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FLASH-RT: Using High-Dose Radiation for Clinical Radiation Therapy

By

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An Undergraduate Honors Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of

Bachelor of Science

in

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and

Biology

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Abstract

This paper is a literature review on the possible mechanisms behind the FLASH effect and why such research can advance the world of radiology treatment by modifying current clinical linear accelerators to produce ultra-high doses of radiation. Radiation Therapy, also known as external Beam Radiation Therapy, is a common type of cancer treatment. Globally, cancer is the second-leading cause of death, but has seen an increase in survival rates over the past couple of years. Cancer can develop in almost any part of the body since *cancer* is defined as uncontrolled cell growth. The FLASH Effect is used when treating a tumor with ultra-high dose radiation (UHDR).One of the advantages of the FLASH Effect is it spares healthy tissue during treatment. The sparring of healthy tissue perplexed researchers (and still does) as to why this phenomenon occurs. It is hypothesized that oxygen depletion, the role of the immune system, and interactions with the cell's mitochondria are the reasons why healthy tissue is spared via the use of UHDR. Rat studies and as of this paper, two human studies, have been conducted to analyze the FLASH effect. It has been seen that FLASH has consistently spared healthy tissue and has treated tumor cases that have not previously been treatable with standard treatment.

Introduction

Radiation Therapy, also known as External Beam Radiation Therapy, is a common type of cancer treatment. Globally, cancer is the second-leading cause of death, but has seen an increase in survival rates over the past couple of years (Mayo Foundation, 2022). As of recent times, physicians have seen an increase in the number of patients diagnosed with cancer in the United States. The reason for this is due to (1) the growth and age of its population and (2) the advancements in medical technology that allow for earlier detection times (Miller K.D et al., 2022). As of January 1st, 2022, there were 18 million living Americans with a history of cancer. 53% of that population was diagnosed in the past ten years, with 65% being over 65. Alongside that, the three (3) most prevalent cancer types were identified to be breast cancer (22.5%), prostate (19.6%), and melanoma of the skin (4.2%) (Miller K.D et al., 2022).

Cancer can develop in almost any part of the body since it is characterized as uncontrolled cell growth. The human body consists of trillions of cells produced through cellular mitosis, a process resulting in the formation of two genetically identical daughter cells. Normally, the body typically goes through this process to replace old or damaged cells. However, errors can arise during cellular mitosis. Damaged and or mutated cells typically undergo a process called apoptosis, programmed cell death, to prevent their reproduction. Cancer cells then contain a mutation that bypasses the apoptosis selection process. The most commonly mutated gene in cancer cells is P53 (or mutp53), a tumor suppressor gene. Its mutated form, mutp53, is inhibited or “turned off” and leads to tumorigenesis (tumor growth) (Chen X. et al., 2022).

As cancer development deals with a mutation in one’s DNA, it can be assumed that cancer is a genetic disease. However, inherited mutations from one’s parents only account for a small percentage of cancers. The majority of cancer cases occur after birth and heavily depend on one’s environment and lifestyle, such as radiation exposure (UV Rays from the sun), viruses, carcinogens, smoking, hormones, and obesity (Mayo Foundation, 2022). This complexity makes it challenging for researchers to find a cure. Additionally, cancer can occur in almost any part of the body, so it is not limited to a localized position. Despite these challenges, significant

advancements in technology have improved cancer detection and treatment, including the use of "FLASH Radiotherapy" or "Ultra-High Dose Radiation Therapy".

Background

Radiation Therapy

To gain a better understanding of what exactly radiation therapy is and how it works, it is important to delve into its nature and functionality. As previously mentioned, radiation therapy is a common type of treatment for cancer that uses radiation to target and eliminate tumor cells that can be located almost anywhere in the body. Exploring the origins of radiation therapy provides us valuable context.

