Dijlah Journal of Medical Sciences (DJMS) Vol. 3, No. 3, September, 2026, pp. 274-289
P-ISSN: 3078-3178, E-ISSN: 3078-8625, paper ID: 24

REVIEW ARTICLE

Cold Atmospheric Plasma as a Selective Strategy for Targeted Cancer Cell Apoptosis: Integrating Plasma Physics with Molecular Oncology

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Article Info

Article history:

Received June, 09, 2026
Revised June, 23, 2026
Accepted July, 02, 2026

Keywords:

Cold Atmospheric Plasma (CAP),
Cancer Cell Apoptosis,
Ros and/or Ram-Systematic Cancer Therapy-Reactive,
Oxygen And Nitrogen,
Species (RONS)

ABSTRACT

Cold Atmospheric Plasma (CAP) is a recent and exciting non-thermal technology with great promise in precision oncology as it can selectively trigger apoptosis of malignant cells while mostly sparing normal tissues. In this review, we cover the physical and biological mechanisms for CAP-induced anticancer activity focusing on synergistic action of plasma-treated reactive oxygen and nitrogen species (RONS), transient electric fields, ultraviolet photons or charged particles. These plasma/blood-derived components disrupt intracellular redox homeostasis, promote mitochondrial dysfunctions, induce DNA damages and activate the intrinsic/ extrinsic apoptotic pathways in cancer cells. It discusses the biological base of CAP selectivity through many characteristics, such as increased basal oxidative stress and changes in the composition of membranes which classically exist for malignant cells; mitochondrial alterations associated with malignancy; failure of antioxidant defense systems. This review also provides a summary of recent advances in this field, including plasma-activated liquids (PALs), nanotechnology-assisted delivery systems, immune-modulatory effects and combination therapies. We outline challenges in plasma dosimetry, device standardization, treatment reproducibility and long-term clinical safety. In conclusion, CAP provides a cross-disciplinary treatment paradigm bridging diverse fields within plasma physics, chemistry and molecular biology with the oncology domain. Although strong experimental evidence demonstrates its effects as an anticancer modality, it requires further large scale preclinical and clinical studies before CAP can be adopted clinically for use in precision cancer medicine.

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1- INTRODUCTION

Despite the remarkable advances in surgery, chemotherapy and radiotherapy, as well as targeted therapy and immunotherapy [1], cancer continue to be one of the leading causes of morbidity and mortality worldwide. These therapeutic strategies, although much improved in terms of patient outcomes remain limited clinically by issues such as non-selective cytotoxicity, drug resistance, tumor recurrence and collateral damage to surrounding healthy tissue [1, 2]. Therefore, great effort has been given to explore efficacious therapeutic methods that only

radicalized malignant cells but spare healthy tissues from collateral damage. Cold Atmospheric Plasma (CAP) has only more recently proven to be among the most viable non-thermal technologies in plasma medicine. Thermal plasma operates at high temperature, and CAP on the other hand is running at near-room temperature. CAP generates high concentrations of reactive oxygen and nitrogen species (RONS), charged particles, ultraviolet photons, transient electric fields, and other labile reactive components during plasma generation that ultimately modulate multiple cellular processes [3, 4] as shown in Fig. 1 with possible applications for cancer therapy. One of the most astonishing aspects of CAP is its preferential cytotoxicity against neoplastic cells. Basal oxidative stress, mitochondrial dysfunction, membrane composition alteration and impairment of antioxidant defense systems is frequently noticed in cancer cells along with a metabolic reprogramming [8]. These biological abnormalities render tumor cells far more susceptible to plasma-induced oxidative damage than normal tissues. This leads to mitochondrial dysfunction, DNA damage, permeabilization of membrane (especially the hepatic mitochondrial membranes), caspase cascade activation and programmed cell death while inducing apoptosis in a high proportion of healthy cells [5, 6].

Plasma oncology the field of research that integrates aspects from plasma physics, chemistry, molecular biology and immunology as well as bioengineering with clinical studies so far has been evolving over the past decade. In addition to its direct cytotoxic activity, the potential applications of CAP include immunomodulation as well as combined use with plasma-activated liquids (PALs), nanotechnology-assisted drug delivery and various combinations strategies along conventional anticancer agents. These developments have significantly broadened the possible scope of CAP in precision oncology and personalized cancer therapy [7]. While many review articles have focused on either physical property of CAP or biological effects, advanced integration between plasma physics and molecular mechanisms inducing selective cancer cell apoptosis is only briefly mentioned in few comprehensive reviews that also cover the latest technological developments, translational hurdles and clinical relevance towards future therapeutic applications. This knowledge gap underscores an urgent need for a state-of-the-art and comprehensive review to critically summaries contemporary evidence while also defining research priorities. Thus, the focus of this review is to present an overview of those physical and biological mechanisms that lead to selective apoptosis in CAP-treated cancer cells. Moreover, it summarizes recent advances in technology and provides an overview of the current state-of-the-art biomedical applications including translational barriers and future perspectives to facilitate CAP being implemented as a new modality for precision cancer therapy [8, 9].

This review presents a multidisciplinary and comprehensive overview of the current state-of-the-art in applying Cold Atmospheric Plasma (CAP) for novel targeted cancer treatment. This review differs from previous ones, as they focused on either the plasma physics or biological responses of CAP and here, we combine these two areas to detail molecular mechanisms explaining apoptosis caused by CAP. Moreover, it reviews and critically summarizes a wide range of recent advances in plasma couple medicine including their applications to plasmas activated liquids (PAL), nanomaterials-assisted drug delivery, immunomodulatory effects through live cancer biomarkers profiling and cocktails-based combinatorial therapies while also discussing the important challenges associated with plasma standardization/dosimetry regimes as well as long-term safety along with clinical translatability aspects. This review combines a diverse array of experimental and translational evidence to deliver an integrated scientific framework aimed at future research directions as well as the challenges that may support clinical utilization of CAP-based precision oncology.

2- PLASMA DEFINITION AND CLASSIFICATION

Cold Atmospheric Plasma (CAP) partially ionized non-equilibrium plasma formed at atmospheric pressure. CAP is different from thermal plasma as there are energetic electrons coexisting with relatively low gas temperatures (ranging normally between 30 and 40 °C), providing a direct interaction with biological tissues without causing severe hot injury to it [10, 11]. This characteristic property has positioned CAP as one of the most promising technologies in plasma medicine, especially for biomedical and oncological applications. Plasma is generally accepted as the fourth state of matter composed of: free electrons, positive and negative ions, neutral atoms, radicals (neutral particles that have either lost or gained an electron during a chemical reaction), excited molecules ultra-violet light range energy photons electromagnetic fields transients. These elements all ultimately determines the physical and chemical properties of plasma, determining its biological activity. According to thermodynamic equilibrium, plasma can be either thermal or non-equilibrium (cold) [3]. Whereas thermal plasma, with temperatures reaching thousands of degrees Celsius (which is why the technique is often employed in industry) would not make contact to living tissues; compared to this cold atmospheric pressure operates at a near-physiological gas temperature [12, 13].

