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Review

Medical Gas Plasma Treatment in Head and Neck Cancer—Challenges and Opportunities

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Abstract: Despite progress in oncotherapy, cancer is still among the deadliest diseases in the Western world, emphasizing the demand for novel treatment avenues. Cold physical plasma has shown antitumor activity in experimental models of, e.g., glioblastoma, colorectal cancer, breast carcinoma, osteosarcoma, bladder cancer, and melanoma in vitro and in vivo. In addition, clinical case reports have demonstrated that physical plasma reduces the microbial contamination of severely infected tumor wounds and ulcerations, as is often seen with head and neck cancer patients. These antimicrobial and antitumor killing properties make physical plasma a promising tool for the treatment of head and neck cancer. Moreover, this type of cancer is easily accessible from the outside, facilitating the possibility of several rounds of topical gas plasma treatment of the same patient. Gas plasma treatment of head and neck cancer induces diverse effects via the deposition of a plethora of reactive oxygen and nitrogen species that mediate redox-biochemical processes, and ultimately, selective cancer cell death. The main advantage of medical gas plasma treatment in oncology is the lack of adverse events and significant side effects compared to other treatment modalities, such as surgical approaches, chemotherapeutics, and radiotherapy, making plasma treatment an attractive strategy for the adjuvant and palliative treatment of head and neck cancer. This review outlines the state of the art and progress in investigating physical plasma as a novel treatment modality in the therapy of head and neck squamous cell carcinoma.

Keywords: kINPen; HNSCC; plasma medicine; reactive oxygen and nitrogen species; RNS; ROS

1. Introduction

Using the properties of physical plasma, medical gas plasma technology has been successfully investigated for the treatment of several types of diseases. Known and upcoming applications include decontamination, wound healing, blood coagulation, surface modifications, dentistry, and the treatment of various medical conditions, including cancer [1–5]. The latter is among the most investigated in the past few years because cancer mortality is still not significantly declining, current treatment schemes often come with severe side effects, and therapy resistance is an urgent issue, even with new treatment modalities. Therapeutic efficacy is not only vital in curative approaches but also in the palliation of

patients where the quality of life during the remaining weeks and months of life is the therapeutic goal [6]. Many cancer studies concerning this topic indicate that physical plasma, a partially ionized gas that contains numerous active components, including electrons and ions, free radicals, reactive molecules, and photons [7], might be an attractive tool in adjuvant cancer treatment [8–17]. Plasma selectively affects cancer cells with no or little harm to the surrounding non-malignant cells in the tissue. The selectivity of plasma toward cancer cells can be attributed to differences in their stages in the cell cycle compared to non-malignant cells; their varying characteristics, such as the redox state; and altered antioxidant defense mechanisms [18–20]. The detailed mechanism of action by which plasma induces the various cancer-cell-specific effects is not well understood at the molecular level [18]. These traits make plasma a promising technology for the minimally invasive treatment of topical tumors [19] that are easily accessible for multiple gas plasma treatment cycles [21]. Head and neck cancer belongs to these type of cancers, which has been investigated post plasma treatment in several studies during the past few years. Not only in vitro and in vivo investigations have been performed, successfully demonstrating the efficacy of physical plasma toward selectively inactivating head and neck cancer cells (Table 1), but also several clinical investigations were conducted with so far promising results (Table 2).

Table 1. Biological effects of physical plasma in head and neck cancer cells in vitro and in vivo. DBD: dielectric barrier discharge; direct: immediate exposure of tissue or cells in culture vessels to gas plasma system; indirect: exposure of a liquid to gas plasma treatment and either immediate or delayed addition of this plasma-conditioned liquid to cells in culture or injection into the tissue. ATM: ataxia-telangiectasia mutated, EGFR: epidermal growth factor receptor, MAPK: mitogen-activated protein kinase, MMP: mitochondrial membrane potential, OSCC: oral squamous cell carcinoma, ROS: reactive oxygen species, TfR: transferrin-receptor.