The genesis of this therapeutic approach started in 1896 with the discovery of a new type of radiation by a German physician by the name of Wilhelm Conrad Roentgen. Coining this new discovery as "X-Ray," with the "X" symbolizing an algebraic representation of an unknown quantity (ACS, 2014). Within three months, the medical community started utilizing this new high-energy ray for X-ray diagnosis, and then for cancer treatment in the following three years. However, as the early stages of X-ray experimentation progressed, it was also noted that these same rays could cause cancer.

Radiation

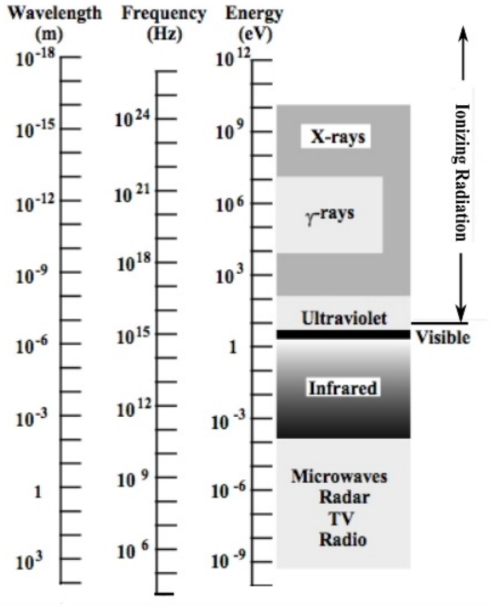

Figure 1 | Electromagnetic spectrum. Note this figure is consistent with the nomenclature used in the medical community, where γ-rays are high-energy photons originating from the nucleus of an unstable atom. All other high-energy photons are classified as X-rays.[1]

[1]Adapted from https://commons.wikimedia.org/wiki/File:Dr1_emSpectrum.jpg

Radiation can be defined as the transport of energy using particles such as electrons, protons, neutrons, and photons. The energies associated with radiation vary from low to high energies, ranging from radio waves to gamma waves. The waves typically seen in the lower region tend to have lower frequencies and longer wavelengths, for example, radio waves,

microwaves, and infrared (IR) would fall under this category. Radio waves are typically used for AM and FM radio, microwaves are used for radar systems, and IR for thermal motion. As for high energy waves, they tend to have higher frequencies and shorter wavelengths. Ultraviolet (UV) radiation typically comes from the sun and has cancer causing capabilities, much like most high energy radiation. X-rays, the topic of this paper, is commonly used to treat cancer due to its ability to damage the rapidly dividing tumor cells and the ability to penetrate deep into the skin. Lastly, the most energetic form of radiation would be gamma waves which are typically used for nuclear medicine. However, radiation is like a double-edged sword. On the one hand, it could damage and eliminate tumors/cancer cells, but on the other hand, it could also damage healthy cells, leading to mutations and, ultimately, cancer. With this in mind, radiation therapy has gone to great lengths to minimize unnecessary radiation exposure to both patient and provider.

Treatment

Radiation therapy is a widely chosen treatment option due to its notable success rate and non-invasive nature. After a patient's initial cancer diagnosis, they are provided with a healthcare team to help plan their treatment accordingly. The specifics of the treatment depend on three crucial factors: (1) the severity of the disease, (2) the location(s) of cancer, (3) and the condition of the patient (how radiation exposure might affect them). To precisely determine the tumor's location, healthcare providers order CT (computed tomography) scans, which provide detailed information about its depth and size. Once the tumor is located, a simulation process is conducted. This involves placing the patient inside a linear accelerator (LINAC), a machine used for delivering radiation treatment, to explore various positions that prioritize comfort while also targeting the tumor. Simulations are also utilized to aid in the measurement and creation of restraints that prevent uncalculated movement done by the patient (Mayo Foundation, 2022)