CAP induces the aforementioned biological response of cells due to a multimodal mechanism-of-action whereby reactive oxygen and nitrogen species (RONS), ultraviolet radiation, charged particles, transient electric fields, and metastable species are produced simultaneously in air plasmas. Those plasma-derived components are known to interact synergistically with both extracellular and intracellular targets through modulation of redox homeostasis, membrane integrity as well as mitochondrial function, DNA stability also multiple intracellular signaling pathways that control cell survival versus apoptosis [14, 15]. This is why, with recent technological advances such as the development of dielectric barrier discharge (DBD), atmospheric-pressure plasma jets (APPJs) [15], corona discharge devices or radiofrequency plasma sources [16, 17, 18], many CAP generation systems have been built. These platforms modulate gas mixture, voltage and frequency, as well as treatment duration or plasma chemistry to improve reproducibility of treatments for subsequent translation into the clinical setting [16, 17].

Although a major influence of CAP biology accredited to plasma generation technology, treatment parameters such as exposure time, environmental humidity and carrier gas composition need further investigation based on individual tissue characteristics. Therefore, standardization and optimization of plasma devices are still necessary for reproducible experimental results as well as in the future clinical application regarding precision oncology [18].

2.1 Publications Characteristics of Cold Atmospheric Plasma

Induction of CAPI activity there is a specific set of basic physical properties that collectively defines the mechanism by which cold atmospheric plasmas trigger biological response, differing from standard therapeutic technologies. Its lowest operating temperature is a key feature of its relevance, in the vicinity of room temperature and approximately 40 °C, allowing direct application to living tissues without causing thermal damage [14]. This feature is especially important for cancer treatment, as it allows specific targeting of cells while avoiding damage to the surrounding healthy structures. The next more crucial trait of CAP according to my research is its chemical compound. Actually, when plasmas are generated, energetic electrons collide with surrounding gas molecules forming reactive oxygen and nitrogen species (RONS), ultraviolet radiation, transient electric fields, ions and metastable particles [15]. This group of components from the plasma all contributes to observation biological effects upon exposure. The variety, quantity, and concentration of these reactive species differ depending on a range of operative variables including gas type, voltage, electrode configuration, exposure time and environmental conditions [16]. Additionally, CAP shows high flexibility of electromagnetic and electrochemical activity. Plasma discharge generated electric fields have made some discoveries about the enhanced permeability of cell membrane, intracellular ion transport and signal transduction pathways [17]. At the same time, generated photons and radicals enter biological tissues and interact with surfaces, causing oxidative damage at the cellular level. That synergistic interplay between these physical parameters is believed to be one of the major mechanisms for intrinsic CAP selectivity on malignant cells. In addition, the atmospheric pressure operation of CAP has practical and technologic advantages. In contrast to low-pressure plasma systems, which are bulky and expensive, attention has now turned towards cost-effective cold atmospheric pressure (CAP) devices that can be deployed in clinical practice [18]. This versatility has sped up the research of plasma medicine and increased possibilities for an application in oncology, dermatology, dentistry and regenerative medicine.

2.2 Formation of Reactive Oxygen and Nitrogen Species (RONS)

The biological activity of CAP is mainly mediated by RONS, generated via electron driven ionization excitation dissociation reactions with ambient gas [O2], N2 and H20 vapor) [19, 20]. The exact composition and concentration of these reactive species are dependent on many operational parameters, such as the plasma sources deployment carrier gases electrical power humidity duration of treatment and distance from source to target tissue [21]. The major RD of CAP are hydroxyl radicals (• OH), superoxide anion radicals (O_2 •$^-$), singlet oxygen($^1O_2$), hydrogen peroxide (H_2 O) and ozone [22] with regard to ROS, while among the RNSs include nitric oxide (• NO), nitrogen dioxide (NO 2), dinitrogen trioxide (N 2 O_3), and peroxynitrite ONOO [23]. Together, these reactive species provide a wide range of biological functions through oxidation and/or nitration of lipids, proteins, nucleic acids and intracellular signaling molecules. Peroxynitrite ($ONOO^-$) has received particular attention among these species, as it forms in high concentrations through the fast reaction between nitric oxide radical (•NO), and superoxide anion O_2•$^-$. Peroxynitrite has been detected as the most powerful oxidizer and nitrator generated from activating CAP exposure. It causes protein nitration, mitochondrial dysfunction, DNA strand breaks and lipid peroxidation as well as activates apoptosis related signal pathways which is a major mechanism that contribute to selective killing of malignant cells [23].

On the physiological side, mild levels of RONS are involved in normal cell signaling and immune regulation as well as tissue repair. Nevertheless, plasma-derived reactive species in abundant amounts can easily saturate the capacity of intracellular antioxidant systems and contributing to systemic redox homeostasis discontents with concurrent oxidative stress [24]. Due to the higher basal oxidative stress and insufficient antioxidant system in cancer cells, they are much more sensitive towards next CAP-induced oxidative damage than normal cells. Differences in these redox balances are one of the major mechanisms by which CAP exerts selective anticancer activity [25]. In addition, these RONS generated by plasma act as second messengers with the potential to regulate multiple signaling pathways (p53 activation, MAPK signaling transduction pathway and NF-κB inhibition of PI3K/Akt mitochondrial membrane depolarization cytochrome c release and caspase). The integration of these distinct molecular events ultimately leads to cell death by suicide while reducing damage in neighboring healthy tissues [26].

2.3 Formation of Reactive Oxygen and Nitrogen Species (RONS)

Cold Atmospheric Plasma (CAP) interacts with biological tissues in a complex, and highly dynamic process of simultaneous physical, chemical, and biological events. Immediately after exposure, plasma-derived reactive oxygen and nitrogen species (RONS), charged particles, ultraviolet photons [6] and transient electric fields interact with the extracellular environment before starting a cascade of intracellular molecular responses [27, 28]. These reactions vary in magnitude according to plasma working conditions, treatment time student and tissue type composition made up of dose-dependent negative mods on cellular antioxidant capability. CAP is mainly viewed at the plasma–cell membrane interface (figure 1). Reactive species oxidize membrane lipids and proteins while transient electric fields cause reversible electroporation-like phenomena of the cellular membranes. These mechanisms form a highly dynamic structural connection between the outer surface and internal components, which promote intracellular diffusion of reactive molecules, calcium ions and signaling mediators that contribute to augmenting CAP biological activity without leading immediate thermal damage [29]. Once membrane is penetrated, plasma-derived reactive species gather in the cytoplasm where they interact with intracellular organelles. Mitochondria represent one of the most important intracellular sites since they are central to cellular energy metabolism, redox balance and apoptotic signaling. Abnormally excessive oxidative stress is known to be able to sensibly disintegrate mitochondrial membrane potential ($\Delta\Psi m$), inhibit ATP synthesis, induce cytochrome c release and subsequently stimulate downstream signaling pathways participating in regulated death of cells [30].