Main Findings and Mechanism of Action	Treatment Modality; Plasma Gas and Source	Model	Cell Type/Animal Model	Ref.
The ability to form colonies and cell viability was selectively reduced in a dose–response manner in head and neck squamous cell carcinoma cells through non-apoptotic mechanisms due to plasma treatment, whereas normal oral epithelial cells remained unaffected.	Direct; helium plasma jet	In vitro	JHU-022, JHU-028, JHU-029, SCC25	Guerrero-Preston et al. [19]
Physical plasma reduces cell viability, induces DNA-damage and apoptosis in head and neck squamous cell carcinoma.	Direct; surface micro discharge (SMD) in air (MiniFlatPlaSter)	In vitro	FaDu, OSC 19	Welz et al. [22]
Treatment with plasma-conditioned liquid induces transcriptomic changes and highly activates p53 pathway-related genes in oral squamous cell carcinoma.	Indirect; DC-powered air–water plasma jet	In vitro	SCC15	Shi et al. [23]
Inactivation effect of plasma-derived active species on oral cancer cells is higher through a small amount of medium than through a high amount of medium.	Direct; DBD in air	In vitro	HSC2	Ono et al. [24]
Plasma-derived NO radicals lead to selective killing of oral squamous cell carcinoma cells by targeting dysfunction of EGFR.	Direct; nitrogen plasma jet	In vitro	SCC-15, HSC-2	Lee et al. [25]
Physical plasma treatment of OSCC induces cell death by triggering the apoptosis pathway and increases intracellular ROS levels.	Direct; helium plasma jet	In vitro	OSCC	Ramireddy et al. [26]

Table 1. *Cont.*

Main Findings and Mechanism of Action	Treatment Modality; Plasma Gas and Source	Model	Cell Type/Animal Model	Ref.
The killing effects of physical plasma on oral cancer cells depends on the amounts of catalytic Fe(II). Physical plasma inhibits the migration, invasion activities, and colony-forming abilities of oral squamous cell carcinoma cells.	Direct; argon Habahiro non-equilibrium atmospheric pressure plasma device	In vitro	SAS, Ca9-22, HSC-2, HSC-3, HSC-4, Sa3, Ho-1-u-1	Sato et al. [27]
Plasma-treatment induced cell death of head and neck squamous cell carcinoma cells due to increased gene expression of mitochondrial E3 ubiquitin-protein ligase 1 (MUL1), which inhibited the level of AKT and p-AKT. Plasma-conditioned liquid inhibited tumor progression by increasing the MUL1 level and reducing p-AKT levels in in vivo head and neck tumor models.	Direct and indirect; helium–oxygen spray-type atmospheric pressure non-thermal plasma system	In vitro and in vivo	SCC15, FaDu, SCC-QLL1, SCC1483, SNU1041, SCC7, AMC-HN6 C3H/HeJ mice, BALB/c nu/nu mice	Kim et al. [28]
Physical plasma treatment leads to the inactivation of oral cancer cells through the activation of apoptosis-related proteins, such as p53, c-Jun-N-terminal kinase (JNK), and p38.	Direct; air-oxygen torch-type DBD	In vitro	HSC3	Mine et al. [29]
Physical plasma exposure generates a large amount of oxidative stress in oral cancer cells and has a significant inhibitory effect on cancer cell growth due to the promoted activation of caspases, a compromised redox state, and a loss of MMP that results in the decline of cellular viability.	Direct; DBD in air	In vitro	KB	Kaushik et al. [30]
Oral squamous cancer cell migration and invasion is inhibited through a decreased focal adhesion kinase expression and matrix metalloproteinase-2/9 activity caused by physical plasma depending on the gas-type. Thereby, N2-plasma inhibited cell migration and invasion the most efficiently.	Direct; nitrogen/helium/ argon micro-nozzle plasma jet system	In vitro	SCC1483, MSKQLL1	Kang et al. [31]
Physical plasma-induced apoptosis of head and neck cancer cells by a mechanism involving MAPK-mediated mitochondrial ROS and inhibited the growth of cancer cells in a nude mouse xenograft model, resulting in the accumulation of intracellular ROS.	Direct; helium–oxygen spray-type atmospheric pressure non-thermal plasma system	In vitro and in vivo	FaDu, SNU1041, SNU899, HN9 BALB/c nu/nu mice	Kang et al. [32]
Apoptosis of head and neck cancer cells is induced by the activation of MAPK, p53 proteins, and caspase due to physical plasma.	Direct; oxygen torch-type DBD	In vitro	HSC3	Hayashi et al. [33]
Physical plasma induces secondary double-strand breaks (DSB) during a short treatment time in oral cancer cells and the number of cells with plasma-mediated DSB decreases farther from the irradiation center.	Direct; nitrogen plasma jet	In vitro	SCC-25	Han et al. [34]
Plasma treatment specifically kills oral squamous cell carcinoma cells via antibody-conjugated gold nanoparticles, and the efficiency can be enhanced through conjugation with anti-EGFR and anti-TfR antibodies.	Direct; copper-polytetrafluoroethylene-DBD in air	In vitro	SCC25	Kim et al. [35]

