applied sciences

MDPI

Review

Molecular Biomarkers for Predicting Cancer Patient Radiosensitivity and Radiotoxicity in Clinical Practice

Angeliki Gkikoudi [1,2], Spyridon A. Kalospyros [1], Sotiria Triantopoulou [2], Stella Logotheti [1], Vasiliki Softa [3], Constantin Kappas [3], Kiki Theodorou [3], Evagelia C. Laiakis [4,5], Gina Manda [6], Georgia I. Terzoudi [2] and Alexandros G. Georgakilas [1,*]

1 DNA Damage Laboratory, Physics Department, School of Applied Mathematical and Physical Sciences, National Technical University of Athens (NTUA), Zografou Campous, 15780 Athens, Greece; angelikigkikoudi@mail.ntua.gr (A.G.); spkals@central.ntua.gr (S.A.K.); stella_logotheti@mail.ntua.gr (S.L.)
2 Laboratory of Health Physics, Radiobiology & Cytogenetics, Institute of Nuclear & Radiological Sciences & Technology, Energy & Safety, National Centre for Scientific Research "Demokritos", 15341 Agia Paraskevi, Greece; iro@rrp.demokritos.gr (S.T.); gterzoudi@rrp.demokritos.gr (G.I.T.)
3 Department of Medical Physics, Faculty of Medicine University of Thessaly, 41500 Larissa, Greece; vasilikisofta@gmail.com (V.S.); kappas@uth.gr (C.K.); ktheodor@med.uth.gr (K.T.)
4 Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC 20057, USA; ecl28@georgetown.edu
5 Department of Biochemistry and Molecular & Cellular Biology, Georgetown University, Washington, DC 20057, USA
6 Victor Babeș" National Institute of Pathology, 99-101 Splaiul Independentei, 050096 Bucharest, Romania; gina.manda@ivb.ro
* Correspondence: alexg@mail.ntua.gr; Tel.: +30-210-7724453

Abstract: Radiotherapy (RT) is a major part of cancer treatment. The reported variability in patient response to this modality can interfere with the continuation of best-possible care, promote side effects, and lead to long-term morbidity. Tools to predict a patient's response to radiation could be highly useful in improving therapeutic outcomes while minimizing unnecessary and toxic exposure to radiation. This study investigates the potential of using molecular biomarkers as predictors of radiosensitivity in clinical practice. We review relative studies researching the positive correlation between various molecular biomarkers and patient radiosensitivity, including DNA damage response and repair proteins, inflammation and apoptosis markers, cell cycle regulators, and other biological markers. The clinical perspectives and applicability of these biomarkers in the prediction of radiosensitivity are also critically discussed. Conclusively, we underline the dynamics of molecular biomarkers to improve the efficacy and safety of radiotherapy in clinical practice and highlight the need for further research in this field. Identification of the most prominent markers is crucial for the personalization of therapies entailing ionizing radiation.

Keywords: radiation biology; radiation therapy; patient radiosensitivity; molecular biomarkers; DNA damage response; inflammation; adverse effects

Citation: Gkikoudi, A.; Kalospyros, S.A.; Triantopoulou, S.; Logotheti, S.; Softa, V.; Kappas, C.; Theodorou, K.; Laiakis, E.C.; Manda, G.; Terzoudi, G.I.; et al. Molecular Biomarkers for Predicting Cancer Patient Radiosensitivity and Radiotoxicity in Clinical Practice. *Appl. Sci.* **2023**, *13*, 12564. https://doi.org/10.3390/app132312564

Academic Editor: Chang Ming Charlie Ma

Received: 20 October 2023
Revised: 16 November 2023
Accepted: 18 November 2023
Published: 21 November 2023

1. Introduction

For over a century, radiotherapy (RT) has remained at the forefront of cancer patient treatment. It contributes to ~40% of curative cancer treatments, alone or in combination with chemotherapy, and tends to be less morbid than surgery [1]. The continued and rapid progress in the field of RT includes radiation type and delivery schemes that provide a conformal dose distribution in the tumor niche, leading to the maximum possible efficacy in cancer cell killing. At the same time, emphasis is given to preserving the functional and structural integrity of neighboring healthy tissues. Some advanced RT techniques such as intensity-modulated radiation therapy (IMRT), stereotactic radiosurgery (SRS), and proton/carbon ion therapy (hadron therapy) greatly improve clinical outcomes and

provide better control of cancer progression via precise delivery of radiation doses to the tumor tissue. New modalities, such as FLASH proton radiotherapy, where high doses are delivered in an ultra-high dose rate fashion, are currently under clinical trials [2,3]. Importantly, in the last few years, the interest in RT modalities has been renewed due to the ability of RT to promote systemic immune responses that can overcome resistance to immunotherapy. Combinations of RT with immunotherapeutic agents, such as immune checkpoint inhibitors, are increasingly favored for overcoming the barriers and synergizing the efficacy of each intervention alone [4].

While RT effectively targets and destroys cancer cells, it can also result in toxicity and adverse effects at the level of healthy tissues in the vicinity of the irradiated area, as well as at the systemic level. Along with interfering with the basic biological properties of cancer cells (such as growth signal autonomy, unlimited replicative potential, and evasion from growth inhibitory signals along with angiogenesis, invasion, and metastasis), ionizing radiation can often inflict life-threatening damage to normal cells. Common toxicities associated with RT include acute and chronic radiation dermatitis (erythema, dryness, skin desquamation and telangiectasias, fibrosis, and ulceration, respectively), mucositis, fatigue, diarrhea, dysphagia, and radiation-induced fibrosis among others depending on the site of exposure. Cell injury due to IR comprises a spectrum of sequential events and biological processes involving intricate cellular morphologic, molecular, and functional changes that are triggered by IR within minutes to hours after irradiation (early toxicity), related to oxidative stress, DNA damage, and cell death, as well as inflammation and cell proliferation [5]. During the first few minutes after exposure, part of the tumor cell is committed to cell death (apoptosis), while part attempts to repair the damaged DNA by developing a robust stress response. IR-induced cell alterations progress over weeks, months, and even years (late toxicity), leading to late tumor and organ dysfunction (atrophy and fibrosis) and therapy-induced secondary malignancies in some cases. The mechanisms underlying radiotoxicity in human tissues involve increased cell death and inflammation, as well as defective tissue regeneration [6].

Patient radiosensitivity is emerging as a multi-dimensional problem [7,8], ranging from the microworld of the cell and its environment to the macroworld of patients and their families, including time scale (from minutes to years) and medical physics parameters (dose and RT modalities). The adverse effects exhibit complex patterns across multiple tissues and have unclear boundaries, rather than being a mere and distinct classification to radiosensitive versus radioresistant individuals. The occurrence of radiogenic side effects is a strong limitation of therapeutic radiation, requiring the adjustment of the effective individual dose for the prolongation of the patient's life. Additionally, late effects of RT include radiation-induced secondary malignancies [9]. In view of these clinical complications, cut-offs need to be defined that can guide medical decisions and reliably stratify patients who can tolerate a standardized treatment RT scheme, exhibiting no or mild side effects, from those who show individualized over-sensitivity responses, entailing a combination of adverse effects that counteract therapeutic benefits [10].

The reliable and early prediction of radiosensitivity reactions in the irradiated patient can improve RT planning and optimize RT treatment on a personalized basis. In this review, we summarize the toxicities that are linked with RT and highlight the factors that individualize sensitivity to RT. Given the power of biomarkers in the prediction of patient responses to cancer therapies [11], we particularly focus on molecular biomarkers that can predict radiosensitivity in human patients or human samples. We particularly summarize the progress in the utilization of different molecular biomarkers for RT toxicities and highlight a need for improving the relative knowledge. These research efforts are expected to be significantly facilitated by big data and artificial intelligence in the future and eventually allow the identification of accurate and molecular biomarkers of radiotoxicity toward personalizing RT therapeutic regimens.

The main goal of this paper is to provide a comprehensive review of the existing data on the utilization of molecular biomarkers for predicting radiosensitivity in human

patients. Therefore, we have excluded genomic-based studies reporting on the possible use of gene signatures for predicting tumor radiosensitivity and in general response to radiation therapy (for an extensive review please see [12]) or markers on the response of tumor microenvironment [13]. We retrieved relevant literature from PubMed and Google Scholar. As our focus was the clinical relevance of molecular biomarkers for predicting radiosensitivity and toxicity in RT, we used the 'clinical trial' filter on PubMed, while using the following related keywords: 'patients radiosensitivity', 'patients radiotoxicity', 'radiation induced damage', 'radiosensitivity molecular markers', 'radiosensitivity biomarkers', and 'radiosensitivity in radiotherapy' to discover the Journal articles that fulfill these requirements. According to our literature research, five studies have examined the use of DNA Damage Response (DDR)-related biomarkers in the last five years to predict radiosensitivity/radiotoxicity using patient samples and only one has examined apoptotic markers. Seven articles were found for biomarkers involving secreted soluble proteins, whereas nine studies looked at biomarkers involving cell-free DNA (cfDNA) and two referred to machine-learning algorithms employed for patient radiosensitivity predictions.

2. RT-Induced Toxicities and Their Clinical Management

Radiosensitivity reactions range from inflammation and fibrosis to radiation hypersensitivity syndromes (e.g., ataxia–telangiectasia, Fanconi anemia, radiosensitive severe combined immunodeficiency disease, etc). The effects can be either acute, including erythema, diarrhea, dermatitis, and cystitis, or late, entailing vascular damage, atrophy, infertility, and fibrosis [14]. Currently, the management of adverse reactions follows consensus guidelines that help healthcare professionals provide care to oncology patients before, during, and after cancer treatment with respect to their cardiovascular health status.

The earliest criteria defining radiation toxicity following RT in the clinic are traced to almost five decades ago. In the 1970s, the Radiation Therapy Oncology Group (RTOG) established radiotoxicity criteria, focusing primarily on acute toxicities such as skin reactions, gastrointestinal symptoms, and hematologic alterations. These criteria set the basic framework for evaluating the severity of adverse radiologic effects by providing a grading scale ranging from 0 to 5, where 0 indicates no toxicity and 5 represents severe toxicity. In the 1980s, the RTOG toxicity criteria were updated, incorporating additional organs and systems, and refining the grading scales to provide more precise assessments. Subsequent updates in the 1990s expanded the criteria to include chronic radiation toxicities, also considering long-term effects. In 1995, RTOG collaborated with the European Organization for Research and Treatment of Cancer (EORTC) to develop a joint set of radiotoxicity criteria, known as the RTOG/EORTC criteria [15]. This collaboration aimed to standardize the assessment and reporting of radiation toxicities to ensure consistency in clinical trials and research studies. Over time, advancements in the knowledge and understanding of the mechanisms underlying radiation toxicities led to the development of more comprehensive and sophisticated radiotoxicity criteria, such as the Common Terminology Criteria for Adverse Events (CTCAE) initiated by the National Cancer Institute (NCI) [16], that provide a widely accepted framework for grading and reporting treatment-related toxicities, covering a comprehensive range of organs and systems, with toxicity grades from 1 to 5 according to severity. The criteria include skin reactions, mucositis, gastrointestinal complications, and hematologic and neurologic disturbances. These newest criteria reflect the most up-to-date understanding of radiation toxicities and are widely used in clinical practice and research settings to assess and manage the adverse effects of RT.

In a recent review regarding skin toxicity following RT for breast cancer patients, Hussein et al., 2022 highlighted skin thickening, less echogenic dermis, a poorly visible dermis–subcutaneous fat boundaryand decreased Pearson correlation coefficient of the hypodermis compared with the nonirradiated skin [17]. Mucositis affects the mucous membranes, causing pain, ulceration, and difficulty in swallowing. In a systematic review, Trotti et al., 2003 displayed the elevated rates of mucositis in RT-treated patients with head and neck cancer, a severe form of radiotoxicity that may lead to hospitalization and

treatment interruptions [18]. Fatigue is a frequent side effect associated with physical and cognitive impairment. Radiation-induced fibrosis involves the progressive scarring of irradiated tissues, leading to functional impairment. The severity and occurrence of these toxicities depend on various factors such as radiation dose, fractionation scheme, and the irradiation field location. Proper management and supportive care are essential to mitigate these toxicities and improve the quality of life for cancer patients undergoing RT.

A key concern during the administration of RT is to increase radiosensitization in cancer cells while protecting normal tissues. Current RT guidelines limit the tolerated dose for minimizing the toxicity risk in the tumor-surrounding normal tissues at 5 years after RT treatment. In the case of side effects, the applicable therapeutic 'window' of RT should be re-adjusted. However, lowering the RT alone is not sufficient to reduce side effects and even with low radiation doses, the risk of complications cannot be avoided. For example, among 5-year survivors of childhood cancer, there appears to be no threshold dose below which radiation to the cardiac substructures does not increase the risk of cardiac diseases [19]. Consistently, in breast cancer patients, even lower doses of irradiation are correlated with cardiovascular manifestation [20].

Several measures are considered to alleviate the unwanted damage and side effects among patients undergoing RT. Periodic fasting or fasting-mimicking diets (FMDs), which are plant-based diets, low in protein and sugar but relatively high in fat, have demonstrated potent anticancer effects in mouse cancer models, including enhancing the efficacy of chemoradiotherapy and targeted cancer drugs, as well as triggering anticancer immune responses. Combining periodic FMD cycles with standard cancer treatments holds the highest potential for promoting cancer-free survival in patients, as fasting or FMDs can disrupt alternative metabolic strategies adopted by cancer cells, prevent resistance acquisition, and protect normal cells from the side effects of cancer drugs [21]. Based mostly on the experimental model's data, renin–angiotensin–aldosterone system (RAAS) inhibitors show promise in mitigating the progression of radiation nephropathy (RN), and epoxyeicosatrienoic acids can attenuate renal vascular dysfunction in patients undergoing abdominal or pelvic radiation [22]. To reduce radiation-induced damage to normal tissues, a plethora of radioprotective agents and radiomitigators have been investigated [23]. For example, amifostine is used to protect normal tissues from radiation damage. The level of protection from amifostine varies among different tissues, with higher protection factors observed in the hematopoietic system and salivary glands compared with the lung, kidney, and bladder, while central nervous system is not protected at all due to the blood-brain barrier [24].

3. Factors Regulating Tolerability of RT in Cancer Patients

The current clinical practice for radioprotection is based on medical experience and population outcomes and does not fully address variations in radiobiological responses in individual patients. On the one hand, the clinical cut-offs are not tailored for patients with radio-resistant tumors since they are based mainly on clinical data and population averages. It is of note that a remarkable percentage of patients exhibit a hypersensitive phenotype by developing severe side effects even when subjected to hypo-fractionated RT [6]. In the real world, different individuals may have individualized responses to a given dose protocol in terms of treatment efficacy and toxicity. The occurrence of distinctive effects related to radiosensitivity among individuals depends on a number of parameters, including both the radiation characteristics (type, dose, and dose rate of radiation) and the features of individual patients and/or their tumors.

A prominent factor related to the toxicity risk in RT is genetic predisposition (intrinsic radiosensitivity). This appears to contribute the most to the individual variability in radiosensitivity [25]. According to Barnett et al., 2012, heritability is responsible for radiosensitivity in 60-80% of oncologic patients [26], while Vaisnav et al., 2014, estimate a contribution of around 80% [27]. Other patient-relative critical factors affecting variations in radiosensitivity are age and sex. For instance, children are considered to be the most

sensitive group in terms of radiation-induced cancer, for all the spectrum of dose exposures [28,29]. Sex is also differently related to radiation responses [30], as mostly shown in studies on the survivors of the Hiroshima and Nagasaki atomic bombs [31]. Lifestyle (such as smoking, alcohol drinking, dietary habits, etc.) is also another factor influencing tissue/organ radiosensitivity [32], as well as the body mass index (BMI) and individual metabolism [33]. The primary cancer type and the use of parallel or preceding treatments (e.g., chemotherapy) also modulate radiosensitivity.

Other important factors include the tumor doubling time, along with the redox and hypoxic status of tissues, and have an important contribution to the total dose delivered to the oncologic patient and the dose fractionation scheme. Any likely association between these alterations and the average normal tissue response to IR reaching the limit of toxicity would be a key step for treatment optimization and individualization. Moreover, the tumor microenvironment (TME) plays a critical role in the optimization of the efficacy of RT. According to Byrne et al., 2021, the effects of RT on the TME scale involve changes in immune cells, vasculature, and stromal components [13]. In this regard, precision medicine approaches that consider the genomic signatures of patients and the TME are crucial to optimize the efficacy of RT and minimize adverse effects.

The mechanisms underlying radiosensitivity are multifactorial [34]. Mechanism-driven strategies to predict, monitor, and treat RT-induced side effects are currently missing. This is not only due to the poor understanding of the underlying pathways but also because the type of side effects may vary depending on the cancer type, therapeutic regimen, and individual patient. The identification of radiation injury mechanisms and the specification of the in vivo toxicity caused by several agents remain topics of immense research. The need to evaluate radiosensitivity and radiotoxic risk prediction for an oncologic patient undergoing RT is of leading priority in modern radiation oncology. For this reason, new tools to predict tumor radioresistance and healthy tissue radiosensitivity are highly needed for developing personalized RT strategies. Reliable biomarkers would greatly help to establish the dose–effect relationship in RT for evaluating its efficacy for tumor control and its toxicity for normal tissues in a personalized manner.

4. The Importance of Biomarkers in Monitoring RT Toxicity

A molecular biomarker is any molecular indicator of normal and pathogenic biological processes or the pharmacologic responses to therapeutic interventions [35]. Biomarkers improve prediction, characterization, and treatment of human diseases. In the oncology field, biomarkers guide informed decisions of clinicians regarding chemo- and radiotherapeutic strategies, relative to the risk of potential side effects and tumor recurrence. Biomarkers are measured, if possible, in readily accessible biological samples before, during, and after RT. Medical imaging with new combined techniques and higher definition, together with assays in biological fluids, constitute the current main strategy in the clinical evaluation of RT-induced radiosensitivity and toxicity. In radiation response investigations, blood and its components, serum/plasma and cells, mostly lymphocytes, are considered a readily accessible source of biomarkers to study IR toxicity.