The FLASH Effect

Discovery

The FLASH Effect is used when treating a tumor with ultra-high dose radiation (UHDR). The initial discovery of this phenomenon was documented in 1959 by Dewey and Boag during a study involving irradiating bacteria with 1000 rads/min where rads/min is the unit dose of radiation per minute. As a reference, 1 rad is equivalent to 10^{-2} Gy or 1 cGy (FlipItPhysics, 2014) Through *in vitro* studies, they observed that bacteria treated with high doses of radiation administered over short durations protected the bacteria more than conventional radiation dose rates. Future rat studies involving the FLASH effect noted some key differences in these two treatment types (Lin B et al., 2021). UHDR spared healthy tissue surrounding the tumor and reduced treatment time significantly (Institut Curie, 2022). To provide some perspective, conventional radiation therapy (CONV) typically administers radiation to a patient for about 1 Gray (Gy) per second (Gy/s), where 1 Gray represents 1 joule of energy being deposited into 1 kg of tissue (FlipItPhysics, 2014). This dose rate would have an average treatment time of several months, depending on the tumor's location and severity. On the other hand, UHDR would reduce that administration time tenfold and treat patients with 10+ Gy/s in one-tenth of a second.

Current Understanding

The groundbreaking research conducted in 1959 served as a catalyst that inspired more elaborate experiments on the FLASH Effect in 2014. It was here that it was dubbed "Flash Radiotherapy" or "FLASH-RT," the names stemming from its rapid dose rates. Initially, these

experiments focused on treating tumors in rats. The sparring of healthy tissue puzzled researchers (and still does) as to why this phenomenon occurs. It sparked significant additional research and experimentation aiming to unravel its biological workings. Within the sections of this paper, we will delve into some of the prevailing theories and discuss the potential of FLASH-RT as a substantial advancement in radiation therapy and cancer treatment.

Methods

Finding Relevant Scholarship

As this is a literature review, finding articles related to the FLASH Effect, Radiation Therapy, and LINACS, I used those as "keywords" when searching peer-reviewed databases/journals such as *Medical Physics*, *JAMA Oncology*, and *Clinical Oncology*. The articles found within those databases must contain the keywords "FLASH", "FLASH-RT", "LINAC", "UHDR", and or "Radiotherapy". Some papers were omitted due to accessibility issues in receiving the full article while others were omitted due to lack of relevance to the subjects of this paper, such as "dosimetry rates" and "image guidance". These subjects are important to further understand the exact treatment of a patient, however, these subjects will not be explored within this paper. FLASH is a relatively new subject, so only newer studies were permitted for general use (2014-2023) as some information stretching further back could potentially be invalidated given the more in-depth and current research.

Findings of Literature Review

Clinical Radiation Therapy Linear Accelerators

Linear Accelerators

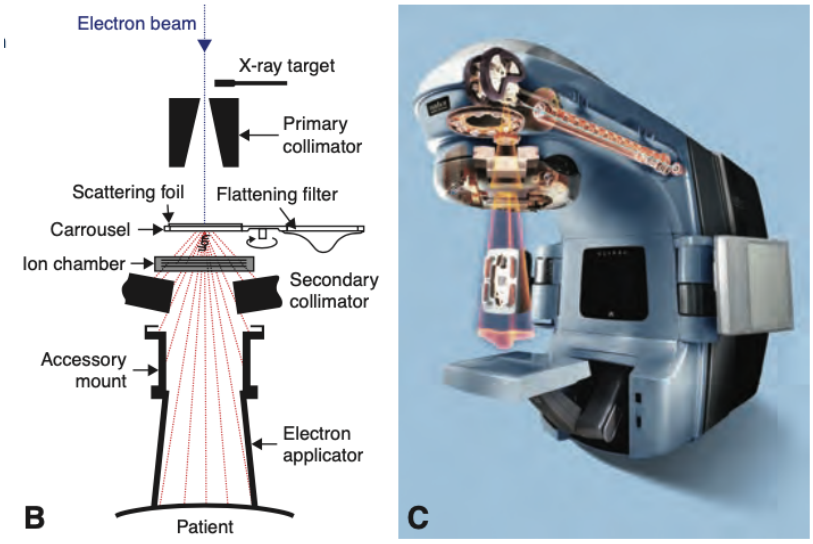

Figure 2 | LINAC Schematics. Karzmark CJ, Morton rJ. *A Primer on Theory and Operation of Linear Accelerators in Radiation Therapy.* Rockville, MD: U.S. Department of Health and Human Services, Bureau of radiological Health; 1981).