Table 1. *Cont.*

Main Findings and Mechanism of Action	Treatment Modality; Plasma Gas and Source	Model	Cell Type/Animal Model	Ref.
Plasma treatment coupled with cancer-specific antibody-conjugated gold nanoparticles significantly decreases cancer cell viability.	Direct; copper-polytetrafluoroethylene-DBD in air	In vitro	SCC25	Choi et al. [36]
Apoptosis and sub-G1 arrest were caused by physical plasma through DNA damage and the ATM/p53 signaling pathway in head and neck cancer cells.	Direct; helium–oxygen spray-type non-thermal atmospheric pressure plasma system	In vitro	MSK QLL1, SCC1483, SCC15, SCC25	Chang et al. [37]
The efficacy of plasma depends on the treatment time, volume, and the cell type, since head and neck cancer cells showed more susceptibility toward plasma treatment than the non-cancer cell line.	Indirect; argon plasma jet	In vitro	SCC-15	Pereira et al. [38]

Table 2. Impact of physical plasma treatment on squamous cell carcinoma of the head and neck region in patients in vivo and of tumor tissue treated with plasma ex vivo.

Mechanism of Action	Treatment Modality; Plasma Source	Study Design/Population	Ref.
Ex vivo plasma treatment of head and neck cancer tissue biopsies increased the number of apoptotic cells and the levels of cytochrome c in the extracellular liquid, indicating apoptotic cell damage. Cell motility significantly decreased in head and neck squamous cell carcinoma cells in vitro after the physical plasma treatment.	Direct and indirect; argon plasma jet kINPen MED	10 patients with squamous cell carcinoma of the head and neck: tissue samples were collected during their preoperative inpatient care at the clinic and subsequently ex vivo plasma-treated. In vitro experiments with HNO97 cells.	Hasse et al. [6]
Application of physical plasma on locally advanced head and neck cancer leads to visible changes in the tumor surface and reduces strong odor and pain.	Direct; argon plasma jet kINPen MED	Six patients with locally advanced (pT4) squamous cell carcinoma of the oropharynx: plasma treatment as part of their palliative program and for microbial decontamination.	Metelmann et al. [21]
Physical plasma treatment causes apoptosis in head and neck cancer tissue and leads to visible effects at the tumor surface.	Direct; argon plasma jet kINPen MED	Group I (n = 12): Plasma treatment as part of their palliative program and to reduce microbiological contamination. Group II (n = 9): curatively surgery and received plasma treatment before total tumor resection.	Schuster et al. [39]
Hyperspectral imaging revealed a relevant increase of superficial and deeper cutaneous oxygen saturation, hemoglobin concentration, and distribution in plasma-treated head and neck cancer.	Direct; argon plasma jet kINPen MED	Patient 1 with T4 squamous cell carcinoma of the oral cavity: plasma treatment for microbial decontamination and as part of the palliative concept. Patient 2 with T3 squamous cell carcinoma of the oral cavity: plasma treatment for microbial decontamination and stimulation of cell proliferation.	Rutkowski et al. [40]
Physical plasma treatment decreased the request for pain medication and the typical odor due to the reduction of microbial contamination in patients with head and neck cancer. Partial plasma-treatment led to superficial partial remission of the tumor and wound healing.	Direct; argon plasma jet kINPen MED	12 patients with advanced squamous cell carcinoma of the head and neck: plasma treatment for decontamination of the infected cancer ulcerations as part of a palliative program.	Metelmann et al. [41]
Physical plasma displayed no severe side effects, except a few mild reactions like bad taste, exhaustion, and bleeding in the treatment of head and neck cancer.	Direct; argon plasma jet kINPen MED	10 female and male patients with locally advanced squamous cell carcinoma of the head and neck area: for palliative decontamination primarily focusing on the possible side effects of plasma treatment.	Schuster et al. [42]