CAP not only has direct intracellular effects but also modulates the tumor microenvironment by regulating inflammatory mediators, cytokine production, immune cell recruitment and redistribution across time of neoplasia progression as well as triggering metabolic reprogramming in cells that can provide extrinsic selective pressures to help promote survival in a dysfunctional redox-sensitive signaling network. Such biological changes lead to both death of tumor cells and the inhibition of progression as well as enhancement of antitumor immune responses [31]
Crucially, the activity of selective CAP arises through intrinsic biological differences between malignant and normal cells. Cancer cells have higher basal oxidative stress, different membrane composition and mitochondrial instability with increased expression of aquaporins and impaired antioxidant defenses. As a result, plasma-derived reactive species accumulate easily in tumor cells generating oxidative damage above the level required for cell survival but largely sparing nearby non-malignant tissues [32, 33]. Together, these coordinated plasma–tissue interactions culminate in the biological basis for CAP-mediated selective cancer therapy and factors that contribute to why plasma medicine is rapidly becoming a new platform for precision oncology.

2.4 Plasma-Tissue Interaction Mechanisms

Cold Atmospheric Plasma (CAP) is known to be a multifaceted process, with various physical, chemical and biological mechanisms occurring simultaneously upon interaction with biological tissues. Upon exposure to plasma, the plasma-derived reactive species, electric fields, charged particles and ultraviolet photons interact with the extracellular environment and cellular structures leading to a cascade of biochemical responses [30]. One of the earliest consequences in rCAP exposed cells is changes in the cellular membrane. Reactive species and transient electric fields could induce non-permanent permeabilization of the cell membrane that allows for reactive molecules to get inside [31]. This phenomenon plays a vital role in ion transport, intracellular signaling, and molecular exchange processes. These plasma-derived effects are enhanced in cancer cells characterized by an aberrant membrane composition and lower antioxidant defenses. Reactive species generated by plasma primarily target important cellular organelles notably mitochondria at the intracellular level. Increased oxidative stress causes loss of mitochondrial membrane potential ($\Delta\Psi m$), cytochrome-c release, and the initiation of apoptotic signaling pathways [32]. At the same time, oxidative damage, such as DNA oxidation and protein modification, leads to genomic instability and dysfunctions of cells. CAP can further exert anti-cancer activity by regulating various inflammatory

mediators, immune signaling pathways, and the tumor microenvironment [33]. Notably, selective targeting of cancer cells by CAP is thought to be a consequence of variability in metabolic pathways, membrane composition and oxidative stress resistance between malignant and normal cells [34]. Such a selective mechanism is one of the key benefits of CAP versus traditional therapies which have poor differentiation between healthy and malignant tissue.

3- CANCER CELL BIOLOGY

One of the hallmarks in metabolic pathways is that cancer cells have additional biological and chemical features to be different with eukaryotes which gives them excessive proliferation capacity, anti-apoptosis capability, genomic instability properties as well as resistance toward therapy [34]. These changes are due to an accumulation of genetic, epigenetic and metabolic abnormalities that together help drive malignant transformation and tumor progression. In comparison to normal cells, cancer cells are more commonly associated with unhealthy cell-cycle regulation, increased proliferative signaling, rewiring of metabolic activity and dysfunctional communication between tumor microenvironment [35]. One of the classical features defining cancer metabolism is a preference for aerobic glycolysis in the presence of oxygen, known as Warburg effect. Such metabolic adaptation enables rapid cellular proliferation by providing biosynthetic intermediates; at the same time, this enhances ROS production within cells. However, the level of oxidative stress is not uniform according to tumor type or malignancy stage and microenvironment so that an imbalance between ROS-generating system and antioxidant defence must be assumed as a general but non-exclusive property of malignant cells [36]. They activate a variety of antioxidant defense systems, such as glutathione metabolism; superoxide dismutase; catalase pathways; and thioredoxin and peroxiredoxin pathways to cope with the oxidative stress. These compensatory mechanisms maintain intracellular redox homeostasis yet put malignant cells near their maximal oxidative threshold. Thus, even this small increase in plasma-derived RONS may overcome this threshold to induce irreversible oxidative damage [37]. In addition to metabolic changes, cancer cells tend towards mitochondrial dysfunction, deficient DNA repair capacity, abnormal membrane composition and fluidity in the plasma membranes as well over- or dysregulated ion transport. Typically, such biological aberrations amplify cell susceptibility to plasma-inducible reactive species and allow triggering of apoptosis-related signaling pathways [38]. Also, the microenvironment of tumors brings in more to this aspect of CAP selectivity. Hypoxia, chronic inflammation, extracellular acidosis and aberrant vascularization and ongoing cytokines signaling together shape oxidative metabolism and treatment response. Reactive species from plasma can alter these pathological conditions by modulating redox-active signaling pathways, inflammatory agents and immune responses to selectively increase the cytotoxicity against tumor tissues [39].

CAP mediates the selective anticancer activity through a complex interaction of plasma-generated reactive species with distinct biological vulnerabilities in tumor cells rather than through any one molecular mechanism. This multifactorial selectivity was the major benefit of CAP vs. many other conventional anticancer therapies [40].

3.1 Redox Imbalance and Oxidative Stress

Redox homeostasis is crucial for normal physiology of cells by establishing a fine balance between the generation and degradation of reactive oxygen (RONS) and nitrogen species respectively as well as intracellular antioxidant defense systems [2, 3]. At physiological conditions, a low concentration of reactive species is recognized as signaling molecules to regulate the cell proliferation, differentiation metabolism and immune process. This residence state is disturbed due to any reason (e.g. exposure with chemical or physical stress) which causes oxidative strain [41] and subsequent permanent damage in proteins, lipids, and nucleic acids e.t.c along cellular organelles.
Tumor cells with mitochondrial dysfunction, oncogene activation, chronic inflammation and metabolic remodeling reflect augment oxidative metabolism pattern. However, the level of oxidative stress is highly variable according to tumor types and molecular subtypes as well as microenvironment. Thus, oxidative stress must be considered a unique biological property and not an inherent universal characteristic of every cancer [42]. Tumor cells activate sophisticated antioxidant defense mechanisms such as glutathione, catalase, superoxide dismutase and thioredoxin to survive at high oxidative stress condition. These adaptive routes preserve intracellular redox homeostasis and yet keep cancer cells vulnerable to oxidative stress. Thus, modest elevations of plasma-derived free radicals may exceed the antioxidant defenses and lead to permanent tissue oxidative damage [43].

Cold Atmospheric Plasma uses this weakness of biology to create a small surge of reactive species that outstrips the antioxidant buffering capacity in malignant cells. Increased intracellular oxidation stimulates lipid peroxidation, protein oxidation as well as mitochondrial dysfunction, DNA strand breaks and calcium dysregulation resulting the activation of apoptosis-associated signaling pathways. Normal cells, on the other hand, have much more effective antioxidant systems and greater redox homeostasis than cancerous cells so that they can tolerate moderate percentage of plasma treatments with significantly less cell damage [44].

Recent evidence also indicates that plasma-derived oxidative stress not only directly induces cytotoxicity but regulates several paths involved in tumor suppression, including p53 activation, MAPK signaling modulation, PI3K/Akt inhibition of AKT pathway and mitochondria apoptotic signaling. These molecular events provide a major insight into the selective anti-cancer therapeutic activity of CAP on malignant cells [45].