An ideal blood biomarker would fulfill the following criteria: (a) precise targeting of a biological process related to the radiosensitivity of the peripheral immune system; (b) specificity for a certain type of cancer with alterations in the systemic immune response; (c) tumor reactivity to RT mirrored in peripheral blood; (d) measurement accuracy in blood samples and validation of the detection method according to international standards; (e) distinct delimitation of the biomarker levels between patients with a certain type and stage of cancer and patients with systemic inflammatory processes; (f) independence from diverse environmental factors (e.g., dietary habits, alcohol intake, etc.) or treatments unrelated to disease therapy; (g) detection of measurable changes during disease evolution and/or therapy; (h) reliability of laboratory measurements and generation of results and; (i) undetectability, if possible, when patients attain remission. In fact, and to the best of our

knowledge, currently, there is no biomarker that fulfills all the criteria mentioned above; rather, several biomarkers are estimated in conjunction with other clinical findings.

The advancement of our understanding of genomic and epigenomic processes, including mutations, gene expression changes, and post-translational modifications, has shifted radiobiology research to gene and protein biomarkers that are indicative of the radiation-induced responses in normal and cancer cells. Deep-sequencing approaches confirmed that complex DNA damage and repair mechanisms, along with chromosomal aberrations and IR-induced cell apoptosis in various irradiated tissue samples can be considered predictors of radiosensitivity and post-irradiation toxicity. Protein biomarkers can also be used for the estimation of the received dose and response of the irradiated biological system (cell/tissue), the assessment of radiosensitivity of different human cell lines, the investigation of susceptibility of an irradiated individual as a whole organism, the detection of early signs arising from radiation-induced disease, and the evaluation of late health effects that are present a long period of time after exposure before the initiation of an associated disease. At the same time, progress in the field of biomarkers that can be widely used in the clinic is rather slow. This is because for a biomarker to qualify as indicative of a clinical outcome, the variation in its values must be strongly correlated with the corresponding changes in disease status. In this process, statistics must prove that there is enough clinical evidence in multiple observations that confirm the hypothesized correlation.

4.1. Cell-Intrinsic Radiosensitivity Biomarkers

Over the years, a variety of assays and endpoints have been developed to measure radiosensitivity, each with its advantages and disadvantages. The clonogenic assay has been widely used and is considered the gold standard for assessing cellular radiosensitivity in vitro [36]. However, it is time-consuming and requires a high level of technical expertise.

In the recent past, several cytogenetic assays have been developed to evaluate radiosensitivity, such as the G2 chromosomal radiosensitivity and the micronucleus assay, along with fluorescence in situ hybridization (FISH) (Table 1). Using these methodologies, several gene and protein biomarkers associated with radiosensitivity have been identified. For example, the expression of DNA repair proteins, such as γH2AX and 53BP1 (Table 1), have been shown to correlate well with radiosensitivity. An overview of these molecular and cytogenetic biomarkers of radiosensitivity is presented below.

4.1.1. Cytogenetic Markers

Cytogenetic assays are extremely effective tools for detecting and analyzing chromosomal abnormalities in cells. Various types of chromosomal aberrations, including deletions, duplications, translocations, and inversions, can be detected by the examination of chromosomes, either at the metaphase stage or the interphase nucleus. Cytogenetic assays, such as the G2 assay, micronucleus (MN) assay, and fluorescence in situ hybridization (FISH), have been used to measure the extent of DNA damage and genetic alterations in cells after exposure to radiation and provide valuable information about an individual's susceptibility to radiation-induced cancer and the effectiveness of RT.

A cell-cycle-based technique that has been utilized by several groups to investigate correlations between human chromosomal radiosensitivity and increased susceptibility to cancer is the G2 chromosomal radiosensitivity assay [37–39]. The G2 assay is a cytogenetic methodology used to evaluate cell radiosensitivity. It assesses the extent of chromosomal aberrations induced by IR at the G2 phase of the cell cycle, which can be visualized at metaphase spreads, where chromosomes are condensed, using standard cytogenetic techniques. In order to assess the conversion of unrepaired DNA lesions into chromosomal damage during the G2 to M phase transition (as chromatid breaks at metaphase), the methodology entails in vitro irradiation of peripheral blood lymphocytes in the G2 phase, followed by repair of DNA damage and a colcemid block of spindle formation during mitosis. Enhanced chromosomal radiosensitivity, manifested by radiation-induced chromatid aberrations in lymphocytes, has been observed in many types of cancer and genomic

instability syndromes. The results of the G2 chromosomal radiosensitivity assay performed by Howe O. L. et al., 2005, on 15 patients with benign prostatic hyperplasia (BPH) [40], 17 patients with prostate cancer (which have not received radio-, chemo-, or immunotherapy for at least 6 months before sampling), and 14 healthy control individuals evidenced an elevated level of radiation-induced chromatid aberrations and enhanced G2 chromosomal radiosensitivity in the first two groups mentioned above, compared with the control group. Howe et al., 2005 applied the G2 assay in heparinized blood samples obtained from 27 breast cancer (BC) patients and confirmed that in a significant proportion, they exhibited elevated G2 chromosomal radiosensitivity in contrast to random controls [41]. Moreover, Chua et al., 2011 observed significantly higher levels of deletion-type aberrations in ex vivo irradiated blood lymphocyte metaphases in a group of BC patients with severely marked radiation-induced change compared with the group with very little/no change after RT; the authors came to the conclusion that chromatid aberration appears to be a suitable biomarker for the prediction of radiation-induced toxicity [42]. Conversely, a comparison of 25 prostate cancer patients with severe side effects and 25 patients without severe side effects after RT, as well as 23 male healthy age-matched donors analyzed in the study performed by Brzozowska et al., 2012 [43] showed that the G2 assay cannot be characterized as a reliable tool for the identification of prostate cancer patients with a high risk for developing severe clinical side effects. This observation was sustained by the study of Pinkawa et al., 2016 [44] on patients with localized T1–3N0M0 prostate cancer who were treated with 3D conformal RT. A predictive value of the G2 assay results after irradiation of lymphocytes with 0.5 Gy could not be established, as no discrimination between urinary and bowel score change could be made.

The existing G2 assay protocols for assessing individual radiosensitivity have limitations, as they can only produce consistent and meaningful results under strict technical conditions [45]. The variability in the yields of radiation-induced chromatid breaks in various samples from the same individual is a well-known issue [46]. Another significant concern related to the appropriateness of the G2 assay for identifying radiosensitive patients is the significant overlap in the yields of G2 chromatid breaks in cancer patients and in healthy individuals [47]. To overcome the above-mentioned limitations, a standardized G2 assay using an ATM/ATR inhibitor as an internal control was established by Pantelias and Terzoudi 2011 [48], minimizing inter-laboratory and intra-experimental variations and estimating the G2 chromosomal radiosensitivity at the individual level.

An alternative method to estimate an individual's radiosensitivity at the cytogenetic level is the G2 cytokinesis-block micronucleus (MN) assay. This assay has been used for almost 40 years and has become one of the most popular methods to assess the genotoxicity of different chemical and physical factors, including radiation-induced DNA damage [49]. Micronuclei are small nuclei formed when chromosomes or chromosome fragments are not incorporated into the daughter nuclei during cell division. Micronuclei can be detected in cells that have completed nuclear division; they are identified by their binucleated appearance after inhibiting the formation of the actin microfilament ring that is required for cytokinesis by adding Cytochalasin-B in the cell culture. Performing the G2 MN assay in the blood samples from 18 BRCA2-mutation carriers and 17 individuals from both BRCA1 and BRCA2 families not showing the familial mutation, Baert et al., 2017 found a higher radiosensitivity in healthy BRCA2-mutation carriers compared with healthy volunteers after exposure of PBL to a dose of 2 Gy γ-rays, while no increased radiosensitivity was observed in non-carrier relatives of BRCA1 and BRCA2 families [50]. The radiosensitivity at the individual level proved to be mild or more severe to a higher proportion in BRCA2-mutation carriers (50%) compared with healthy volunteers (17%) and non-carriers (24%). These results indicate that the MN assay constitutes a valuable tool to identify radiosensitivity in BRCA2-mutation carriers exposed to IR either for diagnostic or therapeutic purposes.

The MN assay is internationally validated due to the ease of scoring and its applicability in different cell types [51]. Despite the clear advantages of the MN assay, some limitations must be considered when performing the test. The MN assay requires exper-

tise in sample preparation, slide preparation, and scoring of micronuclei. However, the scoring criteria and methodology can vary between laboratories and even within the same laboratory, leading to inconsistent results, hence limiting data comparison across studies or institutions. Furthermore, this assay exhibits significant inter-individual variability in control-unexposed donors and their induction can be influenced by various factors other than radiation exposure (such as chemicals, genotoxic agents, and certain physiological conditions) [52], which can affect the specificity of the MN assay as a sole indicator of radiosensitivity due to confounding factors.

Another potent assay utilized for the assessment of individual radiosensitivity the is FISH assay through which certain nucleic acid sequences can be detected and localized. FISH enables the detection of cytogenetic changes, such as translocations or deletions, by using fluorescence microscopy or flow cytometry [53]. Dunst et al., 1995 performed the FISH assay in lymphocytes from 16 different types of cancer patients (12 patients were considered as having normal tolerance to radiotherapy while 4 were considered as having a potentially increased radiosensitivity) [54]. The latter four patients showed increased clinical radiosensitivity, experiencing severe acute reactions and exhibiting chromosomal damage in lymphocytes after irradiation. Particularly, significant differences were found in radiation-induced chromosomal damage, measured as breaks per 1000 mitoses ranging from 70 to 556 after 0.7 Gy and from 420 to 1210 after 2 Gy. Thus, the authors concluded that this technique may be useful to detect patients with severely enhanced radiosensitivity. Huber et al., 2011 quantified chromosomal aberrations by means of FISH using specific probes for chromosomes 1, 4, and 12 in blood samples from 47 BC patients collected 6 weeks post RT. Meanwhile, clinical side effects of RT were evaluated weekly during the therapy, and the scoring was carried out according to the Common Toxicity Criteria (NCI-CTC scale; scale digits 0, 1, 2, 3, 4). The study's findings were increased chromosomal aberrations in patients with more severe side effects, a correlation between the frequency of a specific aberration (painted chromosomes bearing one centromere with a color junction type t(Ba)), and the occurrence of skin side effects. Based on the frequency of t(Ba) aberrations, the authors identified patients with short latency (early side effects) when no correlation was found for other aberrations (painted chromosomes bearing one centromere with a color junction type t(Ab), dicentrics(dic), color junctions(cj)) with side effects or latency. Results have indicated that the estimation of chromosomal radiosensitivity at the translocation level in peripheral blood lymphocytes can be proposed as a predictive assay for the detection of radiosensitive individuals [55]. The 2, 11, and 17 paint probes tested in localized prostate cancer patients treated with 3D conformal RT, with (PS) and without (P0) side effects, as well as in a healthy group, led to negative results in the study of Schmitz et al., 2013 [56] as no pronounced differences in chromosomal radiosensitivity levels between donor groups, after a 2 Gy-irradiation, were mentioned. A dose-dependent increase in aberrations for all analyzed chromosomes, with smaller chromosomes 11 and 17 being less frequently involved in aberrations compared with the larger chromosome 2, was found. Nonetheless, the FISH assay did not manage to demonstrate significant differences in chromosomal radiosensitivity between donor groups when comparing the chromosomes with each other. However, the dose-dependent increase in aberrations suggests that higher radiation doses may be needed to reveal such differences. FISH has some limitations compared with the G2 and MN assay, as it is a laborious technique that requires specialized equipment, while the preparation of FISH probes, hybridization, and image analysis processes can be time-consuming, limiting its widespread implementation and applicability in routine clinical practice. In turn, the utilization of the FISH assay for assessing radiosensitivity offers several advantages, including the detection of chromosomal aberrations with high sensitivity, specificity, and accuracy. These strengths make it a valuable tool in detailing an individual's response to IR toward the development of personalized RT strategies.

Table 1. Biomarkers for assessing the radiosensitivity of blood cells.

Assay (Molecular and Cytogenetic Markers	Sample/Patient Profile	Outcome	Study
γH2AX IF assay	Peripheral lymphocytes of 31 patients with resected head-and-neck cancer with different grades of oral mucositis undergoing adjuvant RT or RCT.	Patients with a proportion of unrepaired DSBs after 24 h higher than the mean value had an increased incidence of severe oral mucositis.	[57]
	Peripheral lymphocytes of 80 post-chemotherapy OR and NOR BC patients and 38 healthy female donors.	Statistical difference between the healthy and NOR groups, the healthy and OR groups, and the NORs and ORs, using PRD (percentage residual damage).	[58]
	PBMCs isolated from 25 prostate cancer patients treated with RT. (Expanded Prostate Cancer Index Composite [EPIC].)	No correlation between the number of γH2AX foci and radiotoxicity at the level of PBMCs.	[44]
	Cancer patient lymphocyte cells were isolated from individuals who did and did not have a severe reaction to RT.	No detectable differences were found between the control group and the RS group by following γH2AX foci kinetics after IR.	[59]
γH2AX FCM assay	PBLs isolated from 12 patients who had experienced severe atypical NTT (Normal Tissue Toxicity) as a consequence of earlier RT were identified. Patients (10) who had experienced little or no NTT acted as one control group, and 7 healthy, non-cancer individuals comprised a second control group.	Reliable differentiation between patients clinically classified as NOR or OR patients.	[60]
γH2AX/53BP1 IF assay	Blood lymphocytes of BC patients who had undergone surgical excision of the primary tumor and postoperative RT to the whole breast. Two groups of patients were identified indicating severely marked radiation-induced change (cases) or very little/no change (controls) in the breast.	Higher levels of residual DSBs and deletion-type aberrations in ex vivo irradiated blood lymphocytes from clinically radiosensitive BC patients provide a suggestion for DSB repair in the development of radiation-induced late normal tissue damage.	[42]
	PBMCs were isolated from a group of unselected BC patients who received RT treatment and a group of apparently healthy donors. The early skin reaction to RT developing in the skin within the radiation field of the breast was controlled at the end of RT and used as an indicator of clinical radiosensitivity	A minor group of retrospectively identified BC patients with an adverse skin reaction to RT showed differences by the γH2AX assay with respect to healthy individuals. The 53BP1 assay was less sensitive than that for histone γH2AX in the case of endogenous (0 Gy) and induced (0.5 Gy, 30 min) foci.	[61]
	Peripheral blood samples from 26 patients who were treated with RT were collected from the BC patients two days before the RT. The acute adverse reactions of all patients are classified according to the Radiation Therapy Oncology Group (RTOG)/European Organization for Research and Treatment of Cancer (EORTC) side effects.	Significantly higher amount of endogenous γH2AX and 53BP1 DNA repair foci in fresh PBL withdrawn prior to RT from the RS patients in comparison with the NOR patients.	[62]
γH2AX/53BP1 co-localization IF assay	PBL from 16 patients who developed severe late radiation toxicity following radiotherapy.	Support for the hypothesis that the RS phenotype is associated with compromised DNA repair.	[63]
G2 chromosomal radiosensitivity assay	Peripheral venous blood was collected from 14 healthy control subjects, 15 patients with benign prostatic hyperplasia (BPH), and 17 patients with prostate cancer.	Enhanced G2 chromosomal radiosensitivity in prostate cancer and BPH patients compared with normal controls.	[40]
	Blood samples were then obtained from 27 breast cancer patients eligible for the study.	A significant proportion of breast cancer patients exhibit elevated G2 chromosomal radiosensitivity in contrast to random controls.	[41]
	Blood lymphocytes of BC patients who had undergone surgical excision of the primary tumor and postoperative RT to the whole breast.	Significantly higher levels of chromosomal aberrations in blood lymphocyte metaphases among women presenting with marked late radiotherapy changes.	[42]
	Blood samples were obtained from 25 prostate cancer patients with severe side effects (S) and 25 patients without severe side effects (0) after radiotherapy as well as from 23 healthy male age-matched donors.	The obtained success rate is not sufficient to validate the G2 assay as a good tool for the identification of prostate cancer patients with a high risk for the development of severe clinical side effects.	[43]
	Blood samples from 25 patients with localized T1–3N0M0 prostate cancer who were treated with three-dimensional conformal radiotherapy.	No predictive value was found after irradiation with 0.5 Gy in the G2 assay.	[44]

Table 1. *Cont.*

Assay (Molecular and Cytogenetic Markers	Sample/Patient Profile	Outcome	Study
G2 micronucleus (MN) assay	Blood samples from 18 *BRCA2* mutation carriers and 17 subjects from both *BRCA1* (n = 9) and *BRCA2* (n = 8) families not showing the familial mutation (non-carriers).	Higher radiosensitivity in healthy *BRCA2* mutation carriers compared with healthy volunteers by means of the G2 MN assay. No increased radiosensitivity was observed in non-carrier relatives of *BRCA1* and *BRCA2* families.	[50]
Fluorescence in situ hybridization (FISH) assay	Lymphocytes obtained from 16 patients that were retrospectively examined 1 to 108 months after radiotherapy.	Detectable differences in interindividual radiation sensitivity.	[54]
	Blood samples from 47 BC patients who received exclusively radiotherapy after surgical lumpectomy.	Significant overall correlation was found between the frequencies of t(Ba) (painted chromosomes bearing one centromere with a color junction) in vitro and the time-dependent occurrence of side effects of the skin.	[55]
	Patients with a localized T1-3N0M0 prostate carcinoma, with and without severe side effects, who were treated with three-dimensional conformal radiotherapy.	No statistically significant difference in the overall aberration yield between healthy donors and patients with and without severe side effects.	[56]

4.1.2. DNA Damage Response

Numerous studies have examined DNA damage response (DDR-related) proteins as radiosensitivity biomarkers. These proteins are indicative of DNA damage and play a critical role in repairing the DNA damage caused by IR, and alterations in their expression levels have been clearly associated with radiosensitivity.