2. Head and Neck Cancer

Head and neck cancer accounts for roughly 4% of all cancers worldwide and is the sixth most common type of cancer with over 800,000 new cases annually [43]. This type of cancer occurs more than twice as often among men than women [44], and the average age of diagnosis is approximately 50 years old [45]. Head and neck cancer arises at several anatomical sites of the upper aerodigestive tract, such as the oral cavity, pharynx, and larynx, whereby the most common histologic type (90%) is the head and neck squamous cell carcinoma (HNSCC) [46]. It is widely accepted that alcohol and tobacco over-use is associated with the majority (72%) [47,48] of this malignancy, partly through the formation of free radicals leading to DNA damage and mutation [49,50]. Another high-risk factor is infection with specific serotypes of human papilloma virus (HPV), especially HSP16 and HSP18. These were established to have an etiological role in the development of HNSCC, primarily of the oropharynx. HPV-associated HNSCC arises in younger patients more often than tobacco- and alcohol-driven HNSCC, and are associated with prolonged survival and better treatment outcomes [51]. Despite the ongoing discovery of new therapies and the optimization of existing therapies, head and neck cancer remains challenging to treat due to resistance development and high genetic heterogeneity [52,53]. Conventional treatment approaches are surgical excision, chemotherapy, and radiotherapy, and therapeutic decisions are based on the tumor location and stage. Approximately one-third of patients display an early-stage disease, while two-thirds suffer from advanced head and neck cancer with lymph node metastases [19]. Early-stage malignancies are predominantly treated using radiation therapy, either alone or in combination with surgery. Chemotherapy in combination with radiation is used as a primary treatment strategy or following surgical resection for patients with advanced disease stages. Treatment options for recurrent disease or distant metastasis are more limited [45], leading to poor survival outcomes and high morbidity [19]. Some of the existing chemotherapeutic agents are poorly selective and associated with toxicities. The surgical removal of oral cancer can result in a facial distortion with physical and psychological consequences [54], and it is sometimes not possible because of the cancer tissue being in close vicinity to vital nerves or vasculature. In addition, patients often develop loco-regional recurrences, distant metastases, and second primary tumors [55]. The emergence of immunotherapy, especially immune checkpoint inhibitors (ICIs), has provided significant clinical improvements, but only about 20% of the patients respond to this kind of cancer therapy [56]. The prognosis for patients with head and neck cancer is mainly determined by the stage at diagnosis. It is devastating to note that survival has not markedly improved in recent decades, with there being an average 5-year survival rate of only 40–50%, and even as poor as 25% for hypopharyngeal cancer [55–57]. This highlights the urgent need for new treatment strategies, experimental investigations, and discoveries of new therapeutic targets in HNSCC.