3.2 Differential Susceptibility of Cancer and Normal Cells to Cold Atmospheric Plasma

The most unique feature of the cold atmospheric plasma (CAP) technique is selective killing effect on tumor cells with less damage to normal tissues. This selective cytotoxicity is not a result of unique biological mechanism, but due to the overall contributions from metabolic and environmental differences along with biochemical, structural and molecular differences between without versus cancerous cells [6, 7]. Cancer cells, due to elevated metabolic activity, mitochondrial defects and dysfunctions associated with oncogenic signaling as well as continuous inflammation stimulation tend to possess higher basal levels of reactive oxygen species (ROS) [15]. These cells nevertheless compensate via the upregulation of affiliating antioxidant defense systems, but are effectively operating at their oxidative tolerance threshold. This leads to significant accumulation of plasma-generated reactive oxygen and nitrogen species (RONS) that exceed intracellular antioxidant capacity, resulting in irreversible oxidative injury followed by apoptotic cell death [6, 7, 10].

Cap selectivity is also an important factor that depends on the changed architecture of the plasma membrane in malignant cells. In cancer cell membranes, lipid peroxidation is usually increased and membrane stability reduced due to alterations in the phospholipid content of cellular membranes with altered cholesterol composition contributing as well plus there are increases in membrane fluidity. These molecular deformation variations, compared to normal cells [5, 9], will help the membrane permeabilization of plasma with reference to their abnormalities tissue by its preferential penetration into intracellular reactive species. Recently, aquaporin membrane channels are known to influence CAP selectivity as well. Many malignant tissues show increased expression of aquaporins, particularly AQP1, AQP3, AQP5 and AQp8 that promote the transport of hydrogen peroxide and other reactive molecules generated by plasma plasmatic to intracellular compartment. Increased aquaporin expression hence intensifies oxidative stress and promotes apoptosis after CAP exposure [7, 10]. Differences in DNA repair capacity then added to selective plasma toxicity. Most tumor cells are defective in DNA damage response pathways, checkpoint regulation and genomic repair capacity. Hence, oxidative DNA lesions generated by CAP are accumulated preferentially in malignant cells over normal tissues leading to cell-cycle arrest, mitochondrial dysfunction and activation of programmed cell death pathways [11, 12]. In addition, emerging evidences suggest that tumor microenvironment may affect the CAP selectivity. The intracellular redox signaling generated by the mechanisms of hypoxia, extracellular acidosis and inflammatory cytokines as well abnormal vascularization alters tumors such that they become more dependent on oxidative adaptation. Plasma derived reactive species disrupt these adaptive pathways, amplifying susceptibility of malignant tissues to oxidative injury while exhibiting far lower toxicity to normal cells [5, 8]. Together these biological differences provide an explanation as to why CAP acts therapeutically, using the multidimensional role of cancer driving cells making this a therapeutic modality that should be classified by mechanisms beyond one molecular target [6, 7].

3.3 Membrane Permeabilization and Mitochondrial Dysfunction Induced by Cold Atmospheric Plasma

The first biological barrier for Cold Atmospheric Plasma (CAP) is represented by the plasma membrane. When implemented, RONS generated from the plasma as well as transient electric fields and charged particles interact with membrane lipids and proteins to induce controlled membrane oxidation-mediated temporary increases in permeability. These reversible modifications increase the intracellular transport of reactive species as well as signaling molecules without completely losing global cell integrity [5, 9]. In addition to the plasma membrane effects, mitochondrial outer membrane permeabilization (MOMP) serves as a critical juncture of CAP-mediated cytotoxicity. Accumulation of oxidative stress within cells may compromise mitochondrial membrane integrity leading to a loss of $\Delta\Psi m$, reduced ATP synthese and increased permeability of mitochondria. Those events finally lead to the release of cytochrome c and other proapoptotic factors in cytosol, starting thereby the intrinsic apoptotic pathway [6, 7, 11]. Members of the B-cell lymphoma-2 (Bcl-2) protein family tightly regulate MOMP. Plasma-induced oxidative stress induces the activation of pro-apoptotic proteins (e.g., Bax and Bak) as well as suppression of anti-apoptotic proteins such as Bcl-2 and Bcl-xL. Activation of apoptosis in malignant cells largely results from this alteration (increase) of Bax/Bcl-2 ratio that promotes acquisition of mitochondrial membrane permeabilization and amplification apoptotic signaling [6, 11]. In addition to mitochondrial injury, oxidative modifications of membrane phospholipids, cytoskeletal proteins and membrane associated receptors are induced by plasma-derived

reactive species. These changes modify intracellular calcium homeostasis, receptor-mediated signaling and ion transport contributing to oxidative damage biasing the cell towards programmed cell death [5, 10].

Significantly, since cancer cells usually have inherited mitochondrial defects and high levels of oxidative metabolism mitochondrial dysfunction is a primary factor that contributes to CAP selectivity. Thus, plasma induced oxidative injury on malignant cells crosses hypoxic-apoptotic threshold much faster than in normal tissues and efficiently kills tumor cells without damaging surrounding healthy tissue [6, 7, 12].

Table (1): Biological Factors Contributing to CAP Selectivity toward Cancer Cells

Biological Factor	Cancer Cells	Normal Cells	Impact on CAP Response
Baseline ROS Levels	Elevated	Low	Increased oxidative vulnerability
Antioxidant Capacity	Unstable / limited	More balanced	Healthy cells better tolerate CAP
Membrane Composition	Altered lipid structure	Stable membrane integrity	Greater membrane damage in tumor cells
Mitochondrial Function	Dysfunctional	Normal	Easier apoptosis induction
DNA Repair Mechanisms	Frequently defective	More efficient	Increased genomic damage in cancer cells
Metabolic Activity	Hyperactive glycolysis	Controlled metabolism	Higher plasma sensitivity
Membrane Permeability	Increased	Regulated	Enhanced RONS penetration
Stress Adaptation	Limited tolerance threshold	Higher adaptive capacity	Selective apoptosis in tumors

4- MECHANISMS OF CAP-INDUCED APOPTOSIS

Apoptosis is a well-controlled form of programmed cell death necessary for normal tissue homeostasis, achieved by eliminating damaged, dysfunctional or aberrantly activated cells without causing significant inflammation. Apoptosis, in contrast to necrosis is characterized by cell shrinkage, chromatin condensation, membrane blebbing and DNA fragmentation with eventual the formation of apoptotic bodies which are then removed from tissues via phagocytic cells. Given that the disruption of apoptotic signaling is one of the hallmarks of cancer, re-establishment or maintenance apoptosis in neoplastic cells has become a major focus area for therapeutic development in modern oncology approaches [6, 12, 13]. Cold Atmospheric Plasma (CAP) is a state of matter that induces apoptosis largely through oxidative stress correlating with plasma-derived reactive oxygen and nitrogen species (RONS). Chronic intracellular buildup of these reactive species deranges redox homeostasis, inhibits proteins, lipids and mitochondrial ATP formation, and as well activates several signaling pathways that mediate programmed cell death. Instead of acting through a single molecular target, CAP simultaneously orchestrates multiple interrelated apoptotic pathways (Fig. 1), which diminishes the potential for therapeutic resistance [4, 6, 7]. Recent evidence has suggested that CAP induces apoptosis via both the intrinsic (mitochondrial) and extrinsic (death receptor-related pathways). All of these pathways converge on the activation of an executioner caspase, causing irreversible cell disassembly with reduced inflammatory response. This coordination of molecular events is what accounts for the distinct selective antitumor efficacy shown by CAP and separates plasma medicine from most conventional anticancer therapies [5, 6, 11].