In order to achieve the FLASH Effect in a clinical/experimental setting, researchers realized that one couldn't produce the effects with conventional radiation therapy methods. Conventional radiation therapy utilizes high-energy radiation to target and eliminate cancerous tumors in various body parts. These high-energy waves are produced through a linear accelerator (LINAC). However, the capabilities of LINAC machines can only do so much in producing high-energy waves in terms of duration and quantity. To provide a clearer understanding of the machines, let us explore the fundamental components of a conventional LINAC and how those components need to be modified to achieve the desired FLASH Effect.

Components of a Convention LINAC

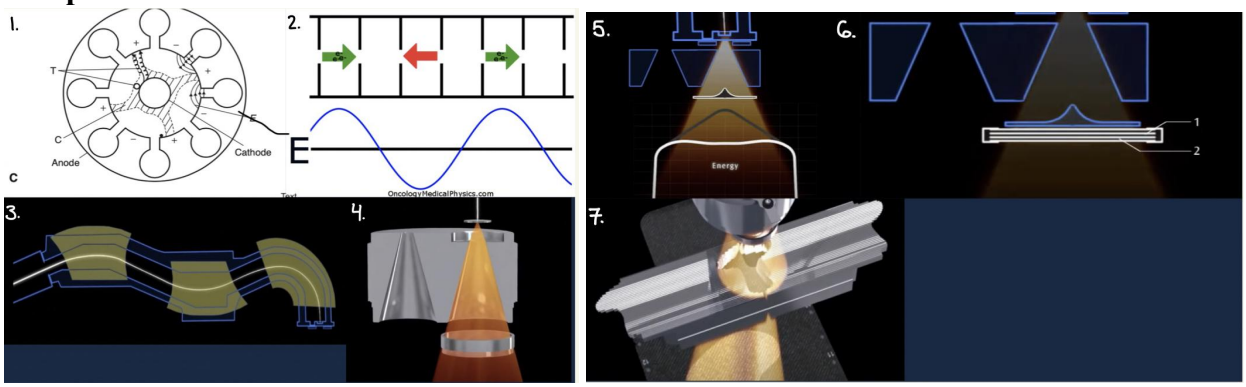

Figure 3 | **(1)** Cross-sectional diagram showing the principle of magnetron operation (BRH, 1981). **(2)** Standing wave diagram (Oncology Medical Physics, 2017). **(3)** Flight Tube diagram. **(4)** Primary collimator. **(5)** Flattening Filter. **(6)** Ion chamber. **(7)** Multi-Leaf Collimator. (Biomedical Engineers TV, 2022).

A conventional LINAC consists of nine (9) different components: an electron gun, magnetron, waveguide, Flight Tube, Tungsten Target, Primary Collimator, Flattening Filter, Ion Chamber, and Multi-Leaf Collimator.

Electron Gun

The electron gun is the central component of a LINAC machine and plays a crucial role in generating X-rays. As the name suggests, the electron gun produces electrons that are accelerated in order to produce X-rays. Electrons are then generated using a metal filament (usually tungsten, given the extreme negative charge that occurs when it's heated) is placed in between an anode and a positive and negative terminal. These terminals will heat the metal to an extreme temperature using voltage from an external power source, making the metal filament increasingly hot. The negative charges (electrons, e^-) start to bounce off of the metal via *thermionic emission*. The anode and/or positively charged plate will attract electrons from the heated filament. It would increase speed (increased kinetic energy) and be ready to be pushed toward the next step, the magnetron (Khan, Faiz M, 2014).

Magnetron

The magnetron is a cylindrical tube surrounding a cathode with empty cavities integrated into the anode of the tube. The primary function is to generate radio waves, which aid in the further acceleration of electrons produced by the electron guns. For the production of high-energy beams like X-ray, the electrons must reach high velocities. The further acceleration

enables the electrons to reach such speeds, marking this step essential as the electron gun alone cannot accelerate the electrons sufficiently to generate ionizing radiation (Khan, Faiz M, 2014).