3. HNSCC and Oxidative Stress

The results of numerous investigations indicate a crucial role of physical plasma-generated reactive oxygen and nitrogen species (ROS) for the inhibitory and antitumoral activity on cancer cells observed with plasma treatment [58]. ROS seem to be important since they trigger the biochemistry and redox signaling pathways necessary to promote oxidation-related changes that lead to altered cellular functions and the activation of apoptosis [30,59]. This is because it is known that ROS are biologically active components that modulate cellular responses in targeted cells [60]. Direct plasma treatment primarily influences the cell physiology through the presence of highly active, short-lived ROS like OH, O_2^-, NO, and HOONO, whereas indirect treatment with plasma-conditioned liquid exhibits toxic cell effects, primarily due to long-lived species like H_2O_2, NO_2^-, and NO_3^- [61]. The impact of both plasma-type-generated ROS has been described mainly for the treatment of head and neck squamous cell carcinoma cells. Kang et al. showed the ROS-mediated inactivation of plasma-treated head and neck cancer cells could be rescued via the addition of antioxidants, which abolished plasma-mediated cytotoxicity through the reduction of apoptosis, levels of intracellular ROS, mitochondrial superoxide, and the loss of mitochondrial membrane potential (MMP) [32]. Besides the initiation of cell death

mechanisms, ROS cause the general elevation of oxidative stress within cancer cells [26]. These ROS or secondary ROS may be able to alter cellular structures, such as DNA and RNA, and proteins could be damaged, potentially leading to autophagy and induction of the DNA-damage response. The latter may be a consequence of the DNA secondary double-strand breaks that have been suggested as a consequence of plasma treatment of head and neck cancer cells [34], but this finding is likely attributed to apoptosis induction [62]. Furthermore, cancer cells might be more prone toward ROS than non-malignant cells since they often display a decreased antioxidant capacity, resulting in higher susceptibility toward ROS-induced signaling responses and cell death [20].

Relating to head and neck cancer, physical plasma-generated ROS have an additional advantage. They inhibit and decrease the microbial growth on advanced-stage tumors, which generally is an issue because the contamination of the tissue produces a hostile odor that negatively affects social interactions and reduces the patients' quality of life [21]. Here, the antimicrobial efficacy of plasma [63] aids in the palliation of the patients. Besides cold physical plasma, other therapy approaches aim for ROS generation to target cancer selectively based on the different redox states of non-malignant and malignant cells. Since malignant cells possess elevated intrinsic ROS generation, they are more dependent on antioxidants for cell survival; therefore, they are more vulnerable to further oxidative stress [20]. The chemotherapeutic agent cisplatin has been used widely in the treatment of head and neck cancer, mostly in combination with other anticancer drugs like docetaxel, fluorouracil (5FU), or 2-deoxy-d-glucose, whereby they produce intracellular ROS and oxidative stress [64,65]. These ROS lead to a shift in the cellular antioxidant defense capacity and the MMP, which initiates anti-proliferative effects and apoptosis in cancer cells [30].

4. In Vitro and In Vivo Studies

Several head and neck cancer cell lines have been investigated regarding the effects of physical plasma on their proliferation and cell activity (Table 1). Most of these experimental in vitro studies used the oral squamous cell carcinoma cell line SCC-15, as well as SCC-25, and highlighted their plasma-induced inactivation due to the reduction of cell growth, induction of apoptosis, and secondary DNA damage, especially through plasma-derived ROS [19,25,28,34–38]. Other HNSCC cell lines were also tested, showing one or more of the tumor-toxic plasma-associated characteristics [6,19,22,24,26–33,37]. In addition, HNSCC has been shown to be more prone to physical plasma treatment than non-malignant cells since the viability of the latter did not significantly decrease after plasma application. The fact that plasma selectively targets cancer cells, leaving non-malignant cell types, such as human fibroblasts (e.g., human gingival fibroblast-1 (HGF-1), HS-K, and IMR-SV-90) or human keratinocytes (HaCaT) unaffected, makes plasma a promising tool for adjuvant head and neck cancer therapy [25,27,29]. Moreover, two studies demonstrated an enhanced selectivity of plasma after the addition of antibody-conjugated gold nanoparticles, which specifically targeted SCC-25 head and neck cancer cells [35,36]. Cancer cell selectivity was achieved through the conjugation of anti-EGFR (epidermal growth factor receptor) or anti-TfR (transferrin-receptor) antibodies to the nanoparticles since this type of cancer usually overexpresses EGFR and TfR. As a result, an 18-fold increased efficacy of the nanoparticle treatment was found compared to the cancer cells not pretreated with plasma [35]. Therapeutic drugs often target the EGFR transmembrane protein since it plays a vital role in the regulation of the cell cycle, proliferation, differentiation, and transformation. Hence, its degradation leads to the inhibition of cell growth [66]. Lee et al. showed that plasma has a similar impact on HNSCC. The plasma-derived ROS induced EGFR dysfunction and degradation in the SCC-15 and HSC-2 cell lines, thereby interrupting the EGFR downstream signaling pathway, consequently killing the squamous cell carcinoma [25].