4.1 Intrinsic (Mitochondrial) Apoptotic Pathway

The intrinsic apoptotic pathway represents the principal mechanism through which Cold Atmospheric Plasma (CAP) induces programmed cell death in malignant cells. This pathway is primarily activated by intracellular oxidative stress resulting from excessive accumulation of plasma-derived reactive oxygen and nitrogen species (RONS). Elevated oxidative stress disrupts mitochondrial homeostasis, damages mitochondrial DNA, proteins, and membrane lipids, ultimately leading to irreversible mitochondrial dysfunction [4, 6, 7]. A critical event in the intrinsic pathway is mitochondrial outer membrane permeabilization (MOMP). Under physiological conditions, mitochondrial membrane integrity is maintained by anti-apoptotic members of the Bcl-2 protein family, including Bcl-2 and Bcl-xL. Following CAP exposure, oxidative stress activates the pro-apoptotic proteins Bax and Bak, which oligomerize within the mitochondrial outer membrane and promote pore formation. This shift in the

Bax/Bcl-2 balance destabilizes mitochondrial integrity and permits the release of apoptogenic proteins into the cytoplasm [6, 11]. Among the released mitochondrial proteins, cytochrome c plays a central role in apoptotic signaling. Once released into the cytosol, cytochrome c associates with Apaf-1 (Apoptotic Protease Activating Factor-1) in the presence of ATP, leading to the formation of the apoptosome complex. This multiprotein complex subsequently activates initiator caspase-9, which in turn cleaves and activates executioner caspases, particularly caspase-3 and caspase-7. Activation of these proteases initiates the irreversible biochemical events responsible for chromatin condensation, DNA fragmentation, cytoskeletal degradation, and apoptotic body formation [6, 11, 13]. The tumor suppressor protein p53 further amplifies CAP-mediated apoptosis by regulating multiple components of the mitochondrial pathway. Oxidative DNA damage induced by CAP activates p53, which promotes transcription of pro-apoptotic genes such as BAX, PUMA, and NOXA, while suppressing anti-apoptotic proteins including Bcl-2. Consequently, p53 functions as a molecular switch that enhances mitochondrial membrane permeabilization and accelerates apoptosis in cancer cells carrying functional p53 signaling pathways [12, 13]. Importantly, cancer cells are considerably more susceptible to mitochondrial dysfunction than normal cells because of their elevated oxidative metabolism, impaired antioxidant defenses, and pre-existing mitochondrial abnormalities. Therefore, CAP-induced oxidative injury preferentially activates the intrinsic apoptotic pathway in malignant cells while causing substantially less damage to healthy tissues, thereby contributing to the selective therapeutic potential of plasma medicine [6, 7, 12].

4.2 Genotoxic Stress and Damage to the DNA

The integrity of DNA is needed for the normal life or death decision made at the cell level and therefore genome stability. On the other hand, increased oxidative stress produced during CAP exposure penetrates into cancer cells and can lead to direct nucleic acids damage which are responsible for strong genotoxic effects [33]. Reactive species from plasma can oxidize DNA molecules, break strands, or modify nucleobases. Cold Atmospheric Plasma promotes single-strand and double-strand DNA breaks by hydroxyl radicals, hydrogen peroxide, and other reactive species released from the discharge area of plasma [24]. Such oxidative modifications disrupt DNA replication and transcription machinery, harm chromosomal stability, and bring irreversible cell dysfunctions [32]. This makes tumor cells particularly susceptible to plasma-induced genomic damage due to many cancer types having defective DNA repair systems and/or failures in cell-cycle checkpoint regulation [34]. Consequently, malignant cells are often unable to effectively repair oxidative DNA damage events leading down the road to activation of apoptosis or mitotic catastrophe. Tumor suppressor proteins such as p53, which is essential for DNA damage detection, cell-cycle arrest and apoptosis, are also implicated in CAP-induced genotoxic stress [33]. Persistent oxidative DNA damage additionally triggers cellular stress response pathways and increased pro-apoptotic signaling cascades.

Table (2): Major Mechanisms of CAP-Induced Apoptosis in Cancer Cells

Mechanism	Plasma-Induced Effect	Biological Outcome
Oxidative Stress	Excessive RONS generation	Cellular redox imbalance
Mitochondrial Dysfunction	Membrane depolarization and cytochrome-c release	Intrinsic apoptosis activation
DNA Damage	Oxidative strand breaks and genomic instability	Cell-cycle arrest and apoptosis
Caspase Activation	Caspase-3 and caspase-9 signaling	Programmed cell death
Membrane Permeabilization	Increased membrane permeability	Enhanced intracellular reactive species penetration
Molecular Signaling Alteration	p53 activation and PI3K/Akt inhibition	Suppression of tumor survival
Immunogenic Cell Death	DAMP release and immune activation	Anti-tumor immune response
Tumor Microenvironment Modulation	Cytokine and inflammatory regulation	Reduced tumor progression

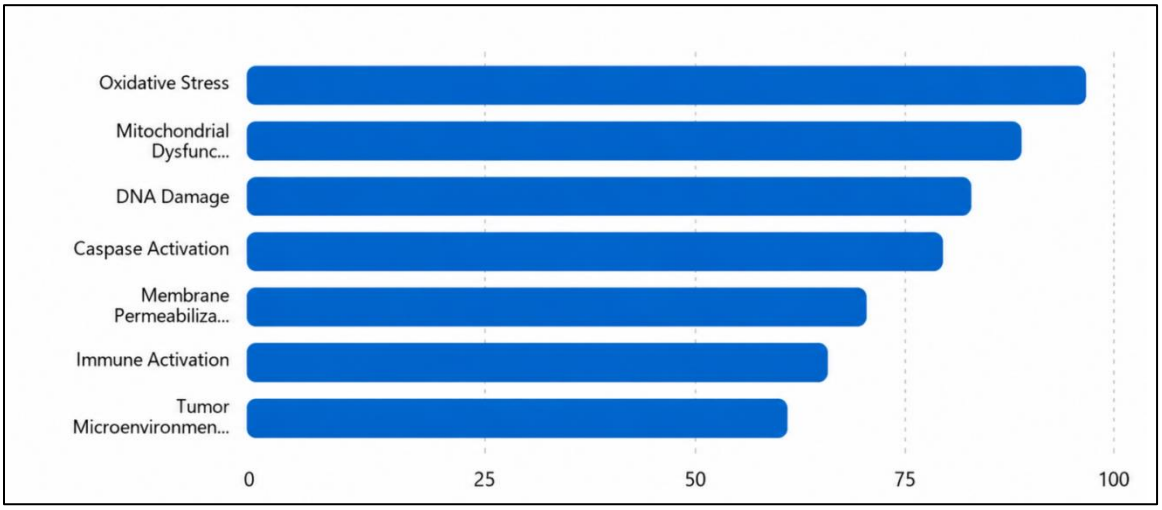

Fig (1): Relative Contribution of Major CAP-Induced Apoptotic Mechanisms in Cancer Cells