In the magnetron, the electrons emitted by the electron gun are placed into an electric and magnetic field. These combined forces establish a circular path for the electrons to travel. Referring to **Figure 4 (1)**, we can see that the electrons start to circulate the cathode and begin to bounce within the surrounding cavities of the anode. These collisions within those cavities produce microwaves that would then be ejected into a waveguide to push and maximize the velocity of the electrons as it travels toward the tungsten target (Khan, Faiz M, 2014).

Waveguide

The waveguide serves as a lengthy tube designed to guide and maintain the trajectory of electrons until they reach the point of contact with the tungsten target. The interactions between the high-energy electrons and the tungsten target generate X-rays.The waveguide is then composed of multiple components: the steering coils, focusing coils, copper cells, and a water cooling system. As the electrons are ejected from the electron gun and propelled into the waveguide along with the microwaves produced from the magnetron, they undergo further interaction with an electromagnetic field produced by the focusing and steering coils, leading to a sinusoidal effect (Khan, Faiz M, 2014).

The sinusoidal effect arises when the microwaves from the magnetron produce standing waves within the waveguide. These standing waves can only change their amplitude (the height of the wave) and not their position, unlike traveling waves. In this case, standing waves are preferred as traveling waves would require a longer waveguide given their change in position. For simplicity and compactness, the standing waves would allow for smaller machines and reduce the need for another component called the coupling chambers (Khan, Faiz M, 2014).

Flight Tube

A flight tube serves as an additional component commonly found in LINACs, playing a role to help narrow the high-velocity electron beam further before it reaches the tungsten target. This component consists of three magnets placed diagonally from each other, enabling the electron beam to be narrowed down to about 1 mm in diameter (Khan, Faiz M, 2014).

Tungsten Target & Primary Collimator

Finally, the electron beam can optimally strike the target to produce the desired X-rays for clinical treatment. This portion of the LINAC does not necessarily have to be tungsten, but this metal is commonly used as the electrons need to hit a high atomic number metal. This is important for production of X-rays using electrons via *Bremsstrahlung*, where the high-speed electrons decelerate around the nuclei of the high atomic number metal, resulting in the emission of energy in the form of X-rays. This energy is then propagated through space via the electromagnetic field. As the electron approaches the nucleus, it is then deflected, with a portion of its energy being converted into a photon (Khan, Faiz M, 2014).

Upon hitting the tungsten target, the generated photons are scattered in all directions. The job of the primary collimator is to catch only forward-facing rays, the rays traveling downward to strike the patient. This component, typically a metal ring, shapes the photons into a cone-shaped beam. By doing so, this helps prevent the leakage of radiation and helps reduce additional unnecessary exposure to the patient and medical team.

Flattening Filter & Ion Chamber

The flattening filter is another preventative component to help reduce unnecessary radiation exposure further by narrowing the photon beam, making it more uniform, and absorbing any other photon rays. This narrowed beam would then travel into the ion chamber.

The ion chamber consists of three (3) individual chambers. The first chamber, also called the *Primary Dosimeter*, allows the radiologist and or medical team to control and monitor the beam quality simultaneously. The second chamber, the *Secondary Dosimeter*, is a backup if chamber one fails. Lastly, the third chamber consists of seven (7) different electrodes that can monitor different sections of the radiation field to help replicate the beams modeled in the medical team's planning system before patient treatment (Khan, Faiz M, 2014).

Multi-leaf collimator

Before the photons are ready to strike the patient; they must go through one more component within the LINAC. The multi-leaf collimator eventually shapes the beam to the exact form needed to match the tumor's shape. This component comprises fine tungsten or other high atomic number metal leaves to create the complex treatment shape (Khan, Faiz M, 2014).