Selectivity, however, is not necessarily a universal feature of gas plasma applications in cancer treatment. Several studies have found non-malignant cells to be more vulnerable when compared to their malignant counterparts or cancer cells in general [67–69]. This is also known from the field of redox biology, where, for instance, human leukemia cells were less prone to ROS-induced cell death

compared with non-malignant myeloid cells [70]. The many studies in murine animal models and the few studies in patients (see below) nevertheless suggest that gas plasma treatment at least eradicates tumor cells while leaving the host intact. Systemic toxicity was also not observed either in mice or humans. Although it is unknown whether gas plasma treatment might be killing non-malignant cells in the tumor microenvironment, the overall decline of tumor mass will, in the end, indicate whether this technology can be usefully applied in oncology or not.

Besides EGFR, studies suggested a crucial role of the p53-signaling pathway in plasma-induced head and neck cancer cell death [23,29,33,37]. This protein contributes to the redox balance within the cells via regulation of the antioxidant gene expression and further senses DNA damage, initiates signaling pathways for the DNA repair, and fulfills the role of a cell-cycle regulator [20]. Subsequently, plasma-induced secondary DNA damage resulted in p53-mediated cell-cycle arrest in head and neck squamous cell carcinoma cells, ultimately impairing cell viability. Thereby, p53 and its downstream pathway are activated via phosphorylation through the ataxia-telangiectasia mutated (ATM) kinase, inducing cellular responses, such as apoptosis, indicating the importance of the ATM/p53 pathway for plasma-mediated head and neck cancer (HNC) cell inactivation [37]. Moreover, Hayashi et al. and Mine et al. demonstrated the contribution of p53 during plasma-induced apoptosis. They showed that plasma-mediated activation of mitogen-activated protein kinase (MAPK) proteins, like c-Jun N-terminal kinase (JNK) and p38, induces p53 phosphorylation, which resulted in apoptosis of the HNSCC cell line HSC3 [29,33].

In addition to the different signaling pathways that are induced through the physical plasma application, the redox state of the cancer cell lines is also influenced by the plasma treatment. It has been reported that the antioxidative activity of cancer cells is generally lower than in non-malignant cells due to a compromised redox state, showing decreased levels of the two major redox state regulators glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH) [30,33]. The additional intracellular oxidative stress that is generated by plasma-derived ROS leads to further imbalances in the cellular redox state and the loss of the MMP. This results in cell damage of squamous carcinoma cells after a plasma-mediated increase of intracellular ROS levels [26,28,30,32]. Furthermore, physical-plasma-induced ROS selectively enhances the iron-dependent lipid peroxidation and mitochondrial superoxide formation in HNSCC cells since they harbor more catalytic Fe(II) than non-malignant cells. Consequently, the amount of catalytic Fe(II) matters for the killing efficacy of physical plasma, suggesting the presence of ferroptosis processes during cancer cell inactivation [27]. Besides inactivation, several studies also point to a decrease in the motility of squamous cell carcinoma cells after plasma treatment [6]. Kang et al. revealed that the migration and invasion of the head and neck cancer cell lines MSKQLL1 and SCC1483 were significantly reduced after plasma treatment through the downregulated expression of the focal adhesion kinase (FAK), integrin, and paxillin, especially when utilizing N_2 as a feed gas [31]. The ability to form colonies was also impaired by plasma treatment in various HNSCC cell lines [19,27,30]. The fact that the application of different feed gases influences the effectivity of plasma, as shown by Kang et al., demonstrates the possibility to optimize physical plasma further for head and neck cancer treatment [31].