4.3 Immunogenic Cell Death and Immune Activation

Beyond its direct cytotoxic effects, Cold Atmospheric Plasma (CAP) has emerged as a potent inducer of immunogenic cell death (ICD), a specialized form of regulated cell death capable of activating adaptive antitumor immune responses. Unlike conventional apoptosis, which is generally considered immunologically silent, ICD promotes the release and surface exposure of danger-associated molecular patterns (DAMPs) that stimulate immune recognition and enhance tumor immune-surveillance [6, 8]. Following CAP exposure, oxidative stress induces the translocation of calreticulin (CRT) from the endoplasmic reticulum to the plasma membrane, where it functions as an "eat-me" signal for dendritic cells and macrophages. Simultaneously, extracellular release of adenosine triphosphate (ATP) serves as a chemoattractant for antigen-presenting cells, while the liberation of high mobility group box-1 (HMGB1) promotes antigen processing and presentation through Toll-like receptor signaling. These coordinated molecular events facilitate efficient recognition of dying tumor cells by the immune system [5, 8]. Activation of dendritic cells following CAP-induced ICD enhances tumor antigen presentation to $CD8^+$ cytotoxic T lymphocytes, leading to expansion of tumor-specific adaptive immune responses. In parallel, CAP has been reported to modulate macrophage polarization, increase natural killer (NK) cell activity, and influence cytokine production within the tumor microenvironment. These immunomodulatory effects complement the direct apoptotic activity of CAP and may contribute to durable antitumor immunity [6, 8].

Previous studies further indicate that CAP-mediated immune activation may improve the therapeutic efficacy of immune checkpoint inhibitors and other immunotherapeutic strategies. Consequently, combining CAP with immune-based therapies has become an active area of investigation in translational oncology, with the potential to overcome immune resistance and improve long-term clinical outcomes [7, 8]. Overall, the ability of CAP to simultaneously induce selective apoptosis and stimulate antitumor immunity distinguishes plasma medicine from many conventional therapeutic supports its development as a multifunctional platform for precision cancer treatment [6, 7].

4.4 Crosstalk between Intrinsic and Extrinsic Apoptotic Pathways

Apoptotic signaling induced by Cold Atmospheric Plasma (CAP) does not occur through isolated molecular pathways; rather, the intrinsic and extrinsic apoptotic pathways function as an integrated signaling network that collectively determines cellular fate. Plasma-derived reactive oxygen and nitrogen species (RONS) initiate multiple oxidative and stress-related responses that activate both mitochondrial dysfunction and death receptor signaling simultaneously, thereby maximizing apoptotic efficiency in malignant cells [4, 6, 7]. A key point of convergence between the two pathways is the activation of Bid, a BH3-only member of the Bcl-2 protein family. Following stimulation of death receptors, activated caspase-8 cleaves Bid into its truncated form (tBid), which subsequently translocates to the mitochondria. There, tBid cooperates with Bax and Bak to induce mitochondrial outer membrane permeabilization (MOMP), resulting in cytochrome c release and amplification of the intrinsic apoptotic pathway [6, 11]. The tumor suppressor p53 serves as another critical regulator linking oxidative stress with apoptosis. CAP-induced DNA damage activates p53, which simultaneously promotes expression of pro-apoptotic

proteins such as Bax, PUMA, and NOXA while suppressing anti-apoptotic proteins including Bcl-2. Through these coordinated molecular events, p53 enhances mitochondrial dysfunction and sensitizes tumor cells to both intrinsic and extrinsic apoptotic stimuli [12, 13]. The convergence of both apoptotic pathways ultimately results in activation of the executioner caspases, particularly caspase-3 and caspase-7, which orchestrate the irreversible biochemical and morphological changes characteristic of apoptosis. These include DNA fragmentation, chromatin condensation, cytoskeletal degradation, membrane blebbing, and formation of apoptotic bodies. Simultaneous activation of multiple apoptotic mechanisms reduces the likelihood of therapeutic resistance and contributes substantially to the selective anticancer efficacy of CAP [7, 11]. Emerging evidence further suggests that CAP-mediated apoptosis is dynamically influenced by intracellular redox signaling, calcium homeostasis, mitochondrial metabolism, and immune-related pathways. Consequently, CAP should be regarded as a multifunctional biological regulator capable of coordinating several interconnected molecular mechanisms rather than acting through a single apoptotic trigger. This integrated mode of action distinguishes plasma medicine from conventional therapies that predominantly target individual signaling pathways [5, 6, 8].

5- PLASMA PHYSICS AND MOLECULAR BIOLOGY INTEGRATION

Cold Atmospheric Plasma (CAP) serves as an interdisciplinary platform connecting plasma physics to molecular and cellular biology in cancer therapy. In contrast to traditional therapy approaches that mainly utilize chemical or radiological mechanisms, CAP provides both physical energy and specifically bioactive reactive species simultaneously, modulating multiple intracellular signals [35]. This interaction leads to a synergistic effect on plasma-induced physical factors and biological responses in tumor cells. Physically, CAP is generated as a multi-component mixture of charged particles, reactive oxygen and nitrogen species (RONS), ultraviolet photons, electromagnetic fields, and transient electric discharges [36]. These plasma-derived constituents directly interact with biological tissues and generate molecular changes related to oxidative stress, attack cell membranes, constitute mitochondrial dysfunction, and initiate apoptosis. At the molecular level, reactive species generated by CAP modulate intracellular signaling pathways related to cell viability, inflammation, immune activation and programmed cell death [37]. The fusion between physical plasma phenomena and cellular molecular biology has played a great thrust to the evolution of plasma oncology as an innovative therapeutic field combining physics, chemistry, biotechnology, and medicine. Reduced unwanted exposure can also help avoid illicit differentiation of malignant tissues and the expression of distant toxic effects. Over the other hand, CAP does not target single molecular pathway but instead modulates multiple biological systems at once and therefore lessens the chance of therapeutic resistance that is very common in conventional cancer treatments [38].

5.1 Electric Fields and Charged Particles

The electric fields and charged particles created by the CAP discharge help to mediate plasma–cell interactions and therapeutic response. Even without direct physical contact between plasma and tissues, initiating response at cellular or subcellular level by the action of plasma-generated electric fields on ion channels/membrane permeability/intracellular ion transport/molecular signaling pathways are possible in the vicinity of bioeffects [39].
This localized membrane destabilization and electroporation occur as transient electric fields produced during plasma exposure. This is associated in increased membrane permeability and intracellular entry of reactive species, ions, and therapeutic molecules [40]. These effects are more pronounced in malignant cells due to the unusual lipid composition and low structural stability of cancer membranes. Charged particles (electrons and ions) also cause the plasma-mediated biochemical responses. High-energy electrons are involved in the ionization and excitation reactions that drive viable production of RONS, and ions influence electrochemical interactions at the cellular interface surface [35]. Collectively these plasma-derived particles act to exacerbate oxidative stress and activate apoptotic signaling in cancer cells. Moreover, electric fields produced by CAP could affect intracellular calcium signaling and mitochondrial activity [41]. Dysregulation of calcium homoeostasis facilitates activation of apoptosis-related pathways and impairs cellular metabolism. What these findings imply is that the physical characteristics of plasma are directly involved in biological healing mechanisms, over and above pure reactive species generation.

