study in breast cancer cells, demonstrated that cisplatin selectively activates Bak in Bcl-2 overexpressing MCF7 cells, upregulates Noxa promoting lipid peroxidation and formation of 4-HNE-Bcl-2 protein adducts, consequently resulting in apoptosis of cancer cells [151]. In gastric cancer cells, cisplatin also induced formation of 4-HNE protein adducts and promoted caspase-3 activity [152]. Doxorubicin elevates 4-HNE, upregulates c-Jun-N-terminal kinases and suppresses Bcl-2 leading to apoptosis of cancer cells [153]. The lipid peroxidation mediated anticancer effects of other currently approved anticancer drugs, such as Arsenic trioxide, Cetuximab, Erastin, Paclitaxel, Sorafenib, Sulfasalazin and Temozolomides have been summarized in a recent review [150].

#### 4. 4-HNE Association with Inflammation in Cancer Regression

Cancer is a complex disease that alters the entire metabolism of the cancer-bearing organism and is also associated with inflammatory response from the early stage of cancer development. For these reasons, each cancer is unique and may vary depending on the relationship between

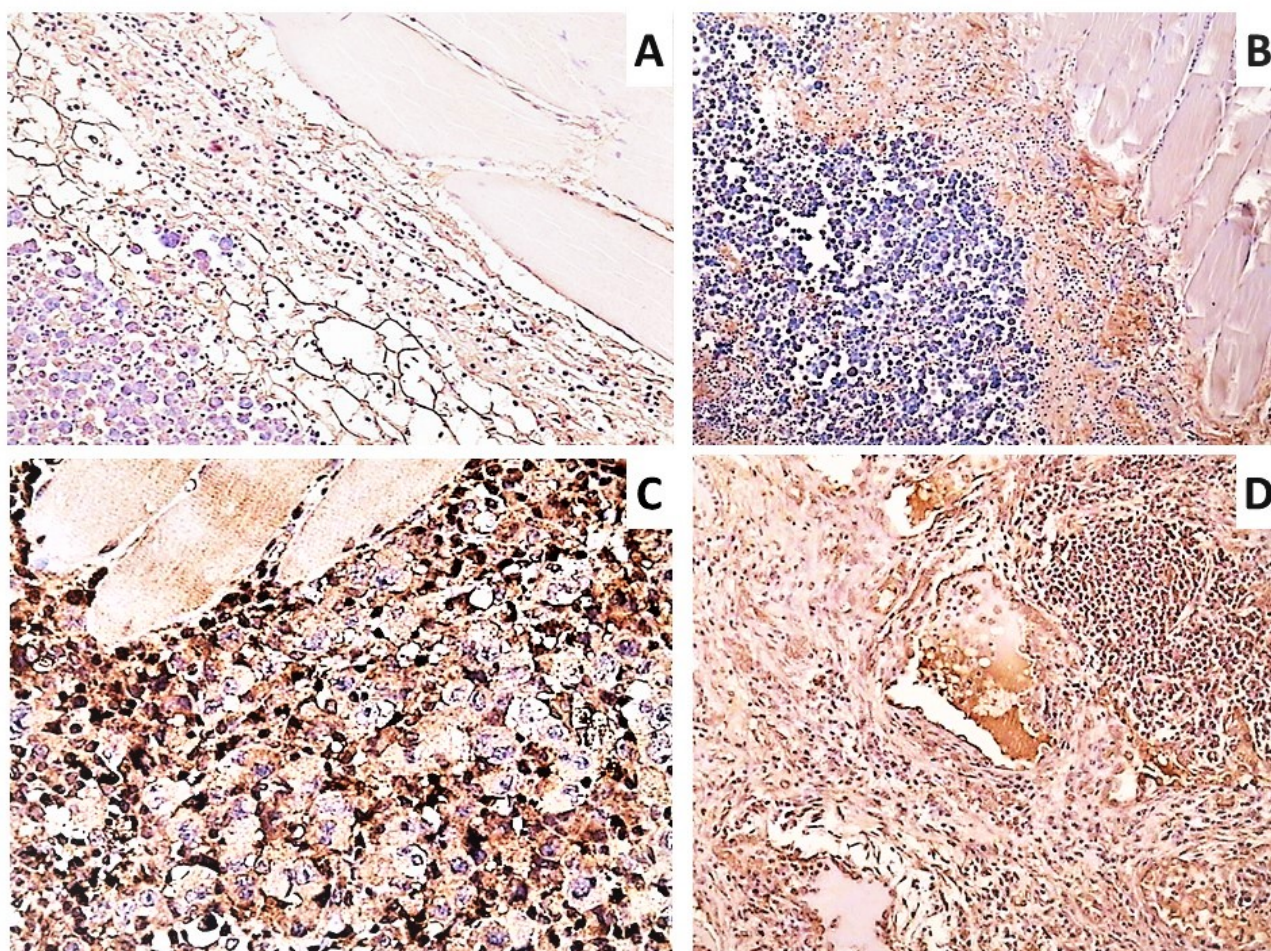
tumor and host. The non-malignant fibroblasts support heterogeneous cancer cells expansion, by producing extracellular matrix and blood vessels, resulting in formation of tumor stroma. They also provide growth enhancing cytokines essential for the growth of cancer cells, in contrast to the immune cells that represent the host response to the malignant cells.