In contrast to the in vitro studies, only two in vivo studies have been performed regarding the physical plasma treatment of HNSCC. The first study confirmed preliminary in vitro results by in vivo experiments using a syngeneic mouse model, as well as a xenograft mouse model. It was demonstrated that the application of a physical-plasma-conditioned liquid applied over one week reduced the head and neck tumor progression through an increase of mitochondrial E3 ubiquitin-protein ligase 1 (MUL1) expression, ultimately resulting in a decline of p-AKT levels and AKT kinase activity. Correspondingly, the tumor weight and volume were significantly reduced in the plasma-treated group compared to the control group [28]. Since AKT contributes to the regulation of cell survival and death in terms of apoptosis and cancer development, it is not surprising that it plays an essential role in plasma-induced HNC cell damage [71]. AKT degradation is mediated by MUL1, which binds to the kinase domain of phosphorylated AKT, thereby inhibiting its downstream signaling. This finally leads to the suppression

of cell proliferation and migration. Since the expression of MUL1 was enhanced by plasma-derived ROS, the reactive species were also assumed to be responsible for the increased MUL1/AKT binding observed. In untreated HNSCC cells, changes in AKT and MUL1 levels were not noticed [28].

The second study investigated the impact of plasma on the growth of FaDu HNC cells in vitro and additionally in vivo using a nude mice xenograft model. Physical-plasma-induced apoptosis was ascertained in the cancer cells through MAPK-mediated mitochondrial ROS and dysfunction. A rise in the expression of p-p38, p-JNK, and p-extracellular-regulated kinase (ERK) was observed, which was presumably intertwined in the plasma-induced cell death. The treatment not only inhibited the tumor growth, volume, and weight after 11 days of application, but also caused increased intracellular ROS and apoptosis within the tumor tissue in comparison to the untreated control group. Therefore, ROS mediated the plasma-induced apoptosis in vivo and in vitro [32].

5. Clinical Studies

All clinical studies that investigated the impact of physical plasma on head and neck cancer (Table 2) were performed with the kINPen MED (Neoplas tools GmbH, Greifswald, Germany), which is licensed for the treatment of infected wounds, as well as infective skin diseases, and has been described in detail before [1]. Except for one trial, the application of plasma was used for the treatment of locally advanced squamous cell carcinoma of the head and neck region, with the primary aim of tumor tissue decontamination as part of the palliative concept. The most apparent plasma-induced effects were a visible change of the tumor surface similar to partial local regression, wound healing of infected ulcerations, and the reduction of the typical fetid odor that is caused by microbial contamination of the tumor. Regarding cancer patient palliation, a decline of requested pain medication was observed, along with an improvement in social interaction, positive emotional effects, and weight gain. These effects led to an overall increase in patients' quality of life [21,39,41]. In a few patients, an 80% reduction of the tumor surface was even observed [21]. The reduction of the tumor mass and inhibition of tumor growth was not only apparent through visible changes, but was also confirmed on a molecular level using TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) staining indicative of apoptosis within the plasma-treated tissue [21,39]. This was confirmed in ex vivo plasma-treated tumor tissue samples, showing increased apoptosis within the tumor tissue [6]. Molecular analysis of the liquid environment that harbored the tissues after plasma treatment moreover revealed elevated cytochrome c levels in malignant over non-malignant samples. This was concomitant with changes in the inflammatory signatures, as seen in elevated levels of tumor necrosis factor-α (TNFα), interleukin-10 (IL-10), and interferon-γ (IFNγ) in plasma-treated head and neck cancer when compared to plasma-treated non-malignant tissue.

Another trial utilized hyperspectral-imaging technology to investigate the impact of plasma on tissue microcirculation parameters in head and neck cancer. An increase of superficial and deeper cutaneous oxygen saturation was demonstrated that correlated with elevated hemoglobin concentration and distribution within the plasma-treated region compared to the non-treated area. Around the spot of plasma treatment, this effect extended locally to the adjacent region not directly being exposed to plasma treatment. Interestingly, the effect also lasted well beyond the time when plasma treatment was stopped. Such a plasma-mediated impact on the microcirculation may not only promote wound healing, but may also reduce microbial contamination through an increased influx of leukocytes [40].