5.2 Modulating Plasma Chemistry and Cellular Microenvironment

Plasma chemistry is one of the most significant contributors to CAP therapeutic action. For instance, in plasma generation, an energetic electron generated through the interaction with atmospheric gases and water molecule contributes to a wide range of chemically unstable species (hydroxyl radicals, ozone singlet oxygen, hydrogen peroxide, nitric oxide and peroxynitrite [42]. These reactive species are critical for modulating the tumor microenvironment featuring hypoxic, acidic pH, chronic inflammation, aberrant vascularization and immune dysregulation [43]. Reactive species derived from CAP can also modify the extracellular balance of redox and pro-

inflammatory signaling pathways and metabolic conditions present in tumor tissues, rendering cancer cells more susceptible to apoptosis. Most notably, plasma chemistry can be modulated using operational parameters such as gas composition, applied electrical voltage, humidity, and exposure time [44]. The ability to control plasma-generated reactive species allows one to optimize specific therapeutic goals and depending on the characteristics of a tumor.

5.3 Synergistic Activity in Selective Cytotoxic

One of the most important therapeutic benefits of CAP is its enhancement of selective cytotoxicity against malignant cells by synergistic physical-biological interactions. CAP-induced apoptosis is not mediated by a singular mechanism but rather occurs due to the participation of multiple mechanisms including oxidative stress, electric field effects, membrane permeabilization, mitochondrial dysfunction and molecular signaling changes [37]. The synergistic effect is especially lethal for cancer cells due to the higher level of oxidative stress, compromised antioxidant defense systems, abnormal metabolism, and less efficient DNA repair mechanisms in these cells [38]. Reactive species generated from plasmas reach levels that exceed the oxidative tolerance of tumor cells, while electric fields and charged particles induce accumulation of intracellular reactive species and disruption of signaling. Reduced therapeutic resistance is also attributed to the synergistic interaction between physical factors derived from plasma and biological responses [40]. Standard cancer therapies typically act on single molecular targets, allowing tumor cells to acquire adaptive resistance mechanisms. In contrast, CAP causes multi-systemic dysfunction in the cells, impairing the capacity of malignant cells to cope with stresses and survive. Moreover, CAP-induced selective cytotoxicity can avoid injury adjacent normal tissues which is underpinned a major limitation of chemotherapy and radiotherapy [39]. This selective therapeutic profile places CAP in a position of potential future application for use in precision oncology.

5.4 CAP: Precision-Based Therapeutic Platform

The wide range of biomedical applications of Cold Atmospheric Plasma has paved the path for establishing CAP as a novel precision-based therapeutic platform in modern oncology. One of the major goals of precision medicine is that individualized treatments can be provided based on specific biological features of either tumors or patients, which increase therapeutic benefit and reduce adverse effects [41]. CAP can satisfy some of the most critical requirements of precision oncology via its controllable plasma chemistry, selective cytotoxicity and tunable operational parameters. The production of reactive species, intensity of electrical field, exposure time and the gas composition within the plasma treatment area can be tailored according to tumor type, tissue tolerance and thus therapeutic goals [44]. In addition, CAP can be combined with some advanced biomedical technologies, including nanotechnology, targeted drug delivery systems (TDDS), immunotherapy and plasma-activated liquids [42]. These integrations facilitate the therapeutic adaptability of plasma medicine and improve anti-cancer potency. Advances in artificial intelligence and computational plasma modeling have also been instrumental in optimizing CAP protocols [44]. Personalized plasma therapy may one day allow clinicians to customize exposure conditions for plasmotherapy according to the molecular profile of the tumor and patient response. As a result, CAP is being unveiled not only as an alternative experimental anti-cancer treatment modality, but also emerging as a platform for precision-based and multidisciplinary oncology treatment solutions of the future.

Table (3): Synergistic Physical-Biological Components of CAP Therapy

Physical Component	Biological Interaction	Therapeutic Outcome
Reactive Oxygen Species (ROS)	Oxidative stress induction	Cancer cell apoptosis
Reactive Nitrogen Species (RNS)	Redox signaling disruption	DNA and protein damage
Electric Fields	Membrane permeabilization	Enhanced intracellular penetration
Charged Particles	Electrochemical interactions	Cellular signaling alteration
UV Radiation	DNA oxidation	Genotoxic stress
Plasma Chemistry	Tumor microenvironment modulation	Reduced tumor progression
Electroporation Effects	Increased drug uptake	Improved therapeutic efficiency
Immune Modulation	Cytokine activation	Anti-tumor immune response

6- CAP APPLICATION IN SOLID TUMORS

Cold Atmospheric Plasma anticancer activity has been proven for various solid tumors, including breast cancer, lung cancer, glioblastoma, melanoma, pancreatic cancer, colorectal cancer and head-and-neck tumors [35]. Experimental investigation has firmly established that CAP exposure causes oxidative stress mediated apoptosis, reduced tumor proliferative activity and inhibition of metastasis in solid tumors. This selective cytotoxicity of CAP is especially beneficial in solid tumors because reactive species generated from plasma preferentially target

malignant tissues while causing minimal damage to surrounding healthy structures and cells [38]. CAP has also been shown useful for tumor vascularization reduction and modulation of tumor-progression-related inflammatory pathways. Moreover, localized plasma therapy allows direct targeting of interaction surfaces reachable for treatment, establishing the postulate that CAP is particularly appropriate for cutaneous and superficial tumors [44]. Plasma-activated liquid has provided an additional opportunity of indirect therapy by penetrating into deeper tumor tissue.

6.1 Hematologic Malignancies and CAP

Besides solid tumors, CAP has been demonstrated its considerable potential for effective treatment against hematological malignancies including leukemia and lymphoma [40]. The apoptosis induction of neoplastic blood cells by plasma-generated reactive species follows an oxidative stress and mitochondrial dysfunction mechanism akin to that seen with solid tumor cell types. CAP has also proven effective against therapy-resistant cancer cells. We note that most resistant tumors display increased antioxidant capacity and modifications in signaling pathways, which contributes to an ineffective chemotherapeutic or radio therapeutic response [44]. Nonetheless, the multi-target mechanisms implicated in CAP-induced cytotoxicity provide a lower opportunity for resistance and a greater chance for therapeutic sensitivity. Recent studies have indicated that CAP may resensitize drug-resistant tumor models by altering pathways involved with oxidative stress and modulating the membranes properties [37]. In conclusion, these results support the potential role of CAP as an appealing coadjuvant tool in the treatment of patients with refractory malignancy-related disorders.