FLASH-RT LINAC Modifications

Normal CONV-RT does not have the capability to generate high enough dose rates to produce the FLASH Effect. To put it into perspective, CONV-RT uses around 45-60 Gy/min or 0.75-1.0 Gy/s while FLASH uses upwards of >10 Gy/s. These clinical linacs cannot (1) produce that much radiation and (2) administer that much radiation in such a small window. Thus, in order for researchers to study the FLASH Effect, they must first modify the irradiation and the parameters of a CONV linac. As of now, there are only two companies that are authorized to produce such machines. Varian Medical Systems and IntraOP are the only manufacturers of FLASH-related linacs. With that, only certified studies and centers are allowed to use such machines as they are highly complex, expensive, and hard to come by (IntraOp, 2023).

To enable clinical linacs to achieve the FLASH Effect, several modifications are required. These include controlling pulse on a pulse-to-pulse basis (given the high dose rate), software modifications, and changes in filament current (Lempart et al., 2019). Starting with the pulse control, a change in the in-house built electrical current was needed. For example, the electrical control unit used for some of the Varian machines is a PIN-type, EDD2-3G Diode (Lempart et al., 2019). This device consists of two parts, one being the two-stage amplifier, and the other being a conditioning circuit. The purpose of this diode is for it to act as a mediator between the linac and dose delivery. The amplifier is mainly used for detection in part to convert photocurrents into an input signal for the linac to detect. Once this signal is detected, the linac is able to stop its beam pulses since the input signal interrupts and pins inside a microcontroller unit. This step is important to make sure that radiologists are able to consistently deliver the correct dosage to patients without bombarding them with unnecessary amounts of radiation (Lempart et al., 2019).

For the software modification, clinical linacs have restrictive codes in them to make sure CONV-RT treatments stay within a set window that could potentially make the machine unstable. But for FLASH, these windows need to be extended in order to produce higher doses of radiation. Normal coding allows the ionization chamber to monitor beam currents and to control the electron gun, however, the control on the electron gun has a set parameter that is too small to produce the FLASH Effect (Lempart et al., 2019). This led researchers to maximize the output of

electrons via the electron gun by changing and increasing the current flowing through. The current was able to be maximized by changing the flight tube magnets to a combination of 10 MV X-ray and 10 MeV beam energy settings while also increasing the charge rate ever so slightly. However, researchers found that maximizing the electron gun also required a change in the electron gun filament to accommodate such increases. This change depends on the machine, but it was usually a metal with a higher Z than tungsten (Lempart et al., 2019).

Ultimately, these changes allowed for different parameters that allow for greater dose rates. As of the time of this paper, one of the best linacs suited for FLASH treatment is IntrapOP's *Mobetron Clinical Electron IORT FLASH System*. The specs behind the machine consist of being able to produce dose rates greater than 1000 Gy/s, electron energies of 6-9 MV with a field size of ≤ 10 cm, pulse widths of 0.5-4 microseconds, and a pulse repetition frequency of 100-120 Hz. For comparison, the CONV *Mobetron* has specs of 10 Gy/min, electron energies of 6-12 MeV with a field size of ≤ 4 cm, and a frequency of 50-60 Hz (IntraOp, 2023).

The Biological Hypothesis Behind the FLASH Effect

At the time of this paper, there is no solid evidence for why high-dose radiation treatment spares healthy tissue, but researchers continue to test various hypotheses as to why this phenomenon may occur. Some leading theories are oxygen depletion, the role of the immune system, and interactions with the cell's mitochondria.

Oxygen Depletion

Under normal conditions, oxygen is quite sensitive to the effects of radiation. This high-energy radiation can generate free radicals upon the release of an electron from its bond.These free radicals can inflict damage to cellular DNA, although most biological cells possess the ability to repair such damage on their own. However, when these free radicals bind to oxygen molecules, they can induce a change in cellular bonds that hinders the cell's repair mechanisms.