In this respect, the histone H2AX as a radiosensitivity biomarker in various types of cancer, including breast, prostate, and head and neck tumors, was investigated. H2AX is a key protein that signals DNA damage (mainly double-strand breaks, DSBs) by its phosphorylation at serine139. This process is known as γH2AX formation, and practically results in the appearance of γH2AX foci at sites of DNA damage [64]. γH2AX foci help to recruit and coordinate the DDR, and, for this reason, are broadly utilized as a marker for the extent and location of DNA damage, allowing for the quantification and visualization of DNA repair events via immunofluorescence (IF) [65]. The main steps of the γH2AX IF assay protocol involve fixation, permeabilizing, and blocking of the cells. The primary antibody against γH2AX is then added, followed by a fluorescently conjugated secondary antibody and nuclear counterstaining while washing steps are taking place between labeling [66]. The final samples are analyzed via fluorescence microscopy. Moreover, the γH2AX IF assay can be combined with other techniques such as Western blotting or flow cytometry for quantitative analysis for measuring DNA damage.

Fleckenstein et al., 2011 examined peripheral lymphocytes of 31 patients with resected head and neck cancer, undergoing adjuvant RT or radio-chemotherapy (RCT), using the γH2AX assay to evaluate the in vivo impact of individual DNA double-strand break (DSB) repair mechanisms on the incidence of severe oral mucositis. γH2AX foci were quantified before and at 0.5 h, 2.5 h, 5 h, and 24 h after in vivo radiation exposure (the first fraction of RT). World Health Organization scores for oral mucositis were documented weekly and were found to correlate well with DSB repair. An important finding of this study was that, at 24 h after RT, patients with a proportion of unrepaired DSBs higher than the mean value plus one standard deviation had an increased incidence of severe oral mucositis. The study concludes that the in vivo DSB repair by evaluating γH2AX foci loss is reliable in clinical practice, allowing the identification of cancer patients with impaired DSB repair. In turn, the incidence of oral mucositis has not been found to be closely correlated with DSB repair under the evaluated conditions [57].

In a prospective study, Mumbrekar et al., 2014 analyzed DNA DSBs and repair via microscopic γH2AX foci analysis in peripheral lymphocytes from 80 post-chemotherapy breast cancer patients with histologically confirmed cancer, without distant metastasis and

without prior RT, and in 38 healthy female donors without recent diagnostic radiation exposure or any chronic diseases. The aim of the study was the examination of the usefulness of γH2AX foci analysis expressed as a percentage of residual damage (PRD), which referred to measurements at 3 and 6 h after in vitro irradiation of peripheral lymphocytes with 2 Gy of 120 kV X rays for predicting adverse reactions of IR at the level of normal skin. For confirming the in vitro results in a clinical setting, breast cancer patients subjected to RT were categorized as over-responders (ORs) and non-over-responders (NORs) with respect to the extent of acute adverse reactions of clinical irradiation in the skin, according to the Radiation Therapy Oncology Group (RTOG) criteria. A statistical difference between the healthy and the NOR groups, as well as between the healthy and the OR groups, has been found. The authors concluded that the slow rate of foci disappearance with respect to the initial DNA damage may be the cause of clinically radiosensitive phenotypes [58]. Accordingly, the H2AX assay is adequate to quantify PRD while considering the degree of the initial DNA damage and is accordingly an informative predictive assay for monitoring RT. Similar results were obtained by Djuzenova et al., 2013. In this study, the γH2AX assay was performed in peripheral blood mononuclear cells (PBMCs) isolated from the blood samples of 57 breast cancer (BC) patients, collected before and during clinical irradiation (72 h after five radiation fractions), against 12 clinically healthy donors. In this clinical study, six retrospectively identified BC patients with an early adverse skin reaction to RT, developed at the level of the irradiation field, were used as a reference for clinical radiosensitivity according to the RTOG score, and showed striking differences in the results of the γH2AX assay compared with healthy individuals. The data obtained using the γH2AX assay identified BC patients with normal clinical reactions to RT based on the induced and residual DNA damage, but not on the background DNA damage. It was concluded that the γH2AX assay may have the potential for screening individualized radiosensitivity of BC patients, as the mean number of γH2AX foci after five clinical fractions was significantly higher than before RT, especially in clinically radiosensitive patients [61]. Different results were produced in a subsequent study, in which Pinkawa et al., 2016 studied 25 prostate cancer patients treated with 3D conformal RT, with minor and larger score changes (Expanded Prostate Cancer Index Composite (EPIC)) by applying the γH2AX, G2, and apoptosis assays in PBMCs. Patients have been surveyed prospectively before, on the last day of RT, and at a median time of 2 months and 16 months after RT, using a validated QoL questionnaire (EPIC). Contrary to the aforementioned studies, no correlation was found between the number of γH2AX foci and radiotoxicity at the level of PBMCs [44]. Vasireddy et al., 2010 also conducted a study based on lymphocyte cells isolated from the blood of cancer patients with and without severe reaction to RT by following gH2AX foci kinetics after IR, which showed that there is no detectable difference between the control group and the RS group as a whole. Nevertheless, with the utilization of γH2AX foci assay they managed to identify an RS cancer patient cell line with a novel ionizing radiation-induced DNA DSB repair defect and thus concluded that in combination with other predictive assessments, the γH2AX assay may eventually facilitate the tailoring of RT regimes to individuals [59]. The differences between the results of different clinical studies might be attributed to methodological reasons.

Along with γH2AX, the 53BP1 assay is a promising tool for predicting radiosensitivity. 53BP1 is a pivotal DDR protein that plays a critical role in maintaining genomic stability. It is recruited to DSBs and forms discrete nuclear foci that are detected by IF. 53BP1 acts as a scaffold protein that recruits other DDR proteins to the site of DNA damage and promotes the repair of DNA breaks via the non-homologous end joining (NHEJ) pathway. The phosphorylation status of 53BP1 is regulated by several kinases, including ATM and the DNA-PKcs protein kinase involved in NHEJ. 53BP1 foci developed in response to radiation-induced DSBs are scored following similar procedures as the γH2AX assay. Chua et al., 2011 investigated via immunofluorescence both proteins in ex vivo irradiated blood lymphocytes with 0.5 and 4 Gy, using 250 kV X-rays from BC patients who had undergone surgical excision of the primary tumor and postoperative RT to the whole breast, with

late adverse effects. The authors analyzed the scores from post-surgical photographs of breasts collected before RT and at 2 years and 5 years post RT. Two groups of patients were identified. The first group appertained to severely marked radiation-induced change (cases) and the second to very little/no change (controls) in the breast. The authors evidenced higher levels of residual DSBs in ex vivo irradiated blood lymphocytes from clinically radiosensitive BC patients at 0.5 h after the ex vivo irradiation with 0.5 Gy, or in the case of cellular samples irradiated ex vivo with 4 Gy and analyzed at 24 h. The role of DSB repair in the development of late radiation-induced damage of normal tissue was demonstrated [42]. Djuzenova et al., 2013 along with the γH2AX marker, studied the 53BP1 protein as a marker of DSB formation in PBMCs isolated from the blood samples of 57 BC patients and came to the conclusion that the 53BP1 assay was less sensitive than that for histone γH2AX in the case of endogenous (0 Gy) and RT-induced (0.5 Gy, 30 min) foci [61]. In a very recent research study, Durdik et al., 2023 analyzed peripheral blood samples from 26 RS and NOR BC patients treated with RT using the combined γH2AX/53BP1 foci assay. A significantly higher amount of endogenous γH2AX and 53BP1 DNA repair foci was found in peripheral blood lymphocytes (PBL) collected from RS patients prior to RT, in comparison with NOR patients. This effect was not reproduced by analyzing in vitro exposed frozen-thawed cells derived from 2 RS and 2 NOR patients before RT [62].

A measure of the DNA repair machinery efficiency in response to radiation-induced DNA damage can be provided by the co-localization of γH2AX and 53BP1 proteins since the 53BP1 protein recognizes the γH2AX foci and forms nuclear foci at the site of DNA damage, playing a critical role in the repair of DSBs. This co-localization assay showed promising results in the clinical study of Lobachevsky et al., 2016 [63] in which the γH2AX and 53BP1 response was investigated in ex vivo irradiated PBL from 16 patients who developed severe late radiation toxicity following RT (RTOG Grade 3–4 late toxicity), representing the OR group, and from 12 control patients (matched for sex, treatment site and intent, RT dose, use of chemotherapy, and approximate age) constituting the NOR group. By applying the co-localization assay at five different time points up to 24 h post irradiation, the authors concluded that the colocalization of γH2AX and 53BP1 foci support the hypothesis that the RS phenotype is associated with compromised DNA repair. A lower co-localization reflects decreased cooperation of DNA repair factors, which presumably impacts the efficiency of DSB processing.

One of the major strengths of the γH2AX assay is its sensitivity in detecting radiation-induced DSBs [67]. Even at low doses, γH2AX foci can be visualized and quantified using IF, hence enabling the assessment of cellular response with high precision [68]. Furthermore, as activated oncogenes induce the stalling and collapse of DNA replication forks leading to the formation of DSBs in precancerous cells, γH2AX levels may reflect genomic instability in tissues, and serve to detect precancerous lesions. Accordingly, preventive measures can be taken or treatment options can be better informed [69]. In addition, the γH2AX assay can be applied to various biological samples, including peripheral blood that is collected by a non-invasive procedure for radiosensitivity assessment [70]. Although it is accepted that -with some exceptions- almost every DSB forms γH2AX foci, whether every γH2AX focus identifies a DSB still remains controversial. A γH2AX focus may persist over time in some tumor cells after the initiating DSBs have been rejoined [71], as DSB complete repair also involves restoring the original chromatin conformation, which may also be facilitated by the presence of γH2AX foci.

The γH2AX assay is limited by false positives and negatives due to variations in staining protocols and interobserver variability. Standardization of protocols and scoring criteria is essential to ensure accurate and reproducible results between laboratories. Furthermore, the assay primarily detects DNA damage and repair kinetics, overlooking other factors that influence radiosensitivity, such as the cell cycle phase. H2AX is not only phosphorylated in response to DNA damage but also during normal replication and in response to replication stress [72]. Progression of damaged cells through the cell cycle can lead to further breakage in the S phase and, therefore, S-phase cells should be avoided

for analysis. The assessment of radiosensitivity based solely on γH2AX foci formation may not provide a comprehensive understanding of cellular responses to radiation, and a combination of assays involving both non-proliferating and proliferating tissues might be necessary to address this issue. Regarding the 53BP1 assay, since 53BP1 is one of the early recruited repair proteins participating in both NHEJ and HR, it represents a promising candidate to study the architecture and dynamics of repair clusters in relation to their importance for DSB repair [73]. Moreover, despite similar labeling and microscopy parameters, 53BP1 foci can be better separated from one another than γH2AX foci [74], leading to more reliable results. On the other hand, 53BP1 is expressed throughout the cell cycle, leading to its diffuse localization in non-irradiated nuclei [75]. Last but not least, 53BP1 is an early indicator of the starting activity of NHEJ/HR repair machinery. Hence, it is reasonable to investigate the behavior of 53BP1 during DNA repair independently [76].

Immunofluorescence (IF) is commonly used to visualize γH2AX foci and 53BPI, but this technique has some limitations related to a reduced level of quantification and subjectivity in interpretation. Another limitation of IF is that blood cells used for assessing the radiosensitivity of normal tissues are non-adherent cells that must be experimentally manipulated for attaching to slides so as to perform IF. Being dedicated to cells in suspension, flow cytometry appears to be more appropriate to analyze the response of blood leukocytes to IR.

In standard flow cytometry, cells are labeled in suspension with specific functional and phenotypic fluorescent indicators and are analyzed one by one from the point of view of morphology (size and granularity) and surface/intracellular fluorescence intensity. The results are finally processed as a distribution of fluorescence intensity per cell, and the mean fluorescence and various other parameters of this distribution are finally calculated using dedicated software. Besides the analysis of cells in suspension, flow cytometry has several other advantages: (i) it allows gating and differential analysis of particular cell populations or sub-populations within a multi-cellular sample, (ii) it provides comprehensive multi-parametric information on single cells in suspension within a complex cellular sample, combining functional, phenotypic, and molecular parameters; for instance, in the radiosensitivity context, DNA damage evaluation (dsDNA breaks evaluated via flow cytometry with the fluorescent TUNEL and γH2AX foci or other specific assays) can be combined with investigations on leukocyte viability [77], apoptosis (annexin V-propidium iodide assay), senescence (β-galactosidase assay), leukocyte activation (MHCII, CD69), proliferation (CFDA-SE assay), and cell cycle (Vybrant orange stains) [78]. The currently available flow cytometers allow simultaneous measurement of more than eight parameters in the very same sample. Recently, the advent of high-dimensional time-of-flight mass cytometry (CyTOF) using antibodies labeled with metals and not with fluorophores enables broad dimensional and unbiased examination of blood cells [79], allowing simultaneous interrogation of more than 40 parameters in the same cellular sample. Clustering techniques were developed to reduce the dimensionality of the data in order to create a visual representation in a reduced number of dimensions. Altogether, this new investigational approach is highly important for deep biomarker screening in blood exposed to various challenges (such as IR), particularly when sample sizes are limited (i.e., immune cells in tumors). From another perspective, the new imaging flow cytometry platforms [80], which combine statistical power and high fluorescence sensitivity of standard flow cytometry with the spatial resolution and quantitative morphology of digital microscopy, provide a broader image of the IR impact on blood leukocytes.

4.1.3. Other Biomarkers of the DDR Pathway

Promising biomarkers for assessing radiosensitivity in various cancer types are also the MRN complex and the ATM (ataxia–telangiectasiamutated) protein. The MRN complex, consisting of Mre11, Rad50, and Nbs1/Xrs2 proteins, is an important player in DNA repair and maintenance of genome stability. This complex acts as a sensor and mediator of DNA damage and is involved in multiple DNA repair mechanisms, including HR and NHEJ [81]. While the MRN complex has a crucial role in DNA damage sensing and

repair processes, ATM is a key regulator of the cellular response to DSBs. Upon detection of DSBs, ATM is activated by the MRN complex, undergoing autophosphorylation and activation and leading to a signaling cascade initiation that regulates various cellular processes (including DNA repair, cell cycle checkpoint activation, and apoptosis) [82]. Several studies have demonstrated the correlation between a dysfunctional MRN complex, especially the MRE11 protein, and increased radiosensitivity, suggesting its potential utility as a predictive biomarker for RT outcomes for better treatment decisions. The MRN complex and the ATM expression are commonly measured by IF for visualizing their localization and dynamics in response to DNA damage. Seminal contributions have been made by Söderlund et al., 2007 by analyzing the tumor samples from 224 premenopausal women with early breast cancer. The study pointed out that high expression of the MRN complex, but not of the ATM, is necessary for tumor cell killing by RT. Therefore, a reduced expression of the MRN complex predicts a poor therapeutic effect of RT in patients with early breast cancer [83]. On the contrary, Yan et al., 2020 have shown that the methylation status of the ATM promoter in hepatocellular carcinoma (HCC) has a predictive value for RT outcome, as assessed by performing PCR (qPCR) and immunohistochemistry (IHC) in 50 paired HCC and adjacent normal tissues, and 68 locally advanced HCC biopsy tissues [84]. Yuan et al., 2012 performed an immunochemical analysis of breast cancer tissues obtained from 254 surgically treated female patients with confirmed pathology of invasive ductal carcinoma and showed that high MRE11 expression in breast cancer cells was associated with malignant behavior, higher recurrence rates, lymph node metastasis, resistance to RT and chemotherapy, and decreased patient survival [85]. The study by Ho et al., 2018 having analyzed surgical specimens collected from 265 patients treated with CRT or neoadjuvant RT followed by surgery for rectal cancer, concluded that high tissue expression levels of the three MRN complex proteins are prognostic indicators in rectal cancer and in response to preoperative therapy [86]. Therefore, the incorporation of the MRN complex and the ATM protein as biomarkers for the radiosensitivity evaluation holds great promise for improving personalized cancer treatment and for optimizing RT procedures and outcomes.

As the MRN complex is essential for DSB repair and telomere maintenance, it is postulated that defective function and low expression of its components lead to DNA damage and malignant transformation. Also, owing to its ability to repair DSBs, it is likely that the levels of MRN expression influence the response of cancer cells to chemotherapy and RT in terms of apoptosis. Generally, conflicting data were obtained in different studies; some studies have associated high expression of MRN and its components with poorer outcome and treatment resistance to chemo-radiation [87–89], while others have found the opposite [90]. In parallel to MRE11, ATM overexpression has a significant relationship with poor disease-free survival in rectal cancer and lymph node positivity [86], suggesting that the observed poor overall survival was likely due to the aggressiveness and metastatic properties of the tumor; the latter's progression could be reasonably attributed to the increased MRE11 levels that stabilize RAD50 and NBS1 and recruit ATM [91]. Because of the importance of the MRN complex and the ATM protein in DDR, they both remain promising biomarkers for predicting tumor response to RT.

4.2. Apoptosis Biomarkers

Cell death may occur by various mechanisms such as apoptosis, necrosis, mitotic catastrophe, ferroptosis, senescence, and autophagy [92–94]. Apoptosis as a programmed cell death usually occurs in multicellular organisms under normal conditions to maintain homeostasis by eliminating damaged cells. It is mediated by intrinsic and extrinsic mechanisms that activate those members of the proteases family that lead to the initiation and amplification of proteolytic cascades: the *intrinsic* and *extrinsic* ones. Extrinsic apoptosis is triggered by external signal proteins that bind to specific receptors of the cell surface and lead to the activation of the apoptotic process. Intrinsic apoptosis involves the release of mitochondrial proteins into the cytosol, which activates a proteolytic cascade. In other cases,

the two pathways work harmoniously together to activate a member of the pro-apoptotic *Bcl2* proteins for killing the cell. It is of note that when the apoptosis rate among cells deviates from the norm, either excessively or insufficiently, and when there is an inaccurate timing and location of apoptotic death, tumorigenesis and autoimmune mechanisms may be triggered [95]. Since apoptosis is characterized as asynchronous in cells and the persistence of apoptotic cells is relatively short, the in vivo detection of apoptosis biomarkers is complicated. Some of the most studied cells in relation to RT-induced apoptosis are endothelial cells, lymphocytes, and bone marrow progenitor cells [96].