6.2 Complexity in Chemotherapy and Nanomedicine

One of the quickest developing fields in plasma oncology focuses around coupling CAP with chemotherapy, nano technology and targeted drug delivery. [42] CAP-induced membrane permeabilization and oxidative stress can facilitate intracellular drug absorption to improve the therapeutic efficacy of classical anti-cancer agents. Many studies have shown synergism for anti-cancer effects with the use of CAP together with chemotherapeutics like doxorubicin, cisplatin and temozolomide [36]. Tumor cells become chemo-sensitive in the presence of plasma due to impaired antioxidant defense and disruption of survival signaling pathways. The applications of CAP have been further extended by plasma-responsive nano formulations and targeted nanocarrier-enabling the theragnostic potential of nanotechnology [43]. Nanoparticles can the delivery of reactive species, increase selectivity and reduce systemic toxic level to patients. These multidisciplinary approaches mark a great step forward towards precision oncology applications.

6.3 Development of Plasma Medical Devices

Recent technical development has greatly advanced the safety, portability, and controllability of plasma medical tools [44]. Modern CAP systems comprise the following types of devices: plasma jets, dielectric barrier discharge (DBD) devices, plasma needles and plasma-activated liquid technology for biomedical applications. State-of-the-art plasma apparatuses now permit close monitoring and control of voltage, gas composition, frequency, and the treatment time in addition to an output of the generated reactive species [35]. This level of controllability is necessary for achieving optimal therapeutic benefits while simultaneously minimizing detrimental biological effects. Additionally, developments in miniaturization and robotics have further improved the applicability of plasma system into clinical practices [41]. If perfected, portable CAP devices could make bedside plasma therapy and some minimally invasive oncological procedures a clinical reality.

6.4 Current Clinical Trials and Translational Aspects

While the majority of CAP oncology studies are still in the experimental phase, early clinical trials have shown very promising results [39]. They are mostly involved in early-phase clinical trials for wound healing as well as in certain applications such as skin cancers, oral tumors that result after radiotherapy, and some palliative-use oncology indications. Plasma treatment protocols are currently being formulated with large-scale translational research aimed at plasma dosimetry optimization and long-term safety evaluations [44]. There continued to be big barriers to getting approval and mass clinical validation prior widespread implementation in the routine treatment of cancer.

Table (4): Major Biomedical Applications of CAP in Oncology

Application Area	Therapeutic Role of CAP	Clinical Significance
Solid Tumors	Selective apoptosis induction	Reduced tumor growth
Hematological Malignancies	Oxidative stress-mediated cytotoxicity	Leukemia and lymphoma targeting
Drug-Resistant Cancers	Sensitization to therapy	Reduced resistance mechanisms
Combination Chemotherapy	Enhanced drug uptake	Improved therapeutic efficacy
Nanotechnology Integration	Targeted delivery systems	Precision oncology applications
Plasma-Activated Liquids	Indirect tumor treatment	Deeper tissue applicability
Immunotherapy Support	Immune activation	Enhanced anti-tumor response
Medical Plasma Devices	Controlled plasma delivery	Clinical adaptability

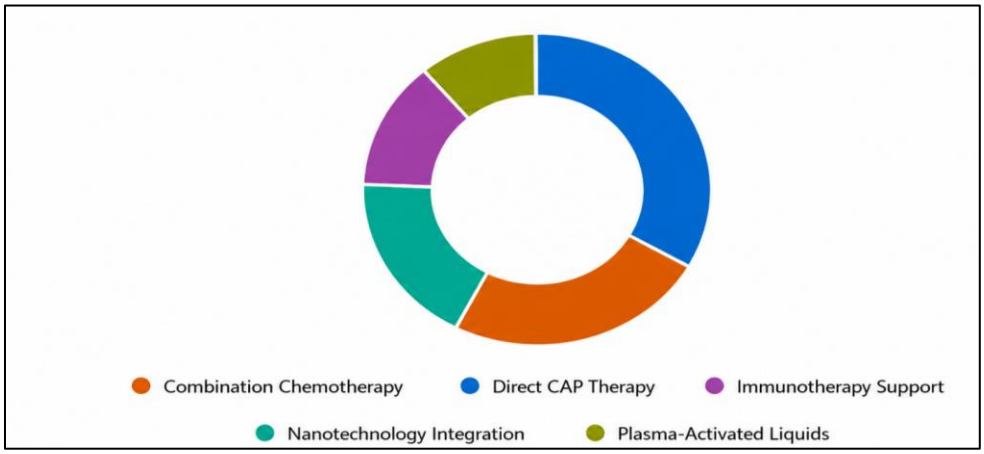

Fig (2): Comparative Therapeutic Efficiency of CAP-Based Oncology Approaches

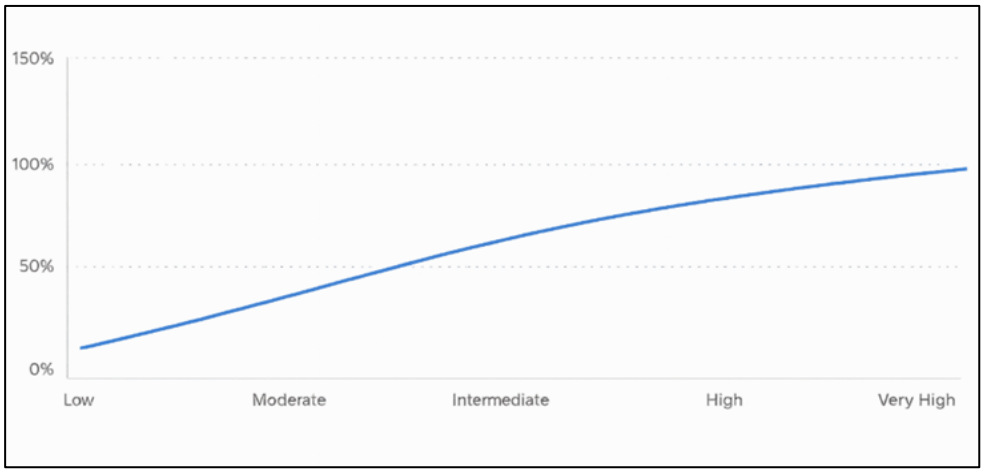

Fig (3): Relationship between Reactive Species Generation and Cancer Cell Apoptosis

7- CONCLUSION

Cold Atmospheric Plasma (CAP) is an emerging treatment approach in precision oncology that brings together plasma physics and molecular oncology. Its therapeutic effect depends on the combined production of RONS, electric fields, and charged particles, which can selectively trigger apoptosis in cancer cells. Malignant cells are especially vulnerable because tumors often have high baseline oxidative stress, dysfunctional mitochondria, altered membrane composition, and weakened antioxidant defenses. CAP activates several overlapping processes: intrinsic mitochondrial apoptosis, responses to genotoxic stress, and immunogenic cell death. It also affects the tumor microenvironment and can strengthen antitumor immunity. Experimental studies have reported promising results across a range of solid tumors and hematologic malignancies, but important barriers remain, including the need to standardize plasma dosimetry, improve device reproducibility, refine treatment conditions, and establish long-term clinical safety. Combining CAP with nanotechnology, plasma-activated liquids, and immunotherapeutic approaches could improve treatment outcomes while reducing resistance mechanisms. To move CAP into clinical practice, however, large-scale preclinical studies, standardized treatment protocols, and well-designed clinical trials will be needed to establish it as a viable precision-based therapeutic platform in modern oncology.

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