Oxidative burst of phagocytes together with increased production of ROS/RNS in case of chronic inflammation could be considered as process of persistent oxidative stress that could eventually be mutagenic and even carcinogenic for normal cells, especially if the primary cause for such inflammatory response was chronic exposure to pro-oxidants and/or cytotoxic stressors, such as asbestos, tobacco smoke, transition metals (iron, copper), toxic pollutants, etc. However, oxidative burst of phagocytes together with increased production of ROS/RNS should eventually end in resolution of inflammation, eliminating its primary cause, if possible. Accordingly, if cancer cells by themselves represent the cause of inflammatory response or the effects of pro-oxidative therapy, such as radiotherapy, chemotherapy (especially if drugs like doxorubicin, cyclophosphamide or cisplatin were used) or surgery resulted in cancer necrosis generating oxidative stress, the inflammatory response could further generate ROS/RNS eliminating cancer. Of course, that should mostly be pronounced in case of acute inflammation, with pronounced production and release of pro-inflammatory cytokines (such as tumor necrosis factor- $\alpha$ ) as well as recruitment of immune-competent inflammatory cells.

Inflammation manifested as response to cancer can activate not only macrophages and lymphocytes but also granulocytes, which act as the innate response cells and could even cause regression of cancer, as already mentioned. Such oxidative burst of granulocytes will be manifested by pronounced MPO activity and consequential generation of 4-HNE. Herewith, we must mention again that thus generated 4-HNE, as well as the 4-HNE generated by nonmalignant cells attacked by cancer cells, can cause necrosis of cancer, without causing damage to the nonmalignant cells, because cancer cells are more sensitive to 4-HNE. Moreover, while 4-HNE can destroy cancer cells by necrosis it can also induce apoptosis, even if present at low concentrations being released the non-malignant cells near invading cancer or perhaps also if coming from the blood [154]. Such options of anti-cancer effects of 4-HNE based on the inflammatory aspects of carcinogenesis were only recently revealed [61,67], while Fig. 2 shows some examples of the 4-HNE appearance in the inflammatory defense against cancer.

As can be seen on Fig. 2, the invasion of W256 cells into the hind limb muscles of the rat is being opposed by inflammatory cells, notably granulocytes recognizable by abundant MPO, which is the enzyme specific for granulocytes. Thus activated granulocytes produce 4-HNE that is





**Fig. 2. Association of 4-HNE with inflammation and tumor regression.** (A) W256 in the hind limb of rat six hours after injection. Tumor cells are present in the lower left corner (negative for 4-HNE cancer cells are contrast-stained by hematoxylin), while muscles bundles are in the upper right corner, with connective tissue in between. There is no sign of inflammation and no immunopositivity of 4-HNE, since there is no prominent brown DAB immunostaining with monoclonal antibodies specific for 4-HNE-protein adducts (100×). (B) The same as A, but 24 hours after injection of W256 cells. Invasion of the muscle by W256 is being blocked by infiltration of the inflammatory cells producing abundant 4-HNE visible by brown DAB staining (100×). Attack of inflammatory cells producing 4-HNE against invading cancer can be noticed also at the lower left corner of the photo. (C) The same as B but showing MPO-immunohistochemistry under high magnification. Decay of W256 carcinoma cells (visible in the center as bigger cells with blue, hematoxylin-stained nuclei) is associated with abundant MPO present in the inflammatory granulocytes (dark brown DAB-stained smaller cells) (400×). (D) Inflammatory cells (upper right corner) within human non-small cell carcinoma with manifested tumor necrosis and edema (center) positive for 4-HNE (100×).

cytotoxic for cancer cells causing necrosis if present at high levels or apoptosis if present at lower levels. Similar process can be seen also on the Fig. 2 in human lung cancer (non-small cell carcinoma), suggesting that even in case of so malignant tumors 4-HNE can act as specific anti-cancer agent, resembling natural cytostatic substance, if produced by inflammatory cells, non-malignant cells attacked by cancer cells or perhaps even if penetrating cancer tissue from the blood due to the oxidative and vascular stress response as was recently revealed in case of aggressive COVID-19 [146,147].