An acknowledged biomarker related to apoptosis is caspase-3, which actively participates in apoptosis as a primary executioner of this process. Caspase-3 initiates DNA fragmentation through the proteolytical inactivation of DEF45 (DNA fragmentation factor-45)/ICAD (inhibitor of caspase-activated DNase), which releases, in turn, the active complex DEF40/CAD (caspase-activated DNase), the endonuclease associated with the inhibitor [97]. Pathways leading to the caspase-3 activation are shown to be either dependent or independent of the cytochrome c release and the caspase-9 synergy [98]. Santos et al., 2017 reported a dose-dependent increase in active caspase-3 expression levels in human blood lymphocytes irradiated with 1, 2, and 4 Gy gamma radiation, which was investigated after different times of in vitro incubation (24, 48, and 72 h). Accordingly, caspase-3 might be used for detecting differences in the radiation sensitivity of patients before undergoing RT [99], as also reported in several other studies [100–102]. A well-documented study by Yang et al., 2005 with irradiated MCF-7 breast cancer cells indicates that caspase-3 plays a critical role in RT-induced apoptosis [103]. It has also been reported that caspase-3 is necessary for the radiation-induced apoptosis of human B-lymphocytes and lymphoblastoid cells [104,105]. The study of Cao et al., 2011 making use of the radiosensitive $CD4^+CD25^+$ T regulatory cells (Treg) from patients with hepatocellular carcinoma, reveals high expression of radiation-induced active caspase-3 [106]. Similar results were independently obtained with ^{137}Cs-irradiation of T cells by Nguyen et al., 2020, who observed an increase in caspase-3 levels due to irradiation [107].

Annexin V has also been shown as a useful biomarker of radiation-induced apoptosis in human T-lymphocytes and as an indicator of radiation toxicity. This anticoagulant protein may detect apoptotic cells exhibiting on their surface negatively charged phospholipids like phosphatidylserine. Several studies emphasized annexin V as a promising candidate biomarker for evaluating radiation toxicity in oncologic patients [108,109].

4.3. Secreted Soluble Protein Biomarkers

Exposure to radiation initiates a programmed cellular response to promote tissue repair, involving the induction and regulation of several proinflammatory cytokines and growth factors [110]. While being beneficial for the rescue of normal cells exposed to RT, the RT-induced inflammatory response also sustains the survival of tumor cells and cancer progression [111], conferring to oncologic patients a poor prognostic outcome.

Cell apoptosis and senescence after RT lead to increased TGF-β release. Their combination with hypoxia in the tumor may amplify this release to a higher degree. Transforming growth factor beta (especially in the isoform TGF-β1) plays a fundamental role in normal tissue injury after RT. This candidate biomarker is one of the most common cytokines released due to cell irradiation, its levels being closely connected to the RT dose, DNA damage, and toxicity due to radiation [112]. TGF-β is a regulator of the physiologic wound healing process, and its overproduction may lead to severe injuries in normal human tissues. Typically, a TGF-β increase in normal tissues may lead to fibrosis, being involved in toxicity related to chronic oxidative stress, genomic instability in tumors and in bystander cells, as well as secondary cancers [113,114]. Additionally, TGF-β has been shown to be a critical suppressor of the anti-tumor immune defense, promoting the proliferation of cancer cells and angiogenesis and tumor metastasis [115–119].

Another promising biomarker belonging to the cytokine family is interleukin-6 (IL-6). This pleiotropic protein is involved in several inflammatory diseases since its axis is asso-

ciated with immunity and metabolism. It also regulates hematopoiesis and acute-phase reactions. The evidence arising from the published literature shows a significant correlation between high levels of IL-6 in serum and a shorter survival/poor prognosis of cancer patients. Reibnegger et al., 1991 reported such a correlation for patients with multiple myeloma [120], together with Pulkki et al., 1996, [121] while Miki et al., 1989 reported IL-6 as a growth factor for renal cancer cells similar to the study of Blay et al., 1992 [122,123]. Van Meir et al., 1990 concluded that high serum IL-6 is a prognostic factor in glioblastoma [124]. Tamn et al., 1989 suggested that IL-6 may promote in vivo tumor metastasis and invasiveness in breast cancer cases [125], while Guo et al., 2023 indicated that IL-6 mediates the crosstalk between breast cancer cells and cancer-associated-fibroblasts in the tumor microenvironment [126]. Scambia et al., 1995 showed that elevated IL-6 serum levels in ovarian cancer patients correlate with poor prognosis [127], as did Berek et al., 1991 [128]. In the same trend, Shariat et al., 2001 reported a general relationship between elevated IL-6 levels and metastatic cancer for prostate cancer patients [129], supporting similar conclusions by other researchers in previous publications [130–134]. The activation of the IL-6 axis has also been reported for patients with colorectal cancer or oesophageal squamous cell cancer [135,136]. The IL-6 signaling pathway was shown to be involved in metastasis formation [137]. Moreover, the results presented by Cao et al., 2011, indicate that increased levels of IL-6 enhance the radiosensitivity of $CD4^+CD25^+$ T cells after low-dose gamma irradiation [106]. Experimental data presented by Chen et al., 2012 demonstrate that IL-6 is crucial in shaping the radiation response of liver tumor cells, suggesting that concurrent treatment with IL-6 inhibitors (such as Tocilizumab) might be a promising therapeutic strategy for increasing the IR response of cancer cells [138]. This observation is sustained by the study of Kopčalić et al., 2022 on 39 patients with localized or locally advanced prostate cancer treated with RT, showing that increased serum levels of IL-6 and TGF-β1 influence the severity of acute genitourinary radiotoxicity and fatigue in these patients [139].

The proinflammatory cytokine interleukin-1beta (IL-1β) shows promise as a cancer biomarker with pleiotropic effects on cancer cell proliferation, angiogenesis, migration, and metastasis, as well as on immune cells [140]. It has been shown that IL-1β induces the expression of the gene encoding the proinflammatory inducible form of cyclooxygenase 2 (COX-1) in several cancer cell lines through mechanisms involving p38 and p44/p42 mitogen-activated protein kinase pathways and enhances the activity of the CDK2 [141,142]. IL-1β was also found to elicit the above-mentioned overproduction of IL-6 through the activation of NF-kB, which has been shown to be crucial for COX-2 induction [143]. IL-1β is also linked to angiogenesis through VEGF production together with IL-6 [144]. It has been shown that increased levels of IL-1β are significantly linked to poor prognosis in patients with gastric and colorectal adenocarcinoma, and are an important survival predictor in patients undergoing RT [145,146]. According to Chen et al., 2011, IL-1β is also an indicator of shorter survival in oesophageal cancer patients [147]. Nguyen et al., 2020, reported an increase in this biomarker in peripheral $CCR6^{neg}$Th lymphocytes after their irradiation with ^{137}Cs [107], and Cao et al., 2011, observed a secretion of IL-1β in $CD4^+CD25^+$ radiosensitive T cells in blood samples collected from patients with hepatocellular cancer in response to low-dose γ-rays [106]. The study of Aggen et al., 2021, has recently shown, via single-cell RNA sequencing, that IL-1β blockade can remodel the myeloid compartment through non-redundant, relatively T-cell-independent mechanisms, concluding that IL-1β represents a potential target for kidney cancer immunotherapy [148].

Another candidate biomarker is tumor necrosis factor α (TNF-α), a proinflammatory cytokine that is a potent apoptosis inducer, having dual roles in pro-tumorigenic and anti-tumorigenic receptors [149,150]. Zhu et al., 2019, showed that TNF-α levels remained high in the cells of lung cancer patients after repeated radiation treatments and proposed the use of this cytokine as a critical biomarker for treatment evaluation [151]. Other studies have also reported high levels of TNF-α after RT [152–156]. On the other hand, Pal et al., 2016 reported a bioinformatic analysis of 982 patients, showing that increased levels of

TNF-α were associated with a low risk of lung cancer progression after RT [157]. Other cytokines, like IL-2, IL-13, IL-23, and interferon-γ have been reported to be associated with RT-induced toxicity [110,158], but there is a need for more studies in this field.

Vascular Endothelial Growth Factor (VEGF) is an angiogenic factor associated with endothelial cell proliferation, cell migration, and autophagy [159]. It is acknowledged that VEGF is a basic mediator of neovascularisation, presenting a high expression in neoplastic cells and especially in the stromal cells in the tumor niche [160]. It has been demonstrated that VEGF also prevents apoptosis induced by serum starvation through the phosphatidylinositol (PI)-3-kinase-Akt pathway and induces the expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells [161]. The detection of VEGF-A in the serum of patients with primary cervical cancer in advanced FIGO stages Ib-IIb before and after RT combined with chemotherapy was reported by the study of Braicu et al., 2013 [162]. More specifically, in this study was shown that a decrease in VEGF-A leads to an increase in patients' survival rate. In this way, VEGF may function as an independent predictor of prognosis in the case of cervical cancer. In another study [163], it was shown that the expression of VEGF was increased in HeLa cells in vivo and in vitro after IR exposure. Increased apoptotic cell death and knockdown of VEGF in these cells increased cellular sensitivity to IR in cervical cancer therapy. Fekete et al., 2015 have reported increased VEGF levels in culture media of non-irradiated multipotent mesenchymal stromal cells (MSCs), but irradiated ones have shown no increase in VEGF [164]. Nguyen et al., 2020, showed that the IR-induced senescence of $CCR6^{+}$Th17 T-cells promotes VEGF-A secretion at 48 h after exposure to 2 Gy irradiation [107]. A meta-analysis for the evaluation of the relationship between serum VEGF levels and radiosensitivity of patients with non-small cell lung cancer (NSCLC) among Asians revealed that these levels were higher than those of the healthy controls. There was also a significant difference in the VEGF levels before and after RT. Meanwhile, no correlations between the tumor node metastasis (TNM)-stage and histologic grade in these patients were found. It was concluded that NSCLC patients with positive VEGF expression had shorter overall survival than those with negative ones [165]. The association between IR exposure and variable VEGF levels has been shown to be cell-type dependent. However, this observation does not exclude the promising perspective of VEGF as a biomarker closely related to radiosensitivity.

High sensitivity C-reactive protein (hs-CRP) was evidenced as an indicator of skin toxicity for breast cancer patients having undergone RT. A well-documented study has found that patients with increased levels of hs-CRP had a significantly higher risk of grade 4 skin toxicity, which was not related to radiation dose or the patient's BMI [166]. hs-CRP may also be a biomarker for radiation-induced cardiac dysfunction, as shown in the study by Canada et al., 2020 in the serum of patients after thoracic RT [167].

Most of the above-mentioned studies have been focused on the variations in the cytokine serum/plasma concentrations before and after RT, correlating them with subsequent radiotoxicity events and different tissue radiosensitivities. Moreover, a clear distinction between cytokine levels and the used dose during RT is necessary, as higher doses delivered in larger volumes around the tumor might be closely associated with different levels of cytokine biomarkers [168]. It is increasingly clear that the reported changes in the concentration of cytokines are strongly related to biochemical mechanisms initiated during or after the RT and not before, and this could be a deterrent for their use as predictive markers, except for the cases when multiple studies have reported strong evidence in this respect (e.g., IL-6, IL-1β, and TGF-β1).

On the other hand, the predictive assays utilized so far for assessing normal tissue radiosensitivity have some major advantages: (i) blood collected non-invasively from patients and controls is an important source of soluble biomarkers; (ii) soluble biomarkers are assessed using validated, easy-to-perform, and cost-efficient methods that are commonly used in clinical practice (i.e., ELISA). Instead, the screening for new biomarkers requires sophisticated high-throughput techniques for protein multiplexing. In the case of the plasma/serum concentration of these biomarkers being too low to be assessed by ELISA

(such as particular soluble stress proteins), the new Quanterix technology for ultrasensitive biomarker detection is now available "https://www.quanterix.com/ (accessed on 2 September 2023)".

Ultimately, there is a high need for extended and well-documented studies to validate for clinical use those soluble protein biomarkers that represent a reliable radiosensitivity signature of normal tissues in connection with the RT regimen and tumor characteristics.

4.4. Cell-Free DNA-Based Biomarkers

There is a tremendous need for biomarker discovery in blood for characterizing the molecular and functional profile of tumors, especially in the case of tumors that are difficult to access for biopsy (such as tumors of the central nervous system) [169]. Blood biomarkers are highly needed for non-invasive early diagnosis as well as for treatment selection and real-time monitoring, avoiding repeated invasive biopsies.

A valuable source of liquid biomarkers is the cell-free DNA (cfDNA), which derives from damaged tumors and blood cells, and circulates in blood and other biological fluids, such as the cerebrospinal fluid [169,170]. In cancer patients, higher levels of tumor cfDNA were detected compared with normal individuals, possibly due to increased tumor cell proliferation and damage or death by necrosis/apoptosis, especially during anti-cancer therapies [171]. This allows tumor cfDNA quantitation via digital polymerase chain reaction during therapy and the detection of cancer-specific or therapy-induced molecular alterations via sequencing [172] if sufficiently high amounts of tumor cfDNA are available in plasma. For example, the evaluation of 200 patients with colorectal, breast, lung, or ovarian cancer detected somatic mutations in the plasma of patients with stage I and II disease (71, 59, 59, and 68%, respectively) [173] that were highly correlated with alterations detected in the tumors of these patients. It was also evident that higher amounts of preoperative tumor cfDNA were associated with disease recurrence and decreased overall survival of patients with resectable colorectal cancers. Considering that RT inflicts profound damage to the tumor and normal cells, it is expected to obtain enough cfDNA to perform a detailed analysis of tumor radiosensitivity and radiation-induced resistance, along with local and systemic alterations of normal tissues, including blood leukocytes.

Various publications in the field have addressed tumor cfDNA in connection with RT-related biomarkers. It has been shown that RT can increase tumor cfDNA levels in plasma, as shown in a study on 17 stage I–II (early) and IV (advanced) non-small cell lung cancer (NSCLC) patients who underwent RT [174]. In this study, tumor cfDNA levels at 24 h after irradiation were significantly higher than in the corresponding pre-RT samples, peaking at 7 days post irradiation, while maximal tumor reduction was observed only later, at 4–8 weeks after RT. Moreover, the post-RT levels of cfDNA were lower than the corresponding pre-RT levels in stage I–II NSCLC patients, indicating a significant tumor reduction. The dynamics of tumor cfDNA released into circulation due to irradiation was investigated in tumor cell lines, showing that mitotic catastrophe dominates the early response to radiation exposure, with a peak in apoptosis and cfDNA release occurring later, at around 3–6 days after irradiation [175].

Several recent studies have demonstrated that the persistence of tumor-derived cfDNA following primary therapy can predict those patients who may benefit from adjuvant therapy or subsequently relapse with early metastatic disease [176,177]. A recent study has shown that the cfDNA I-score, calculated based on low-depth whole-genome sequencing, is a predictor of radiation response in patients with lung, esophageal, and head and neck cancer. The score could detect the minimal residual disease after RT earlier than by using imagistic methods [178].

In addition, the analysis of tumor-associated cfDNA in 43 early-stage non-small cell lung cancer patients subjected to high-dose proton beam therapy (PBT) indicated that cfDNA detection before treatment is an effective biomarker that is able to predict out-of-field recurrence after PBT [179]. In turn, tumor-associated cfDNA levels at the mid-term of chemo-RT detected using the Cancer Personalized Profiling by deep Sequencing

(CAPP-Seq) method could predict treatment outcomes in 41 patients with localized lung cancer [180]. From another perspective, higher amounts of tumor cfDNA obtained after RT allowed the detection of mutations that account for the tumor drug resistance induced by previous treatment with a tyrosine kinase inhibitor [174]. Such data were difficult to obtain using the low tumor cfDNA levels in the absence of RT.

Altogether, cfDNA analysis is of utmost importance for the RT precision that needs personalized irradiation dose adjustment during a fractionated scheme for maximizing therapy efficacy, while limiting radiotoxicity to levels that do not produce prolonged impairment of the life quality [181]. Moreover, the cfDNA approach is also informative on the radiation-induced resistance of tumors, requiring a dose increase in the next irradiation session or an adjunctive therapy to increase the radiosensitivity of tumor cells [182–184]. In addition, a recent study on methylated cfDNA from breast cancer patients identified dose-dependent and specific tissue responses, with right-sided-breast treated patients showing endothelial DNA implicating liver tissue as an affected tissue. Three patients in this study developed a grade 2 skin toxicity, and showed increased breast basal and luminal epithelial damage. This highlights the complexity of the RT responses and the potential of identifying specific tissue interactions in order to design radioprotective methods. For the moment, cfDNA has been used for the detection of the tumor response to RT. It is expected that in the future, extensive studies on leukocyte-derived cfDNA will be developed for assessing immune cells' response to RT as a measure of potential radiotoxic effects. In this context, the differential analysis of cfDNA deriving from tumor and hematopoietic cells is expected to provide helpful information on therapy outcome and the response of patients to therapy in terms of therapy efficacy (mirrored by changes in tumor cfDNA) and radiotoxicity (mirrored by changes in cfDNA derived from blood leukocytes and normal tissues affected by therapy). The main issue lies in distinguishing between the different forms of cfDNA according to their origin, both in basal conditions and during/after therapy.

The field of translational research related to cfDNA is expected to revolutionize precision oncology. A potential technological development may be related to the analysis of DNA fragments across the whole genome [185] using the DNA evaluation of fragments for early interception (DELFI) technology. It was found that the fragmentation patterns of cfDNA across 245 healthy individuals reflected nucleosomal patterns of white blood cells, whereas the fragmentation patterns in the plasma of 236 patients with breast, colorectal, lung, ovarian, pancreatic, gastric, or bile duct cancer had distinctive disease-specific fragmentation profiles. This investigational approach was able to improve diagnostic evaluation by identifying subsets of individuals more likely to have a particular type of cancer. The DELFI technology, combined with mutation-based cfDNA analysis, also shows promise for deeply monitoring the outcome of pre-operative and curative RT in oncologic patients, as well as for evaluating radiotoxicity at the level of immune cells.