It is essential to mention that plasma-mediated tumor regress was only effective in some patients (responders), while others showed no response (non-responders) [21,41]. One particular case showed a dramatic tumor shrinkage with plasma treatment over 7 months before tumor growth relapsed, despite the continuation of the plasma treatment further. This loss of physical plasma efficacy with long-term treatment reveals one of the challenges in the medical application of this innovative tool since the success seems to depend on various parameters ranging from the general genetics of the tumor to the tumor microenvironment and possibly adaption [21]. Clonal evolution and tissue conductivity also contribute to potential therapeutic resistance in head and neck tumors [39]. Theoretically, tissue

conductivity may influence the production of plasma-derived ROS, thereby altering the plasma composition itself and, as a result, its downstream effects in the tumor tissue [21].

In light of the current literature, it is difficult to assess whether gas plasma treatment of HNSCC might be more promising in this type of tumor than other tumor types. A sophisticated comparison with other tumor entities is currently lacking and improbable to achieve because animal models of cancer differ in their susceptibility to treatment agents, as well as in their genetic background. However, the clinical success of plasma treatment in HNSCC patients already demonstrates its principal capability for having therapeutic success. It is conceivable that gas plasma treatment may not only be used for tumor patient palliation, but also as adjuvant or neo-adjuvant therapy during radiotherapy or chemotherapy. This way, the tumor cells might be targeted by several mechanisms simultaneously, as we have recently demonstrated experimentally in other types of cancer cells [72–74].

Besides efficacy, safety is also a key trait of any anticancer treatment. In plasma-treated tumor patients, no severe or serious side effects were reported during the treatment. Some patients complained about a dry mouth, bad taste, exhaustion, and a sharp pain after longer therapy sessions [21,39,42]. Collateral edema, as well as superficial bleeding, occurred in some of the treated tumors, and in one case, sub-mucosal necrosis was observed [41,42]. Nevertheless, unwanted effects were always mild to moderate, but never life-threatening [42]. Even if the squamous cell carcinoma of the head and neck region is among the easiest accessible tumors for physical plasma treatment, some tumor areas remained badly inaccessible. This is partly because the plasma jet effluent is perpendicular to the handpiece, while in some situations, a 60°–90° tilted plasma effluent to the side would be ideal for reaching all tumor surfaces [41]. Nevertheless, HNSCC constitutes a surgical challenge for resection since the tumors emerge close to vital structures important for breathing, vocalization, and swallowing, which is why physical plasma seems to be a promising tool for the treatment of this area [6]. After dozens of plasma treatment sessions, several patients had shown desmoplastic reactions in the tumor tissue [21,41]. This potential side effect leads to a stiff tumor topology and morphology with abundant fibroblasts and collagen that may hamper the penetration of drugs on the one hand [75] and ROS from consecutive plasma treatment on the other. While this is an undesired effect, gas plasma treatment is currently employed only in HNSCC patients in the palliative setting with no therapeutic option left, and these patients benefit substantially from plasma-mediated pain relief, antimicrobial effects, and partial tumor regress until the disease becomes fatal.

6. Conclusions

Contrary to conventional therapeutic approaches, plasma displays nearly no side effects since it is minimally invasive and the treatment is defined to a local region without affecting the whole organism. Preclinical evidence points to a promising role of physical plasma in the treatment of HNSCC. However, the clinical studies demonstrated both plasma-mediated tumor remission in some patients, while others did not respond to the treatment at all. Therefore, further studies are needed that focus on the question of what makes plasma treatment effective against HNSCC. This includes studying the impact of the tumor microenvironment, the tumor microflora, the molecular determinants in HNSCC cells that render them either sensitive or resistant to plasma treatment, and possible adaption processes of HNSCC cells to repeated plasma application. Such findings, along with more elaborated clinical studies, may spur the future utilization of cold physical plasma in the palliation or adjuvant treatment of head and neck cancer patients.

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