However, it has been reported that in the hepatitis C virus mediated hepatocellular carcinoma developed oxidative stress, caused by CD68-positive inflammatory cells, and consequently formed 4-HNE, can together contribute to liver injury deterioration and to cancer progression [155]. On the other hand, it was also suggested that granulocytes may also be involved in the immune reactions against cancer [156], as it was reported that spontaneous regression of or complete resistance to cancer cells is mediated by rapid infiltration of leukocytes [157] and cytotoxicity of granulocytes against tumor cells *in vitro* and *in vivo* conditions [62]. Therefore, we assume that which inflammatory re-

sponse process will occur, the one promoting, or the other suppressing cancer growth is individual, depending on the tumor-host relationship for which 4-HNE might be relevant factor.

Namely, 4-HNE is an important pathophysiological factor that regulates the key cellular processes and signaling pathways. The outcome of so important biological activities of 4-HNE varies depending on the origin of cancer cells, their differentiation and phase of carcinogenesis. Evidently, tumor growth modulated by 4-HNE, either by modulation of immune response or by direct modulation of tumor cell growth and even by the 4-HNE induced cancer cell death. Which of these effects will prevail depends on numerous factors like carbohydrate and lipid metabolism, especially metabolism of cardiolipin, on the levels of antioxidants, notably glutathione and the cancer-specific catalase, as well as on the NF- $\kappa$ B, NRF2, RLIP76 and other signaling pathways and their crosslinking. Fine tuning of each pathway can contribute to the overall outcome of the tumor-host relationship and of the anticancer treatments applied, in which 4-HNE can help defending the organism and directing cancer to the decay.

It should be mentioned here that twenty-five years ago specific process denoted as elimination of transformed cells by normal cells was proposed as a novel concept for the control of carcinogenesis in particular in the early stage of cancer initiation [158]. It implied TGF- $\beta$ -induced signal pathway in normal cells resulting in production of the apoptosis-inducing factor that can, in the ROS-dependent manner, eliminate the transformed cells, thus acting complementary to removal of single transformed cells by phagocytic cells, notably macrophages [159]. It is justified to assume that 4-HNE could also be involved in such elimination of transformed cells by normal cells, because very similar concept was revealed as important for prevention of colon carcinogenesis [105,134] and was proven *in vitro* as effective 4-HNE triggered mechanism of cancer cell elimination acting upon cancer-cell specific membrane associated catalase [154]. The most recent concept of such cancer cell elimination implies competition between cells with different “fitness levels”, which was proven as crucial defense mechanism causing spontaneous cancer regression at its initial stage [160–162]. That means also that spontaneous regression of cancer is more frequent than assumed but is often not realized because it occurs before clinical symptoms of disease are manifested, while cancer elimination by cell competition might also explain the association of spontaneous regression of cancer with tissue regeneration and/or inflammation described at the beginning of this review [41–47]. Moreover, cancer elimination by cell competition probably involves the KEAP1–NRF2 system, which is by many considered as possible molecular target of cancer treatment [83,163], for which 4-HNE is known to be very potent and specific regulator. Taken together, these data suggest that majority of malignant cells are eliminated at the initial stage

of cancer development, spontaneously regressing due to the competition with the neighboring normal cells and their 4-HNE triggered anti-cancer defense mechanisms.

Finally, an option for the effective anti-cancer defense even in case of advanced cancer involving not only small numbers but millions of cancer cells was proposed as adjuvant natural integrative biomedicine approach based on the species-unspecific innate anti-cancer capacity of granulocytes [164]. Although this particular patent submission did not result in medical application of the proposed treatment, we hope that it will eventually lead to development of such adjuvant treatment approach and help curing cancer patients.

## 5. Conclusions

Although too rarely occurring or noticed, spontaneous regression is well documented for different types of human cancer. While several explanations have been offered, none of those can be considered as universal mechanism behind regression of different types of tumors, mostly likely because each cancer is specific as much as each patient is unique person. Nevertheless, translation animal models suggest that systemic stress response to cancer manifested by inflammatory response and altered lipid metabolism, in particular lipid peroxidation, could play fundamental roles in spontaneous regression of cancer. However, since these can also promote cancer development their dual nature should be better revealed. In particular better understanding on the likely involvement of 4-HNE maybe acting as crucial factor for cancer regression, also generated by different anti-cancer therapies, should be further studied to help better monitoring of cancer and develop more effective methods for cancer prevention and regression.

## Author Contributions

NŽ and MJ wrote most of the manuscript. KŽ provided substantial support in the pathological expertise in the field and evaluation of translation models of spontaneous cancer regression. AG and ES provided important contribution on lipid metabolism and cell signaling in respect to inflammatory cells, oxidative stress and cell growth control. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## Ethics Approval and Consent to Participate

Not applicable.

## Acknowledgment

This manuscript is dedicated to Milan Kubović and Mislav Jurin, who were pioneers and strong supporters of research in the field.



## Funding

This review paper received no external funding except collaboration support through Polish National Agency for Academic Exchange NAWA as a part of the International Academic Partnerships (PPI/APM/2018/00015/U/001).

## Conflict of Interest

The authors declare no conflict of interest. NŻ is serving as the editorial board member of this journal. We declare that NŻ had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to GP.

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