5. Challenges and Opportunities for Cancer Radiosensitivity Biomarkers in the Era of Artificial Intelligence

For over a decade, there has been a growing interest in cancer radiosensitivity/toxicity biomarker research due to the important role of RT in cancer treatment. At the moment, there is a restricted application of the aforementioned biomarkers (Figure 1) for clinical use, with an experimental character among medical laboratories and university hospitals. This multi-sided difficulty originates from the fact that enough evidence should be provided to persuade the international radiation therapy and oncology communities to start using such biomarkers in the clinical routine. On the other hand, the low patient numbers in the proof-of-concept studies are still a critical issue for the validity of these biomarkers, since there is still an urgent need for randomized control trials with large comparable cohorts. Another important disadvantage in such investigations is the absence of patient groups having undergone RT under the variation in other factors affecting potentially the same investigated biomarker. Last but not least, most studies addressing radiosensitivity biomarkers do not discern those cases where cancer patients have undergone only RT (not

combined with chemotherapy), so as to observe promptly the response of the cells/tissues to irradiation and conclude the pure connection of biomarkers to IR. When RT takes place following chemotherapy, the latter therapy affects the whole biochemistry of the patient and complicates the study of possible biomarkers related to radiosensitivity. It is true that in most oncological cases, nowadays, a combined therapeutical strategy is applied, comprising both RT and chemotherapy. Accordingly, a priority in the search for radiosensitivity biomarkers would be to conduct large studies in the cases of cancer patients who undergo only RT. Another future trend in this field should be the use of highly reproducible assays capable of providing rapidly reliable results. Moreover, the use of easy-to-collect tissues (e.g., blood or other readily accessible biological fluids) and economically affordable assays and equipment limits the clinical translation of radiosensitivity biomarkers.

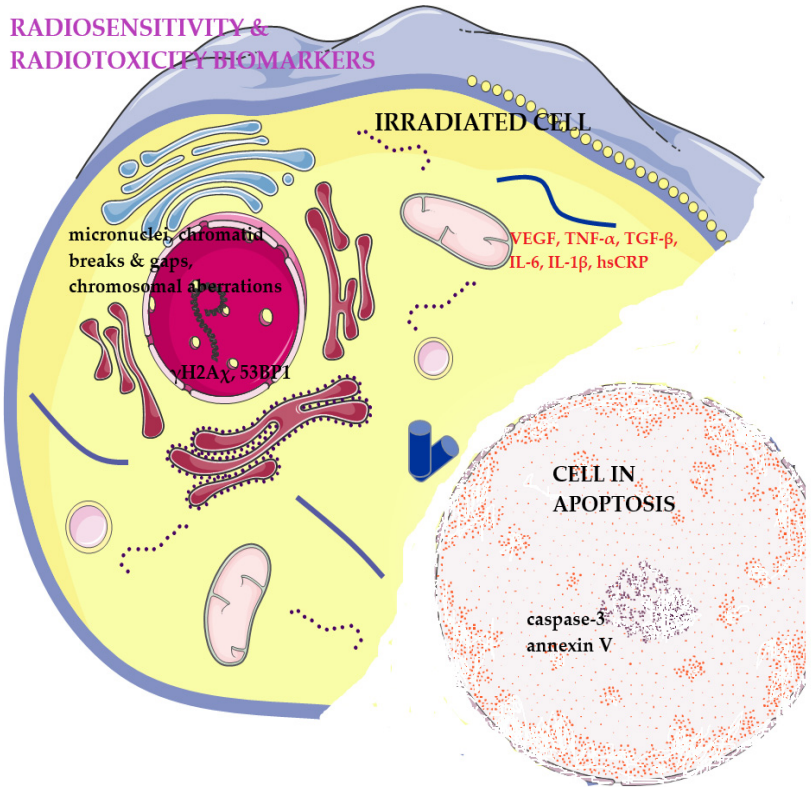

Figure 1. Biomarker overview for assessing radiosensitivity and radiotoxicity. On the bottom right-hand corner, the nucleus is depicted in apoptosis (the nuclear envelope is shown disassembled and the chromatin broken up into fragments) (the figure was created using Servier Medical Art, available at "http://smart.servier.com/ (accessed on 10 September 2023)".

Computational approaches and Artificial Intelligence (AI) have revolutionized biomarker identification, although such approaches in the field of radiotherapy and responses are still evolving. Transcriptomics, genomics, proteomics, metabolomics, and glycomics are some of the '-omics' data that are converging to identify critical bioindicators and biomarkers that can be used to train models. These can be further combined with radiomics to identify populations that could be radiosensitive [186–188]. For example, Zhang et al. combined gene expression, DNA methylation, and clinical data to identify eight radiosensitivity-related genes (AR, WBP1, AKR1E2, FANCG, NR2C2AP, CXCR4, SYNE4, and WFDC2) [189], while Liu et al. identified 12 genes (BEST2, TMPRSS15, FGF19, ALP1, KCNB2, CLDN6, IL17REL, RORB, DDX25, TDRD9, CELF3, and FABP7) that can aid in identifying population responses in head and neck cancer patients [190].

Big data from clinical patient cohorts are becoming publicly available. At the same time, machine learning algorithms are employed for radiosensitivity predictions [191,192], while artificial neural networks are increasingly being utilized to uncover new patterns

and increase radiosensitivity predictions [193]. Starting in 2020, the Department of Energy (DOE) Office of Science directed National Laboratories in the United States to explore the potential of machine learning and AI capabilities based on models generated through a DOE—National Cancer Institute (NCI) collaboration, termed CANDLE (CANcer Distributed Learning Environment) that focused on precision medicine [194,195]. This particular field is gaining considerable momentum and may provide significant guidance in the field of radiotherapy for effective personalized therapeutic regimens that are dynamic in nature based on daily responses to the treatments.

Research on genome and proteome deregulation in relation to radiosensitivity vs toxicity is the key to the future development of 'smart' and adaptive oncologic therapies. The potential of genomics in guiding the delivery of radiotherapy and the importance of commercializing genomic-based tools for implementation is highlighted [12]. Collaborative efforts toward identification, standardization, and validation of RNA-and DNA-based gene signatures can overcome past challenges and provide robust biomarkers suitable for clinical practice and genomics-guided radiotherapy.

6. Conclusions

Identification and validation of biomarkers closely related to radiosensitivity/radiotoxicity is a critical step in oncology for identifying those patients with potential intolerability to RT. Cellular radiosensitivity is influenced by various treatment-specific, as well as patient-specific factors. The γH2AX protein emerged as one prominent marker for radiosensitivity prediction. A practical and efficient method for assessing an individual's global DSB repair capacity has been measuring γH2AX via flow cytometry, which holds promise to enable the prediction of an individual's radiosensitivity and the risk of adverse effects to RT [196]. Moreover, ELISA-based assays against H2AX and TP53 have been proposed for monitoring the efficacy of radiotherapy in cancer patients [197]. The findings of the study of Mahmoud et al., 2022, showed a decrease in H2AX levels after radiotherapy, making it a useful indicator of DNA damage response and radiation sensitivity, and activation of TP53 via radiotherapy, as its expression increases in cancer patients, indicating its role as a marker for DNA damage response in early radiation exposure. Furthermore, interleukin-6 (IL-6) and interleukin-1beta (IL-1β) are promising biomarkers for common clinical practice, especially in the format of ELISA assays, as they are easily operated and cost-efficient. Finally, the still ongoing research in cell-free DNA (cfDNA) shows that it is a propitious liquid biomarker for non-invasive diagnosis, treatment selection, tumor response to radiation therapy, predicting treatment outcomes and evaluating radiotoxicity. Modern oncology is entering an era of individualized onco-treatment and risk assessment. Therefore, the discovery of biomarkers in accessible biological samples such as blood (serum, plasma, blood cells, etc.) is of particular importance. The accurate identification of such protein molecules as novel predictive biomarkers, associated with normal tissue radiosensitivity and toxicity, will open new avenues for the optimization of personalized RT therapies against cancer.

The incorporation of radiosensitivity biomarkers and in general radiobiology approaches into clinical routine represents an upcoming pivotal advancement in the field of radiation oncology and personalized medicine. The aforementioned biomarkers have demonstrated, in many cases, their ability to predict patients' radiosensitivity and individual patient responses to radiation therapy, thereby providing clinicians with the tools to tailor treatment regimens with unprecedented precision.

All of the above explain the rationale for this work, i.e., toward a concise and critical review of such molecular markers. By harnessing the power of these biomarkers, healthcare professionals can minimize the risks of overexposure or undertreatment, optimizing therapeutic outcomes while minimizing unnecessary side effects. Moreover, the integration of these biomarkers into routine clinical practice holds the promise of opening new avenues for innovative therapeutic strategies, ultimately enhancing the quality of care for cancer patients. As we continue to refine our understanding of radiosensitivity biomarkers for

clinical applications, their importance in the field cannot be overstated, offering only a beacon of hope for more effective and individualized cancer treatments.

Author Contributions: Conceptualization, A.G.G.; methodology, A.G., S.A.K. and A.G.G.; investigation, A.G., S.A.K., E.C.L. and G.M.; resources, A.G.G.; data curation, A.G., S.A.K., S.T., S.L. and V.S.; writing—original draft preparation, S.A.K., A.G., A.G.G., G.M., E.C.L. and S.T.; writing—review and editing, A.G., S.L., S.A.K., G.I.T., K.T., S.T., C.K. and A.G.G.; supervision, A.G.G. and G.I.T.; project administration, A.G.G.; funding acquisition, A.G.G. All authors have read and agreed to the published version of the manuscript.

Funding: The author(s) would like to acknowledge funding from the project 21GRD02 BIOSPHERE, from the European Partnership on Metrology and is co-financed by the European Union's Horizon Europe Research and Innovation Programme.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sagkrioti, E.; Biz, G.M.; Takan, I.; Asfa, S.; Nikitaki, Z.; Zanni, V.; Kars, R.H.; Hellweg, C.E.; Azzam, E.I.; Logotheti, S.; et al. Radiation Type- and Dose-Specific Transcriptional Responses across Healthy and Diseased Mammalian Tissues. *Antioxidants* **2022**, *11*, 2286. [CrossRef]
2. Mascia, A.E.; Daugherty, E.C.; Zhang, Y.; Lee, E.; Xiao, Z.; Sertorio, M.; Woo, J.; Backus, L.R.; McDonald, J.M.; McCann, C.; et al. Proton FLASH Radiotherapy for the Treatment of Symptomatic Bone Metastases: The FAST-01 Nonrandomized Trial. *JAMA Oncol.* **2023**, *9*, 62–69. [CrossRef]
3. Zou, W.; Zhang, R.; Schüler, E.; Taylor, P.A.; Mascia, A.E.; Diffenderfer, E.S.; Zhao, T.; Ayan, A.S.; Sharma, M.; Yu, S.J.; et al. Framework for Quality Assurance of Ultrahigh Dose Rate Clinical Trials Investigating FLASH Effects and Current Technology Gaps. *Int. J. Radiat. Oncol. Biol. Phys.* **2023**, *116*, 1202–1217. [CrossRef]
4. Galluzzi, L.; Aryankalayil, M.J.; Coleman, C.N.; Formenti, S.C. Emerging evidence for adapting radiotherapy to immunotherapy. *Nat. Rev. Clin. Oncol.* **2023**, *20*, 543–557. [CrossRef]
5. Azzam, E.I.; Jay-Gerin, J.P.; Pain, D. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Lett.* **2012**, *327*, 48–60. [CrossRef]
6. Chistiakov, D.A.; Voronova, N.V.; Chistiakov, P.A. Genetic variations in DNA repair genes, radiosensitivity to cancer and susceptibility to acute tissue reactions in radiotherapy-treated cancer patients. *Acta Oncol.* **2008**, *47*, 809–824. [CrossRef]
7. Pavlopoulou, A.; Bagos, P.G.; Koutsandrea, V.; Georgakilas, A.G. Molecular determinants of radiosensitivity in normal and tumor tissue: A bioinformatic approach. *Cancer Lett.* **2017**, *403*, 37–47. [CrossRef]
8. Pavlopoulou, A.; Oktay, Y.; Vougas, K.; Louka, M.; Vorgias, C.E.; Georgakilas, A.G. Determinants of resistance to chemotherapy and ionizing radiation in breast cancer stem cells. *Cancer Lett.* **2016**, *380*, 485–493. [CrossRef]
9. Sachs, R.K.; Brenner, D.J. Solid tumor risks after high doses of ionizing radiation. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13040–13045. [CrossRef]
10. Barnett, G.C.; West, C.M.L.; Coles, C.E.; Pharoah, P.D.P.; Talbot, C.J.; Elliott, R.M.; Tanteles, G.A.; Symonds, R.P.; Wilkinson, J.S.; Dunning, A.M.; et al. Standardized Total Average Toxicity Score: A Scale- and Grade-Independent Measure of Late Radiotherapy Toxicity to Facilitate Pooling of Data From Different Studies. *Int. J. Radiat. Oncol. Biol. Phys.* **2012**, *82*, 1065–1074. [CrossRef]
11. Ben-Hamo, R.; Jacob Berger, A.; Gavert, N.; Miller, M.; Pines, G.; Oren, R.; Pikarsky, E.; Benes, C.H.; Neuman, T.; Zwang, Y.; et al. Predicting and affecting response to cancer therapy based on pathway-level biomarkers. *Nat. Commun.* **2020**, *11*, 3296. [CrossRef]
12. Price, J.M.; Prabhakaran, A.; West, C.M.L. Predicting tumour radiosensitivity to deliver precision radiotherapy. *Nat. Rev. Clin. Oncol.* **2023**, *20*, 83–98. [CrossRef]
13. Byrne, N.M.; Tambe, P.; Coulter, J.A. Radiation Response in the Tumour Microenvironment: Predictive Biomarkers and Future Perspectives. *J. Pers. Med.* **2021**, *11*, 53. [CrossRef]
14. Bouffler, S.D. Evidence for variation in human radiosensitivity and its potential impact on radiological protection. *Ann. ICRP* **2016**, *45*, 280–289. [CrossRef]
15. Cox, J.D.; Stetz, J.A.; Pajak, T.F. Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC). *Int. J. Radiat. Oncol. Biol. Phys.* **1995**, *31*, 1341–1346. [CrossRef]
16. Trotti, A.; Colevas, A.D.; Setser, A.; Rusch, V.; Jaques, D.; Budach, V.; Langer, C.; Murphy, B.; Cumberlin, R.; Coleman, C.N.; et al. CTCAE v3.0: Development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin. Radiat. Oncol.* **2003**, *13*, 176–181. [CrossRef]
17. Hussein, F.A.; Manan, H.A.; Mustapha, A.; Sidek, K.; Yahya, N. Ultrasonographic Evaluation of Skin Toxicity Following Radiotherapy of Breast Cancer: A Systematic Review. *Int. J. Environ. Res. Public Health* **2022**, *19*, 13439. [CrossRef]

18. Trotti, A.; Bellm, L.A.; Epstein, J.B.; Frame, D.; Fuchs, H.J.; Gwede, C.K.; Komaroff, E.; Nalysnyk, L.; Zilberberg, M.D. Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: A systematic literature review. *Radiother. Oncol.* **2003**, *66*, 253–262. [CrossRef]
19. Bates, J.E.; Shrestha, S.; Liu, Q.; Smith, S.A.; Mulrooney, D.A.; Leisenring, W.; Gibson, T.; Robison, L.L.; Chow, E.J.; Oeffinger, K.C.; et al. Cardiac Substructure Radiation Dose and Risk of Late Cardiac Disease in Survivors of Childhood Cancer: A Report From the Childhood Cancer Survivor Study. *J. Clin. Oncol.* **2023**, *41*, 3826–3838. [CrossRef]
20. Little, M.P.; Azizova, T.V.; Richardson, D.B.; Tapio, S.; Bernier, M.O.; Kreuzer, M.; Cucinotta, F.A.; Bazyka, D.; Chumak, V.; Ivanov, V.K.; et al. Ionising radiation and cardiovascular disease: Systematic review and meta-analysis. *BMJ (Clin. Res. Ed.)* **2023**, *380*, e072924. [CrossRef]
21. Nencioni, A.; Caffa, I.; Cortellino, S.; Longo, V.D. Fasting and cancer: Molecular mechanisms and clinical application. *Nat. Rev. Cancer* **2018**, *18*, 707–719. [CrossRef]
22. Klaus, R.; Niyazi, M.; Lange-Sperandio, B. Radiation-induced kidney toxicity: Molecular and cellular pathogenesis. *Radiat. Oncol.* **2021**, *16*, 43. [CrossRef]
23. Obrador, E.; Salvador, R.; Villaescusa, J.I.; Soriano, J.M.; Estrela, J.M.; Montoro, A. Radioprotection and Radiomitigation: From the Bench to Clinical Practice. *Biomedicines* **2020**, *8*, 461. [CrossRef]
24. Kouvaris, J.R.; Kouloulias, V.E.; Vlahos, L.J. Amifostine: The First Selective-Target and Broad-Spectrum Radioprotector. *Oncol.* **2007**, *12*, 738–747. [CrossRef]
25. Marcu, L.G. Photons—Radiobiological issues related to the risk of second malignancies. *Phys. Medica* **2017**, *42*, 213–220. [CrossRef]
26. Barnett, G.C.; Coles, C.E.; Elliott, R.M.; Baynes, C.; Luccarini, C.; Conroy, D.; Wilkinson, J.S.; Tyrer, J.; Misra, V.; Platte, R.; et al. Independent validation of genes and polymorphisms reported to be associated with radiation toxicity: A prospective analysis study. *Lancet Oncol.* **2012**, *13*, 65–77. [CrossRef]
27. Vaisnav, M.; Xing, C.; Ku, H.C.; Hwang, D.; Stojadinovic, S.; Pertsemlidis, A.; Abrams, J.M. Genome-wide association analysis of radiation resistance in Drosophila melanogaster. *PLoS ONE* **2014**, *9*, e104858. [CrossRef]
28. Preston, R.J. Children as a sensitive subpopulation for the risk assessment process. *Toxicol. Appl. Pharmacol.* **2004**, *199*, 132–141. [CrossRef]
29. Pearce, M.S.; Salotti, J.A.; Little, M.P.; McHugh, K.; Lee, C.; Kim, K.P.; Howe, N.L.; Ronckers, C.M.; Rajaraman, P.; Sir Craft, A.W.; et al. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: A retrospective cohort study. *Lancet* **2012**, *380*, 499–505. [CrossRef]
30. Narendran, N.; Luzhna, L.; Kovalchuk, O. Sex Difference of Radiation Response in Occupational and Accidental Exposure. *Front. Genet.* **2019**, *10*, 260. [CrossRef]
31. Grant, E.J.; Brenner, A.; Sugiyama, H.; Sakata, R.; Sadakane, A.; Utada, M.; Cahoon, E.K.; Milder, C.M.; Soda, M.; Cullings, H.M.; et al. Solid Cancer Incidence among the Life Span Study of Atomic Bomb Survivors: 1958–2009. *Radiat. Res.* **2017**, *187*, 513–537. [CrossRef]
32. Belli, M.; Ottolenghi, A.; Weiss, W. The European strategy on low dose risk research and the role of radiation quality according to the recommendations of the "ad hoc" High Level and Expert Group (HLEG). *Radiat. Environ. Biophys.* **2010**, *49*, 463–468. [CrossRef]
33. Bentzen, S.M.; Overgaard, J. Patient-to-Patient Variability in the Expression of Radiation-Induced Normal Tissue Injury. *Semin. Radiat. Oncol.* **1994**, *4*, 68–80. [CrossRef]
34. Burger, H.; Loos, W.J.; Eechoute, K.; Verweij, J.; Mathijssen, R.H.; Wiemer, E.A. Drug transporters of platinum-based anticancer agents and their clinical significance. *Drug Resist. Updates* **2011**, *14*, 22–34. [CrossRef]
35. Menetski, J.P.; Hoffmann, S.C.; Cush, S.S.; Kamphaus, T.N.; Austin, C.P.; Herrling, P.L.; Wagner, J.A. The Foundation for the National Institutes of Health Biomarkers Consortium: Past Accomplishments and New Strategic Direction. *Clin. Pharmacol. Ther.* **2019**, *105*, 829–843. [CrossRef]
36. Franken, N.A.; Rodermond, H.M.; Stap, J.; Haveman, J.; van Bree, C. Clonogenic assay of cells in vitro. *Nat. Protoc.* **2006**, *1*, 2315–2319. [CrossRef]
37. Scott, D.; Spreadborough, A.R.; Jones, L.A.; Roberts, S.A.; Moore, C.J. Chromosomal radiosensitivity in G2-phase lymphocytes as an indicator of cancer predisposition. *Radiat. Res.* **1996**, *145*, 3–16. [CrossRef]
38. Terzoudi, G.I.; Jung, T.; Hain, J.; Vrouvas, J.; Margaritis, K.; Donta-Bakoyianni, C.; Makropoulos, V.; Angelakis, P.; Pantelias, G.E. Increased G2 chromosomal radiosensitivity in cancer patients: The role of cdk1/cyclin-B activity level in the mechanisms involved. *Int. J. Radiat. Biol.* **2000**, *76*, 607–615. [CrossRef]
39. Vral, A.; Thierens, H.; Baeyens, A.; De Ridder, L. Induction and disappearance of G2 chromatid breaks in lymphocytes after low doses of low-LET gamma-rays and high-LET fast neutrons. *Int. J. Radiat. Biol.* **2002**, *78*, 249–257. [CrossRef]
40. Howe, O.L.; Daly, P.A.; Seymour, C.; Ormiston, W.; Nolan, C.; Mothersill, C. Elevated G2 chromosomal radiosensitivity in Irish breast cancer patients: A comparison with other studies. *Int. J. Radiat. Biol.* **2005**, *81*, 373–378. [CrossRef]
41. Howe, O.; O'Malley, K.; Lavin, M.; Gardner, R.A.; Seymour, C.; Lyng, F.; Mulvin, D.; Quinlan, D.M.; Mothersill, C. Cell death mechanisms associated with G2 radiosensitivity in patients with prostate cancer and benign prostatic hyperplasia. *Radiat. Res.* **2005**, *164*, 627–634. [CrossRef]

42. Chua, M.L.; Somaiah, N.; A'Hern, R.; Davies, S.; Gothard, L.; Yarnold, J.; Rothkamm, K. Residual DNA and chromosomal damage in ex vivo irradiated blood lymphocytes correlated with late normal tissue response to breast radiotherapy. *Radiother. Oncol.* **2011**, *99*, 362–366. [CrossRef]
43. Brzozowska, K.; Pinkawa, M.; Eble, M.J.; Müller, W.U.; Wojcik, A.; Kriehuber, R.; Schmitz, S. In vivo versus in vitro individual radiosensitivity analysed in healthy donors and in prostate cancer patients with and without severe side effects after radiotherapy. *Int. J. Radiat. Biol.* **2012**, *88*, 405–413. [CrossRef]
44. Pinkawa, M.; Brzozowska, K.; Kriehuber, R.; Eble, M.J.; Schmitz, S. Prediction of radiation-induced toxicity by in vitro radiosensitivity of lymphocytes in prostate cancer patients. *Future Oncol.* **2016**, *12*, 617–624. [CrossRef]
45. Smart, V.; Curwen, G.B.; Whitehouse, C.A.; Edwards, A.; Tawn, E.J. Chromosomal radiosensitivity: A study of the chromosomal G2 assay in human blood lymphocytes indicating significant inter-individual variability. *Mutat. Res./Fundam. Mol. Mech. Mutagen.* **2003**, *528*, 105–110. [CrossRef]
46. Vral, A.; Thierens, H.; Baeyens, A.; De Ridder, L. The Micronucleus and G2-Phase Assays for Human Blood Lymphocytes as Biomarkers of Individual Sensitivity to Ionizing Radiation: Limitations Imposed by Intraindividual Variability. *Radiat. Res.* **2002**, *157*, 472–477. [CrossRef]
47. Lisowska, H.; Lankoff, A.; Wieczorek, A.; Florek, A.; Kuszewski, T.; Góźdź, S.; Wojcik, A. Enhanced chromosomal radiosensitivity in peripheral blood lymphocytes of larynx cancer patients. *Int. J. Radiat. Oncol. Biol. Phys.* **2006**, *66*, 1245–1252. [CrossRef]
48. Pantelias, G.E.; Terzoudi, G.I. A standardized G2-assay for the prediction of individual radiosensitivity. *Radiother. Oncol.* **2011**, *101*, 28–34. [CrossRef]
49. Sommer, S.; Buraczewska, I.; Kruszewski, M. Micronucleus Assay: The State of Art, and Future Directions. *Int. J. Mol. Sci.* **2020**, *21*, 1534. [CrossRef]
50. Baert, A.; Depuydt, J.; Van Maerken, T.; Poppe, B.; Malfait, F.; Van Damme, T.; De Nobele, S.; Perletti, G.; De Leeneer, K.; Claes, K.B.; et al. Analysis of chromosomal radiosensitivity of healthy BRCA2 mutation carriers and non-carriers in BRCA families with the G2 micronucleus assay. *Oncol. Rep.* **2017**, *37*, 1379–1386. [CrossRef]
51. Kirsch-Volders, M.; Plas, G.; Elhajouji, A.; Lukamowicz, M.; Gonzalez, L.; Vande Loock, K.; Decordier, I. The in vitro MN assay in 2011: Origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. *Arch. Toxicol.* **2011**, *85*, 873–899. [CrossRef]
52. Krupina, K.; Goginashvili, A.; Cleveland, D.W. Causes and consequences of micronuclei. *Curr. Opin. Cell Biol.* **2021**, *70*, 91–99. [CrossRef]
53. Huber, D.; Voith von Voithenberg, L.; Kaigala, G.V. Fluorescence in situ hybridization (FISH): History, limitations and what to expect from micro-scale FISH? *Micro Nano Eng.* **2018**, *1*, 15–24. [CrossRef]
54. Dunst, J.; Gebhart, E.; Neubauer, S. Can an extremely elevated radiosensitivity in patients be recognized by the in-vitro testing of lymphocytes? *Strahlenther. Onkol. Organ Dtsch. Rontgenges* **1995**, *171*, 581–586.
55. Huber, R.; Braselmann, H.; Geinitz, H.; Jaehnert, I.; Baumgartner, A.; Thamm, R.; Figel, M.; Molls, M.; Zitzelsberger, H. Chromosomal radiosensitivity and acute radiation side effects after radiotherapy in tumour patients—A follow-up study. *Radiat. Oncol.* **2011**, *6*, 32. [CrossRef]
56. Schmitz, S.; Brzozowska, K.; Pinkawa, M.; Eble, M.; Kriehuber, R. Chromosomal radiosensitivity analyzed by FISH in lymphocytes of prostate cancer patients and healthy donors. *Radiat. Res.* **2013**, *180*, 465–473. [CrossRef]
57. Fleckenstein, J.; Kühne, M.; Seegmüller, K.; Derschang, S.; Melchior, P.; Gräber, S.; Fricke, A.; Rübe, C.E.; Rübe, C. The impact of individual in vivo repair of DNA double-strand breaks on oral mucositis in adjuvant radiotherapy of head-and-neck cancer. *Int. J. Radiat. Oncol. Biol. Phys.* **2011**, *81*, 1465–1472. [CrossRef]
58. Mumbrekar, K.D.; Fernandes, D.J.; Goutham, H.V.; Sharan, K.; Vadhiraja, B.M.; Satyamoorthy, K.; Bola Sadashiva, S.R. Influence of double-strand break repair on radiation therapy-induced acute skin reactions in breast cancer patients. *Int. J. Radiat. Oncol. Biol. Phys.* **2014**, *88*, 671–676. [CrossRef]
59. Vasireddy, R.S.; Sprung, C.N.; Cempaka, N.L.; Chao, M.; McKay, M.J. H2AX phosphorylation screen of cells from radiosensitive cancer patients reveals a novel DNA double-strand break repair cellular phenotype. *Br. J. Cancer* **2010**, *102*, 1511–1518. [CrossRef]
60. Bourton, E.C.; Plowman, P.N.; Smith, D.; Arlett, C.F.; Parris, C.N. Prolonged expression of the γ-H2AX DNA repair biomarker correlates with excess acute and chronic toxicity from radiotherapy treatment. *Int. J. Cancer* **2011**, *129*, 2928–2934. [CrossRef]
61. Djuzenova, C.S.; Elsner, I.; Katzer, A.; Worschech, E.; Distel, L.V.; Flentje, M.; Polat, B. Radiosensitivity in breast cancer assessed by the histone γ-H2AX and 53BP1 foci. *Radiat. Oncol.* **2013**, *8*, 98. [CrossRef]
62. Durdik, M.; Markova, E.; Kosik, P.; Vigasova, K.; Gulati, S.; Jakl, L.; Vrobelova, K.; Fekete, M.; Zavacka, I.; Pobijakova, M.; et al. Assessment of Individual Radiosensitivity in Breast Cancer Patients Using a Combination of Biomolecular Markers. *Biomedicines* **2023**, *11*, 1122. [CrossRef]
63. Lobachevsky, P.; Leong, T.; Daly, P.; Smith, J.; Best, N.; Tomaszewski, J.; Thompson, E.R.; Li, N.; Campbell, I.G.; Martin, R.F.; et al. Compromized DNA repair as a basis for identification of cancer radiotherapy patients with extreme radiosensitivity. *Cancer Lett.* **2016**, *383*, 212–219. [CrossRef]
64. Rogakou, E.P.; Pilch, D.R.; Orr, A.H.; Ivanova, V.S.; Bonner, W.M. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.* **1998**, *273*, 5858–5868. [CrossRef]
65. Paull, T.T.; Rogakou, E.P.; Yamazaki, V.; Kirchgessner, C.U.; Gellert, M.; Bonner, W.M. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr. Biol. CB* **2000**, *10*, 886–895. [CrossRef]

66. Rogakou, E.P.; Boon, C.; Redon, C.; Bonner, W.M. Megabase chromatin domains involved in DNA double-strand breaks in vivo. *J. Cell Biol.* **1999**, *146*, 905–916. [CrossRef]
67. Mah, L.J.; El-Osta, A.; Karagiannis, T.C. γH2AX: A sensitive molecular marker of DNA damage and repair. *Leukemia* **2010**, *24*, 679–686. [CrossRef]
68. Bonner, W.M.; Redon, C.E.; Dickey, J.S.; Nakamura, A.J.; Sedelnikova, O.A.; Solier, S.; Pommier, Y. GammaH2AX and cancer. *Nat. Rev. Cancer* **2008**, *8*, 957–967. [CrossRef]
69. Halazonetis, T.D.; Gorgoulis, V.G.; Bartek, J. An oncogene-induced DNA damage model for cancer development. *Science* **2008**, *319*, 1352–1355. [CrossRef]
70. Ivashkevich, A.; Redon, C.E.; Nakamura, A.J.; Martin, R.F.; Martin, O.A. Use of the γ-H2AX assay to monitor DNA damage and repair in translational cancer research. *Cancer Lett.* **2012**, *327*, 123–133. [CrossRef]
71. Marková, E.; Schultz, N.; Belyaev, I.Y. Kinetics and dose-response of residual 53BP1/gamma-H2AX foci: Co-localization, relationship with DSB repair and clonogenic survival. *Int. J. Radiat. Biol.* **2007**, *83*, 319–329. [CrossRef]
72. Ward, I.M.; Chen, J. Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. *J. Biol. Chem.* **2001**, *276*, 47759–47762. [CrossRef]
73. Kakarougkas, A.; Ismail, A.; Klement, K.; Goodarzi, A.A.; Conrad, S.; Freire, R.; Shibata, A.; Lobrich, M.; Jeggo, P.A. Opposing roles for 53BP1 during homologous recombination. *Nucleic Acids Res.* **2013**, *41*, 9719–9731. [CrossRef]
74. Bobkova, E.; Depes, D.; Lee, J.-H.; Jezkova, L.; Falkova, I.; Pagacova, E.; Kopecna, O.; Zadneprianetc, M.; Bacikova, A.; Kulikova, E.; et al. Recruitment of 53BP1 Proteins for DNA Repair and Persistence of Repair Clusters Differ for Cell Types as Detected by Single Molecule Localization Microscopy. *Int. J. Mol. Sci.* **2018**, *19*, 3713. [CrossRef]
75. Penninckx, S.; Pariset, E.; Cekanaviciute, E.; Costes, S.V. Quantification of radiation-induced DNA double strand break repair foci to evaluate and predict biological responses to ionizing radiation. *NAR Cancer* **2021**, *3*, zcab046. [CrossRef]
76. Depes, D.; Lee, J.-H.; Bobkova, E.; Jezkova, L.; Falkova, I.; Bestvater, F.; Pagacova, E.; Kopecna, O.; Zadneprianetc, M.; Bacikova, A.; et al. Single-molecule localization microscopy as a promising tool for γH2AX/53BP1 foci exploration. *Eur. Phys. J. D* **2018**, *72*, 158. [CrossRef]
77. Johansson, P.; Fasth, A.; Ek, T.; Hammarsten, O. Validation of a flow cytometry-based detection of γ-H2AX, to measure DNA damage for clinical applications. *Cytom. Part B Clin. Cytom.* **2017**, *92*, 534–540. [CrossRef]
78. Sun, J.; Kroeger, J.L.; Markowitz, J. Introduction to Multiparametric Flow Cytometry and Analysis of High-Dimensional Data. *Methods Mol. Biol.* **2021**, *2194*, 239–253. [CrossRef]
79. Gadalla, R.; Noamani, B.; MacLeod, B.L.; Dickson, R.J.; Guo, M.; Xu, W.; Lukhele, S.; Elsaesser, H.J.; Razak, A.R.A.; Hirano, N.; et al. Validation of CyTOF Against Flow Cytometry for Immunological Studies and Monitoring of Human Cancer Clinical Trials. *Front. Oncol.* **2019**, *9*, 415. [CrossRef]
80. Ricklefs, F.L.; Maire, C.L.; Reimer, R.; Dührsen, L.; Kolbe, K.; Holz, M.; Schneider, E.; Rissiek, A.; Babayan, A.; Hille, C.; et al. Imaging flow cytometry facilitates multiparametric characterization of extracellular vesicles in malignant brain tumours. *J. Extracell. Vesicles* **2019**, *8*, 1588555. [CrossRef]
81. Stracker, T.H.; Petrini, J.H. The MRE11 complex: Starting from the ends. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 90–103. [CrossRef]
82. Lavin, M.F.; Kozlov, S. ATM activation and DNA damage response. *Cell Cycle* **2007**, *6*, 931–942. [CrossRef]
83. Söderlund, K.; Stål, O.; Skoog, L.; Rutqvist, L.E.; Nordenskjöld, B.; Askmalm, M.S. Intact Mre11/Rad50/Nbs1 complex predicts good response to radiotherapy in early breast cancer. *Int. J. Radiat. Oncol. Biol. Phys.* **2007**, *68*, 50–58. [CrossRef]
84. Yan, X.; Wu, T.; Tang, M.; Chen, D.; Huang, M.; Zhou, S.; Zhang, H.; Yang, X.; Li, G. Methylation of the ataxia telangiectasia mutated gene (ATM) promoter as a radiotherapy outcome biomarker in patients with hepatocellular carcinoma. *Medicine* **2020**, *99*, e18823. [CrossRef]
85. Yuan, S.-S.F.; Hou, M.-F.; Hsieh, Y.-C.; Huang, C.-Y.; Lee, Y.-C.; Chen, Y.-J.; Lo, S. Role of MRE11 in Cell Proliferation, Tumor Invasion, and DNA Repair in Breast Cancer. *JNCI J. Natl. Cancer Inst.* **2012**, *104*, 1485–1502. [CrossRef]
86. Ho, V.; Chung, L.; Singh, A.; Lea, V.; Abubakar, A.; Lim, S.H.; Ng, W.; Lee, M.; de Souza, P.; Shin, J.-S.; et al. Overexpression of the MRE11-RAD50-NBS1 (MRN) complex in rectal cancer correlates with poor response to neoadjuvant radiotherapy and prognosis. *BMC Cancer* **2018**, *18*, 869. [CrossRef]
87. Chen, C.; Wang, Y.; Mei, J.F.; Li, S.S.; Xu, H.X.; Xiong, H.P.; Wang, X.H.; He, X. Targeting RAD50 increases sensitivity to radiotherapy in colorectal cancer cells. *Neoplasma* **2018**, *65*, 75–80. [CrossRef]
88. Ebi, H.; Matsuo, K.; Sugito, N.; Suzuki, M.; Osada, H.; Tajima, K.; Ueda, R.; Takahashi, T. Novel NBS1 heterozygous germ line mutation causing MRE11-binding domain loss predisposes to common types of cancer. *Cancer Res.* **2007**, *67*, 11158–11165. [CrossRef]
89. Altan, B.; Yokobori, T.; Ide, M.; Bai, T.; Yanoma, T.; Kimura, A.; Kogure, N.; Suzuki, M.; Bao, P.; Mochiki, E.; et al. High Expression of MRE11-RAD50-NBS1 Is Associated with Poor Prognosis and Chemoresistance in Gastric Cancer. *Anticancer Res.* **2016**, *36*, 5237–5247. [CrossRef]
90. Ihara, K.; Yamaguchi, S.; Ueno, N.; Tani, Y.; Shida, Y.; Ogata, H.; Domeki, Y.; Okamoto, K.; Nakajima, M.; Sasaki, K.; et al. Expression of DNA double-strand break repair proteins predicts the response and prognosis of colorectal cancer patients undergoing oxaliplatin-based chemotherapy. *Oncol. Rep.* **2016**, *35*, 1349–1355. [CrossRef]

91. Takemura, H.; Rao, V.A.; Sordet, O.; Furuta, T.; Miao, Z.H.; Meng, L.; Zhang, H.; Pommier, Y. Defective Mre11-dependent activation of Chk2 by ataxia telangiectasia mutated in colorectal carcinoma cells in response to replication-dependent DNA double strand breaks. *J. Biol. Chem.* **2006**, *281*, 30814–30823. [CrossRef]
92. Sia, J.; Szmyd, R.; Hau, E.; Gee, H.E. Molecular Mechanisms of Radiation-Induced Cancer Cell Death: A Primer. *Front. Cell Dev. Biol.* **2020**, *8*, 41. [CrossRef]
93. Beretta, G.L.; Zaffaroni, N. Radiotherapy-induced ferroptosis for cancer treatment. *Front. Mol. Biosci.* **2023**, *10*, 1216733. [CrossRef]
94. Kim, J.H.; Brown, S.L.; Gordon, M.N. Radiation-induced senescence: Therapeutic opportunities. *Radiat. Oncol.* **2023**, *18*, 10. [CrossRef]
95. Elmore, S. Apoptosis: A review of programmed cell death. *Toxicol. Pathol.* **2007**, *35*, 495–516. [CrossRef]
96. Paris, F.; Fuks, Z.; Kang, A.; Capodieci, P.; Juan, G.; Ehleiter, D.; Haimovitz-Friedman, A.; Cordon-Cardo, C.; Kolesnick, R. Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science* **2001**, *293*, 293–297. [CrossRef]
97. Wolf, B.B.; Schuler, M.; Echeverri, F.; Green, D.R. Caspase-3 Is the Primary Activator of Apoptotic DNA Fragmentation via DNA Fragmentation Factor-45/Inhibitor of Caspase-Activated DNase Inactivation. *J. Biol. Chem.* **1999**, *274*, 30651–30656. [CrossRef]
98. Yang, J.; Liu, X.; Bhalla, K.; Kim, C.N.; Ibrado, A.M.; Cai, J.; Peng, T.I.; Jones, D.P.; Wang, X. Prevention of apoptosis by Bcl-2: Release of cytochrome c from mitochondria blocked. *Science* **1997**, *275*, 1129–1132. [CrossRef]
99. Santos, N.; Silva, R.F.; Pinto, M.; Silva, E.B.D.; Tasat, D.R.; Amaral, A. Active caspase-3 expression levels as bioindicator of individual radiosensitivity. *An. Acad. Bras. Cienc.* **2017**, *89*, 649–659. [CrossRef]
100. Erasmus, W.L.; Slabbert, J.P.; Crompton, N.E.A.; Meehan, K.A. The leukocyte apoptosis assay: A clinical predictor of radiosensitivity. *Med. Technol. SA* **2005**, *19*, 9–14.
101. Schnarr, K.; Dayes, I.; Sathya, J.; Boreham, D. Individual radiosensitivity and its relevance to health physics. *Dose-Response* **2007**, *5*, 333–348. [CrossRef]
102. Pinar, B.; Henríquez-Hernández, L.A.; Lara, P.C.; Bordon, E.; Rodriguez-Gallego, C.; Lloret, M.; Nuñez, M.I.; De Almodovar, M.R. Radiation induced apoptosis and initial DNA damage are inversely related in locally advanced breast cancer patients. *Radiat. Oncol.* **2010**, *5*, 85. [CrossRef]
103. Yang, X.H.; Edgerton, S.; Thor, A.D. Reconstitution of caspase-3 sensitizes MCF-7 breast cancer cells to radiation therapy. *Int. J. Oncol.* **2005**, *26*, 1675–1680. [CrossRef]
104. Hallan, E.; Blomhoff, H.K.; Smeland, E.B.; Lømo, J. Involvement of ICE (Caspase) family in gamma-radiation-induced apoptosis of normal B lymphocytes. *Scand. J. Immunol.* **1997**, *46*, 601–608. [CrossRef]
105. Yu, Y.; Little, J.B. p53 Is Involved in But Not Required for Ionizing Radiation-induced Caspase-3 Activation and Apoptosis in Human Lymphoblast Cell Lines1. *Cancer Res.* **1998**, *58*, 4277–4281.
106. Cao, M.; Cabrera, R.; Xu, Y.; Liu, C.; Nelson, D. Different radiosensitivity of $CD4^+CD25^+$ regulatory T cells and effector T cells to low dose gamma irradiation in vitro. *Int. J. Radiat. Biol.* **2011**, *87*, 71–80. [CrossRef]
107. Nguyen, H.Q.; Belkacemi, Y.; Mann, C.; Hoffschir, F.; Kerbrat, S.; Surenaud, M.; Zadigue, P.; de La Taille, A.; Romeo, P.-H.; Le Gouvello, S. Human CCR6+ Th17 Lymphocytes Are Highly Sensitive to Radiation-Induced Senescence and Are a Potential Target for Prevention of Radiation-Induced Toxicity. *Int. J. Radiat. Oncol. Biol. Phys.* **2020**, *108*, 314–325. [CrossRef]
108. Kiani, A.; Mohebat, L.; Javanmard, S.; Kazemi, M.; Tavakoli, M.; Kheirollahi, M. Annexin V FITC conjugated as a radiation toxicity indicator in lymphocytes following radiation overexposure in radiotherapy programs. *Adv. Biomed. Res.* **2015**, *4*, 119. [CrossRef]
109. Mochizuki, T.; Kuge, Y.; Zhao, S.; Tsukamoto, E.; Hosokawa, M.; Strauss, H.W.; Blankenberg, F.G.; Tait, J.F.; Tamaki, N. Detection of Apoptotic Tumor Response In Vivo After a Single Dose of Chemotherapy with ^{99m}Tc-Annexin V. *J. Nucl. Med. January* **2003**, *44*, 92–97.
110. Stanojković, T.P.; Matić, I.Z.; Petrović, N.; Stanković, V.; Kopčalić, K.; Besu, I.; Đorđić Crnogorac, M.; Mališić, E.; Mirjačić-Martinović, K.; Vuletić, A.; et al. Evaluation of cytokine expression and circulating immune cell subsets as potential parameters of acute radiation toxicity in prostate cancer patients. *Sci. Rep.* **2020**, *10*, 19002. [CrossRef]
111. Coussens, L.M.; Werb, Z. Inflammation and cancer. *Nature* **2002**, *420*, 860–867. [CrossRef]
112. Lee, J.; Kim, M.-R.; Kim, H.-J.; An, Y.S.; Yi, J.Y. TGF-β1 accelerates the DNA damage response in epithelial cells via Smad signaling. *Biochem. Biophys. Res. Commun.* **2016**, *476*, 420–425. [CrossRef]
113. Anscher, M.S. Targeting the TGF-beta1 pathway to prevent normal tissue injury after cancer therapy. *Oncologist* **2010**, *15*, 350–359. [CrossRef]
114. Shao, C.; Folkard, M.; Prise, K.M. Role of TGF-β1 and nitric oxide in the bystander response of irradiated glioma cells. *Oncogene* **2008**, *27*, 434–440. [CrossRef]
115. Ahmadi, A.; Najafi, M.; Farhood, B.; Mortezaee, K. Transforming growth factor-β signaling: Tumorigenesis and targeting for cancer therapy. *J. Cell. Physiol.* **2019**, *234*, 12173–12187. [CrossRef]
116. Biswas, S.; Guix, M.; Rinehart, C.; Dugger, T.C.; Chytil, A.; Moses, H.L.; Freeman, M.L.; Arteaga, C.L. Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression. *J. Clin. Investig.* **2007**, *117*, 1305–1313. [CrossRef]
117. Zhang, M.; Kleber, S.; Röhrich, M.; Timke, C.; Han, N.; Tuettenberg, J.; Martin-Villalba, A.; Debus, J.; Peschke, P.; Wirkner, U.; et al. Blockade of TGF-β signaling by the TGFβR-I kinase inhibitor LY2109761 enhances radiation response and prolongs survival in glioblastoma. *Cancer Res.* **2011**, *71*, 7155–7167. [CrossRef]

118. Bouquet, F.; Pal, A.; Pilones, K.A.; Demaria, S.; Hann, B.; Akhurst, R.J.; Babb, J.S.; Lonning, S.M.; DeWyngaert, J.K.; Formenti, S.C.; et al. TGFβ1 Inhibition Increases the Radiosensitivity of Breast Cancer Cells In Vitro and Promotes Tumor Control by Radiation In Vivo. *Clin. Cancer Res.* **2011**, *17*, 6754–6765. [CrossRef]
119. Formenti, S.C.; Lee, P.; Adams, S.; Goldberg, J.D.; Li, X.; Xie, M.W.; Ratikan, J.A.; Felix, C.; Hwang, L.; Faull, K.F.; et al. Focal Irradiation and Systemic TGFβ Blockade in Metastatic Breast Cancer. *Clin. Cancer Res.* **2018**, *24*, 2493–2504. [CrossRef]
120. Reibnegger, G.; Krainer, M.; Herold, M.; Ludwig, H.; Wachter, H.; Huber, H. Predictive value of interleukin-6 and neopterin in patients with multiple myeloma. *Cancer Res.* **1991**, *51*, 6250–6253.
121. Pulkki, K.; Pelliniemi, T.T.; Rajamäki, A.; Tienhaara, A.; Laakso, M.; Lahtinen, R. Soluble interleukin-6 receptor as a prognostic factor in multiple myeloma. Finnish Leukaemia Group. *Br. J. Haematol.* **1996**, *92*, 370–374. [CrossRef]
122. Miki, S.; Iwano, M.; Miki, Y.; Yamamoto, M.; Tang, B.; Yokokawa, K.; Sonoda, T.; Hirano, T.; Kishimoto, T. Interleukin-6 (IL-6) functions as an in vitro autocrine growth factor in renal cell carcinomas. *FEBS Lett.* **1989**, *250*, 607–610. [CrossRef]
123. Blay, J.Y.; Negrier, S.; Combaret, V.; Attali, S.; Goillot, E.; Merrouche, Y.; Mercatello, A.; Ravault, A.; Tourani, J.M.; Moskovtchenko, J.F.; et al. Serum level of interleukin 6 as a prognosis factor in metastatic renal cell carcinoma. *Cancer Res.* **1992**, *52*, 3317–3322.
124. Van Meir, E.; Sawamura, Y.; Diserens, A.-C.; Hamou, M.-F.; de Tribolet, N. Human Glioblastoma Cells Release Interleukin 6 in Vivo and in Vitro1. *Cancer Res.* **1990**, *50*, 6683–6688.
125. Tamm, I.; Cardinale, I.; Krueger, J.; Murphy, J.S.; May, L.T.; Sehgal, P.B. Interleukin 6 decreases cell-cell association and increases motility of ductal breast carcinoma cells. *J. Exp. Med.* **1989**, *170*, 1649–1669. [CrossRef]
126. Guo, Z.; Zhang, H.; Fu, Y.; Kuang, J.; Zhao, B.; Zhang, L.; Lin, J.; Lin, S.; Wu, D.; Xie, G. Cancer-associated fibroblasts induce growth and radioresistance of breast cancer cells through paracrine IL-6. *Cell Death Discov.* **2023**, *9*, 6. [CrossRef]
127. Scambia, G.; Testa, U.; Benedetti Panici, P.; Foti, E.; Martucci, R.; Gadducci, A.; Perillo, A.; Facchini, V.; Peschle, C.; Mancuso, S. Prognostic significance of interleukin 6 serum levels in patients with ovarian cancer. *Br. J. Cancer* **1995**, *71*, 354–356. [CrossRef]
128. Berek, J.S.; Chung, C.; Kaldı, K.; Watson, J.M.; Knox, R.M.; Martínez-Maza, O. Serum interleukin-6 levels correlate with disease status in patients with epithelial ovarian cancer. *Am. J. Obstet. Gynecol.* **1991**, *164*, 1038–1042, discussion 1042–1043. [CrossRef]
129. Shariat, S.F.; Andrews, B.; Kattan, M.W.; Kim, J.; Wheeler, T.M.; Slawin, K.M. Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. *Urology* **2001**, *58*, 1008–1015. [CrossRef]
130. George, D.J.; Halabi, S.; Shepard, T.F.; Sanford, B.; Vogelzang, N.J.; Small, E.J.; Kantoff, P.W. The prognostic significance of plasma interleukin-6 levels in patients with metastatic hormone-refractory prostate cancer: Results from cancer and leukemia group B 9480. *Clin. Cancer Res.* **2005**, *11*, 1815–1820. [CrossRef]
131. Hobisch, A.; Eder, I.E.; Putz, T.; Horninger, W.; Bartsch, G.; Klocker, H.; Culig, Z. Interleukin-6 regulates prostate-specific protein expression in prostate carcinoma cells by activation of the androgen receptor. *Cancer Res.* **1998**, *58*, 4640–4645.
132. Akimoto, S.; Okumura, A.; Fuse, H. Relationship between serum levels of interleukin-6, tumor necrosis factor-alpha and bone turnover markers in prostate cancer patients. *Endocr. J.* **1998**, *45*, 183–189. [CrossRef]
133. Nakashima, J.; Tachibana, M.; Horiguchi, Y.; Oya, M.; Ohigashi, T.; Asakura, H.; Murai, M. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. *Clin. Cancer Res.* **2000**, *6*, 2702–2706.
134. Twillie, D.A.; Eisenberger, M.A.; Carducci, M.A.; Hseih, W.S.; Kim, W.Y.; Simons, J.W. Interleukin-6: A candidate mediator of human prostate cancer morbidity. *Urology* **1995**, *45*, 542–549. [CrossRef]
135. Kinoshita, T.; Ito, H.; Miki, C. Serum interleukin-6 level reflects the tumor proliferative activity in patients with colorectal carcinoma. *Cancer* **1999**, *85*, 2526–2531. [CrossRef]
136. Oka, M.; Yamamoto, K.; Takahashi, M.; Hakozaki, M.; Abe, T.; Iizuka, N.; Hazama, S.; Hirazawa, K.; Hayashi, H.; Tangoku, A.; et al. Relationship between serum levels of interleukin 6, various disease parameters and malnutrition in patients with esophageal squamous cell carcinoma. *Cancer Res.* **1996**, *56*, 2776–2780.
137. Rašková, M.; Lacina, L.; Kejík, Z.; Venhauerová, A.; Skaličková, M.; Kolář, M.; Jakubek, M.; Rosel, D.; Smetana, K., Jr.; Brábek, J. The Role of IL-6 in Cancer Cell Invasiveness and Metastasis-Overview and Therapeutic Opportunities. *Cells* **2022**, *11*, 3698. [CrossRef]
138. Chen, M.F.; Hsieh, C.C.; Chen, W.C.; Lai, C.H. Role of interleukin-6 in the radiation response of liver tumors. *Int. J. Radiat. Oncol. Biol. Phys.* **2012**, *84*, e621–e630. [CrossRef]
139. Kopčalić, K.; Matić, I.Z.; Besu, I.; Stanković, V.; Bukumirić, Z.; Stanojković, T.P.; Stepanović, A.; Nikitović, M. Circulating levels of IL-6 and TGF-β1 in patients with prostate cancer undergoing radiotherapy: Associations with acute radiotoxicity and fatigue symptoms. *BMC Cancer* **2022**, *22*, 1167. [CrossRef]
140. Rébé, C.; Ghiringhelli, F. Interleukin-1β and Cancer. *Cancers* **2020**, *12*, 1791. [CrossRef]
141. Fiebich, B.L.; Mueksch, B.; Boehringer, M.; Hüll, M. Interleukin-1beta induces cyclooxygenase-2 and prostaglandin E(2) synthesis in human neuroblastoma cells: Involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB. *J. Neurochem.* **2000**, *75*, 2020–2028. [CrossRef]
142. Nathe, T.J.; Deou, J.; Walsh, B.; Bourns, B.; Clowes, A.W.; Daum, G. Interleukin-1beta inhibits expression of p21(WAF1/CIP1) and p27(KIP1) and enhances proliferation in response to platelet-derived growth factor-BB in smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* **2002**, *22*, 1293–1298. [CrossRef]
143. Lahm, H.; Petral-Malec, D.; Yilmaz-Ceyhan, A.; Fischer, J.R.; Lorenzoni, M.; Givel, J.C.; Odartchenko, N. Growth stimulation of a human colorectal carcinoma cell line by interleukin-1 and -6 and antagonistic effects of transforming growth factor beta 1. *Eur. J. Cancer* **1992**, *28*, 1894–1899. [CrossRef]

144. Nakahara, H.; Song, J.; Sugimoto, M.; Hagihara, K.; Kishimoto, T.; Yoshizaki, K.; Nishimoto, N. Anti-interleukin-6 receptor antibody therapy reduces vascular endothelial growth factor production in rheumatoid arthritis. *Arthritis Rheum.* **2003**, *48*, 1521–1529. [CrossRef]
145. Wolf, J.S.; Chen, Z.; Dong, G.; Sunwoo, J.B.; Bancroft, C.C.; Capo, D.E.; Yeh, N.T.; Mukaida, N.; Van Waes, C. IL (interleukin)-1alpha promotes nuclear factor-kappaB and AP-1-induced IL-8 expression, cell survival, and proliferation in head and neck squamous cell carcinomas. *Clin. Cancer Res.* **2001**, *7*, 1812–1820.
146. Mukhopadhyay, P.; Ali, M.A.; Nandi, A.; Carreon, P.; Choy, H.; Saha, D. The cyclin-dependent kinase 2 inhibitor down-regulates interleukin-1beta-mediated induction of cyclooxygenase-2 expression in human lung carcinoma cells. *Cancer Res.* **2006**, *66*, 1758–1766. [CrossRef]
147. Chen, M.F.; Lu, M.S.; Chen, P.T.; Chen, W.C.; Lin, P.Y.; Lee, K.D. Role of interleukin 1 beta in esophageal squamous cell carcinoma. *J. Mol. Med.* **2012**, *90*, 89–100. [CrossRef]
148. Aggen, D.H.; Ager, C.R.; Obradovic, A.Z.; Chowdhury, N.; Ghasemzadeh, A.; Mao, W.; Chaimowitz, M.G.; Lopez-Bujanda, Z.A.; Spina, C.S.; Hawley, J.E.; et al. Blocking IL1 Beta Promotes Tumor Regression and Remodeling of the Myeloid Compartment in a Renal Cell Carcinoma Model: Multidimensional Analyses. *Clin. Cancer Res.* **2021**, *27*, 608–621. [CrossRef]
149. Rath, P.C.; Aggarwal, B.B. TNF-induced signaling in apoptosis. *J. Clin. Immunol.* **1999**, *19*, 350–364. [CrossRef]
150. Wang, X.; Lin, Y. Tumor necrosis factor and cancer, buddies or foes? *Acta Pharmacol. Sin.* **2008**, *29*, 1275–1288. [CrossRef]
151. Zhu, R.; Xue, X.; Shen, M.; Tsai, Y.; Keng, P.C.; Chen, Y.; Lee, S.O.; Chen, Y. NFκB and TNFα as individual key molecules associated with the cisplatin-resistance and radioresistance of lung cancer. *Exp. Cell Res.* **2019**, *374*, 181–188. [CrossRef]
152. Hallahan, D.E.; Spriggs, D.R.; Beckett, M.A.; Kufe, D.W.; Weichselbaum, R.R. Increased tumor necrosis factor alpha mRNA after cellular exposure to ionizing radiation. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 10104–10107. [CrossRef]
153. Weichselbaum, R.R.; Beckett, M.A.; Vokes, E.E.; Brachman, D.G.; Haraf, D.; Hallahan, D.; Kufe, D. Cellular and molecular mechanisms of radioresistance. In *Head and Neck Cancer: Basic and Clinical Aspects*; Hong, W.K., Weber, R.S., Eds.; Springer: Boston, MA, USA, 1995; pp. 131–140.
154. Rosen, E.M.; Fan, S.; Rockwell, S.; Goldberg, I.D. The molecular and cellular basis of radiosensitivity: Implications for understanding how normal tissues and tumors respond to therapeutic radiation. *Cancer Investig.* **1999**, *17*, 56–72. [CrossRef]
155. An, H.J.; Seong, S.J. Proteomics Analysis of Apoptosis-regulating Proteins in Tissues with Different Radiosensitivity. *J. Radiat. Res.* **2006**, *47*, 147–155. [CrossRef]
156. Meng, Y.; Beckett, M.A.; Liang, H.; Mauceri, H.J.; van Rooijen, N.; Cohen, K.S.; Weichselbaum, R.R. Blockade of tumor necrosis factor alpha signaling in tumor-associated macrophages as a radiosensitizing strategy. *Cancer Res.* **2010**, *70*, 1534–1543. [CrossRef]
157. Pal, S.; Yadav, P.; Sainis, K.B.; Shankar, B.S. TNF-α and IGF-1 differentially modulate ionizing radiation responses of lung cancer cell lines. *Cytokine* **2018**, *101*, 89–98. [CrossRef]
158. Hall, W.A.; Karrison, T.G.; Rosenthal, S.A.; Amin, M.B.; Gomella, L.G.; Purdy, J.A.; Sartor, A.O.; Michalski, J.M.; Garzotto, M.G.; Bergom, C.; et al. The Influence of the Pretreatment Immune State on Response to Radiation Therapy in High-Risk Prostate Cancer: A Validation Study from NRG/RTOG 0521. *Int. J. Radiat. Oncol. Biol. Phys.* **2022**, *114*, 266–274. [CrossRef]
159. Murphy, J.F.; Fitzgerald, D.J. Vascular endothelial growth factor induces cyclooxygenase-dependent proliferation of endothelial cells via the VEGF-2 receptor. *FASEB J.* **2001**, *15*, 1667–1669. [CrossRef]
160. Ferrara, N.; Gerber, H.P.; LeCouter, J. The biology of VEGF and its receptors. *Nat. Med.* **2003**, *9*, 669–676. [CrossRef]
161. Gerber, H.P.; Dixit, V.; Ferrara, N. Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. *J. Biol. Chem.* **1998**, *273*, 13313–13316. [CrossRef]
162. Braicu, E.I.; Fotopoulou, C.; Chekerov, R.; Richter, R.; Blohmer, J.; Kümmel, S.; Stamatian, F.; Yalcinkaya, I.; Mentze, M.; Lichtenegger, W.; et al. Role of serum concentration of VEGFR1 and TIMP2 on clinical outcome in primary cervical cancer: Results of a companion protocol of the randomized, NOGGO-AGO phase III adjuvant trial of simultaneous cisplatin-based radiochemotherapy vs. carboplatin and paclitaxel containing sequential radiotherapy. *Cytokine* **2013**, *61*, 755–758. [CrossRef]
163. Hu, X.; Xing, L.; Wei, X.; Liu, X.; Pang, R.; Qi, L.; Song, S. Nonangiogenic function of VEGF and enhanced radiosensitivity of HeLa cells by inhibition of VEGF expression. *Oncol. Res.* **2012**, *20*, 93–101. [CrossRef]
164. Fekete, N.; Erle, A.; Amann, E.M.; Fürst, D.; Rojewski, M.T.; Langonné, A.; Sensebé, L.; Schrezenmeier, H.; Schmidtke-Schrezenmeier, G. Effect of high-dose irradiation on human bone-marrow-derived mesenchymal stromal cells. *Tissue Eng. Part C Methods* **2015**, *21*, 112–122. [CrossRef]
165. Fu, Z.Z.; Sun, X.D.; Li, P.; Zhang, Z.; Li, G.Z.; Gu, T.; Shao, S.S. Relationship between serum VEGF level and radiosensitivity of patients with nonsmall cell lung cancer among asians: A meta-analysis. *DNA Cell Biol.* **2014**, *33*, 426–437. [CrossRef]
166. Hu, J.J.; Urbanic, J.J.; Case, L.D.; Takita, C.; Wright, J.L.; Brown, D.R.; Langefeld, C.D.; Lively, M.O.; Mitchell, S.E.; Thakrar, A.; et al. Association Between Inflammatory Biomarker C-Reactive Protein and Radiotherapy-Induced Early Adverse Skin Reactions in a Multiracial/Ethnic Breast Cancer Population. *J. Clin. Oncol.* **2018**, *36*, 2473–2482. [CrossRef]
167. Canada, J.M.; Thomas, G.K.; Trankle, C.R.; Carbone, S.; Billingsley, H.; Van Tassell, B.W.; Evans, R.K.; Garten, R.; Weiss, E.; Abbate, A. Increased C-reactive protein is associated with the severity of thoracic radiotherapy-induced cardiomyopathy. *Cardio-Oncol.* **2020**, *6*, 2. [CrossRef]
168. Bower, J.E.; Ganz, P.A.; Tao, M.L.; Hu, W.; Belin, T.R.; Sepah, S.; Cole, S.; Aziz, N. Inflammatory biomarkers and fatigue during radiation therapy for breast and prostate cancer. *Clin. Cancer Res.* **2009**, *15*, 5534–5540. [CrossRef]

169. Bunda, S.; Zuccato, J.A.; Voisin, M.R.; Wang, J.Z.; Nassiri, F.; Patil, V.; Mansouri, S.; Zadeh, G. Liquid Biomarkers for Improved Diagnosis and Classification of CNS Tumors. *Int. J. Mol. Sci.* **2021**, *22*, 4548. [CrossRef]
170. Yan, Y.Y.; Guo, Q.R.; Wang, F.H.; Adhikari, R.; Zhu, Z.Y.; Zhang, H.Y.; Zhou, W.M.; Yu, H.; Li, J.Q.; Zhang, J.Y. Cell-Free DNA: Hope and Potential Application in Cancer. *Front. Cell Dev. Biol.* **2021**, *9*, 639233. [CrossRef]
171. Anker, P.; Mulcahy, H.; Chen, X.Q.; Stroun, M. Detection of circulating tumour DNA in the blood (plasma/serum) of cancer patients. *Cancer Metastasis Rev.* **1999**, *18*, 65–73. [CrossRef]
172. Raja, R.; Kuziora, M.; Brohawn, P.Z.; Higgs, B.W.; Gupta, A.; Dennis, P.A.; Ranade, K. Early Reduction in ctDNA Predicts Survival in Patients with Lung and Bladder Cancer Treated with Durvalumab. *Clin. Cancer Res.* **2018**, *24*, 6212–6222. [CrossRef]
173. Phallen, J.; Sausen, M.; Adleff, V.; Leal, A.; Hruban, C.; White, J.; Anagnostou, V.; Fiksel, J.; Cristiano, S.; Papp, E.; et al. Direct detection of early-stage cancers using circulating tumor DNA. *Sci. Transl. Med.* **2017**, *9*, eaan2415. [CrossRef]
174. Kageyama, S.I.; Nihei, K.; Karasawa, K.; Sawada, T.; Koizumi, F.; Yamaguchi, S.; Kato, S.; Hojo, H.; Motegi, A.; Tsuchihara, K.; et al. Radiotherapy increases plasma levels of tumoral cell-free DNA in non-small cell lung cancer patients. *Oncotarget* **2018**, *9*, 19368–19378. [CrossRef]
175. Rostami, A.; Lambie, M.; Yu, C.W.; Stambolic, V.; Waldron, J.N.; Bratman, S.V. Senescence, Necrosis, and Apoptosis Govern Circulating Cell-Free DNA Release Kinetics. *Cell Rep.* **2020**, *31*, 107830. [CrossRef]
176. Tie, J.; Cohen, J.D.; Lahouel, K.; Lo, S.N.; Wang, Y.; Kosmider, S.; Wong, R.; Shapiro, J.; Lee, M.; Harris, S.; et al. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. *N. Engl. J. Med.* **2022**, *386*, 2261–2272. [CrossRef]
177. Tie, J.; Wang, Y.; Tomasetti, C.; Li, L.; Springer, S.; Kinde, I.; Silliman, N.; Tacey, M.; Wong, H.L.; Christie, M.; et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. *Sci. Transl. Med.* **2016**, *8*, 346ra92. [CrossRef]
178. Cho, W.K.; Lee, J.; Youn, S.M.; Oh, D.; Lim, D.H.; Yoon, H.G.; Cho, E.H.; Noh, J.M. Liquid biopsy using cfDNA to predict radiation therapy response in solid tumors. *Radiat. Oncol. J.* **2023**, *41*, 32–39. [CrossRef]
179. Nakamura, M.; Kageyama, S.I.; Hirata, H.; Tochinai, T.; Hojo, H.; Motegi, A.; Kanai, A.; Suzuki, Y.; Tsuchihara, K.; Akimoto, T. Detection of Pretreatment Circulating Tumor DNA Predicts Recurrence after High-Dose Proton Beam Therapy for Early-Stage Non-Small Cell Lung Cancer. *Int. J. Radiat. Oncol. Biol. Phys.* **2023**, *116*, 1085–1090. [CrossRef]
180. Chaudhuri, A.A.; Lovejoy, A.F.; Chabon, J.J.; Newman, A.; Stehr, H.; Merriott, D.J.; Carter, J.N.; Azad, T.D.; Padda, S.; Gensheimer, M.F.; et al. Circulating Tumor DNA Analysis during Radiation Therapy for Localized Lung Cancer Predicts Treatment Outcome. *Int. J. Radiat. Oncol. Biol. Phys.* **2017**, *99*, S1–S2. [CrossRef]
181. McLaren, D.B.; Aitman, T.J. Redefining precision radiotherapy through liquid biopsy. *Br. J. Cancer* **2023**, *129*, 900–903. [CrossRef]
182. Tang, L.; Wei, F.; Wu, Y.; He, Y.; Shi, L.; Xiong, F.; Gong, Z.; Guo, C.; Li, X.; Deng, H.; et al. Role of metabolism in cancer cell radioresistance and radiosensitization methods. *J. Exp. Clin. Cancer Res.* **2018**, *37*, 87. [CrossRef]
183. Fukui, R.; Saga, R.; Matsuya, Y.; Tomita, K.; Kuwahara, Y.; Ohuchi, K.; Sato, T.; Okumura, K.; Date, H.; Fukumoto, M.; et al. Tumor radioresistance caused by radiation-induced changes of stem-like cell content and sub-lethal damage repair capability. *Sci. Rep.* **2022**, *12*, 1056. [CrossRef]
184. Busato, F.; Khouzai, B.E.; Mognato, M. Biological Mechanisms to Reduce Radioresistance and Increase the Efficacy of Radiotherapy: State of the Art. *Int. J. Mol. Sci.* **2022**, *23*, 10211. [CrossRef]
185. Cristiano, S.; Leal, A.; Phallen, J.; Fiksel, J.; Adleff, V.; Bruhm, D.C.; Jensen, S.; Medina, J.E.; Hruban, C.; White, J.R.; et al. Genome-wide cell-free DNA fragmentation in patients with cancer. *Nature* **2019**, *570*, 385–389. [CrossRef]
186. Mireștean, C.C.; Iancu, R.I.; Iancu, D.P.T. Simultaneous Integrated Boost (SIB) vs. Sequential Boost in Head and Neck Cancer (HNC) Radiotherapy: A Radiomics-Based Decision Proof of Concept. *J. Clin. Med.* **2023**, *12*, 2413. [CrossRef]
187. Peng, Z.; Wang, Y.; Wang, Y.; Jiang, S.; Fan, R.; Zhang, H.; Jiang, W. Application of radiomics and machine learning in head and neck cancers. *Int. J. Biol. Sci.* **2021**, *17*, 475–486. [CrossRef]
188. Logotheti, S.; Georgakilas, A.G. More than Meets the Eye: Integration of Radiomics with Transcriptomics for Reconstructing the Tumor Microenvironment and Predicting Response to Therapy. *Cancers* **2023**, *15*, 1634. [CrossRef]
189. Zhang, Y.; Qiu, L.; Ren, Y.; Cheng, Z.; Li, L.; Yao, S.; Zhang, C.; Luo, Z.; Lu, H. A meta-learning approach to improving radiation response prediction in cancers. *Comput. Biol. Med.* **2022**, *150*, 106163. [CrossRef]
190. Liu, J.; Han, M.; Yue, Z.; Dong, C.; Wen, P.; Zhao, G.; Wu, L.; Xia, J.; Bin, Y. Prediction of Radiosensitivity in Head and Neck Squamous Cell Carcinoma Based on Multiple Omics Data. *Front. Genet.* **2020**, *11*, 960. [CrossRef]
191. Manem, V.S.K. Development and validation of genomic predictors of radiation sensitivity using preclinical data. *BMC Cancer* **2021**, *21*, 937. [CrossRef]
192. Du, Z.; Zhang, X.; Tang, Z. More evidence for prediction model of radiosensitivity. *Biosci. Rep.* **2021**, *41*, BSR20210034. [CrossRef]
193. Zeng, Z.; Luo, M.; Li, Y.; Li, J.; Huang, Z.; Zeng, Y.; Yuan, Y.; Wang, M.; Liu, Y.; Gong, Y.; et al. Prediction of radiosensitivity and radiocurability using a novel supervised artificial neural network. *BMC Cancer* **2022**, *22*, 1243. [CrossRef]
194. Available online: https://www.energy.gov/articles/candle-illuminates-new-pathways-fight-against-cancer (accessed on 27 September 2023).
195. National Academies of Sciences, Engineering, and Medicine; Division on Earth and Life Studies; Nuclear and Radiation Studies Board; Committee on Developing a Long-Term Strategy for Low-Dose Radiation Research in the United States. *Leveraging Advances in Modern Science to Revitalize Low-Dose Radiation Research in the United States*; National Academies Press (US): Washington, DC, USA, 2022.

196. Lee, Y.; Wang, Q.; Shuryak, I.; Brenner, D.J.; Turner, H.C. Development of a high-throughput γ-H2AX assay based on imaging flow cytometry. *Radiat. Oncol.* **2019**, *14*, 150. [CrossRef]
197. Mahmoud, A.S.; Abdalla, O.M.; Ali, A.A.; Hassan, N.M.; Yousif, A.A.; Elbashir, F.E.; Omer, A.; Mussa, R.; Hassan, A.M. Status of H2AX and TP53 (Non-phosphorylation) for DNA Damage in Cancer Patients on Radiotherapy-A Case-Control Study. *Health Sci.* **2022**, *11*, 51–56.