In the context of FLASH-RT, the rapid irradiation of tumor cells creates a hypoxic or oxygen-depleted environment in the surrounding healthy tissues. This hypoxic tissue becomes approximately two to three times more resistant to radiation. It's important to note that even in CONV-RT, where irradiation occurs at a slower dose rate, oxygen consumption still takes place. The key distinction is that FLASH-RT delivers significantly higher dose rates. Nevertheless, some researchers suggest that oxygen depletion may not be the sole mechanism responsible for the sparing of healthy tissues. It is proposed that other factors may contribute, especially considering that not all of the oxygen is consumed during this process, particularly in larger areas of the body (Bogaerts E et al., 2022).

Peroxides

The hypothesis behind the FLASH using oxygen depletion does not fully explain the characteristics seen. Another hypothesis that supports the oxygen depletion theory while also providing further insight is the act of the metabolization of peroxided compounds and the labile iron content that lies between the tumor and healthy tissue (Bogaerts et al, 2022). Peroxides are the chemical compounds that bind two oxygen atoms using a single covalent bond while labile iron is a catalyst for harmful hydroxyl radical production. In turn, peroxides and labile iron are both classified as hydroperoxides and are ultimately toxic to organic tissue. When comparing the metabolism of the peroxides in healthy tissue to that of tumor cells, normal tissues have a higher metabolism and thus lower peroxidized compounds. This, in turn, reduces the amount of labile

iron content in the space between the tumor and the healthy tissue alongside a reduced amount of peroxides (Bogaerts E et al., 2022).

Role of the Immune System

T lymphocytes

T lymphocytes, a type of white blood cell found in the body's innate immune system, play a crucial role in combating disease-causing pathogens, including bacteria, viruses, fungi, and cancer cells. Under CONV-RT, the irritation of tissue would normally cause tissue complications such as fibrosis, the scarring of fibrous tissue in the body. This scarring is a result of the T lymphocytes being killed off from the bombardment of radiation during treatment. The lack of that immune response allows for an inflammatory response that would damage and scar the tissue cells (Bogaerts E et al., 2022). In contrast, FLASH-RT was found to spare those T lymphocytes from destruction and was better at preventing the inflammatory response needed to induce complications in the tissue. Scientists were able to compare the results of T lymphocyte production in irradiated animals for both CONV-RT and FLASH-RT to determine whether or not their hypothesis was correct. They found that irradiated animals under FLASH-RT were found to have less of an inflammatory response and fewer instances of fibrosis while CONV-RT had more instances of each. These data suggest that FLASH-RT spares the T lymphocytes, and would then agree with the hypothesis made by the authors (Bogaerts E et al., 2022).

The authors speculate the reason behind the sparing of the T lymphocytes would be due to the short delivery time of UDHR (<1s). To further support their hypothesis, the authors conducted a computation study where they were able to model the relationship between the radiation dose and the death of the immune cell (Bogaerts E et al., 2022) . The simulation results revealed a significant reduction in the number of dead T lymphocytes in the irradiated tissue. Approximately 95% of the T-cells in CONV-RT using < 5 Gy/min were found dead while only about 5-10% of the T cells were found dead using UHDR at a whopping > 200 Gy/min. This sparing of lymphocytes during an immune response could lead to a more intact immune system following cancer treatment, as CONV-RT patients tend to become immunocompromised and would ultimately become more susceptible to infections and disease (Bogaerts E et al., 2022).

However, the sparing of the T lymphocytes was not the only thing the authors of the study found. As stated previously, these T cells play a key role in providing an anti-tumor response/immunity. Maintaining a healthy amount of cells would ultimately lead to better treatment outcomes as a patient continues radiation treatment. Numerous studies have reported a strong association between the lack of T lymphocytes and the severity of tumor progression with the survival of the patient with solid tumors. Vice versa, it was also strongly suggested that there is a positive correlation between the higher number of T cells and tumor regression (Bogaerts E et al., 2022).

Expression of TGF-β

Besides the hypothesis with the sparing of T lymphocytes, it was also speculated that there would be an increase in healthy tissue protection during FLASH-RT due to the altered expression of certain cytokines. Cytokines are a kind of small protein that are important for cell signaling within the body. These proteins work specifically with the immune system to help control inflammatory responses to help the body defend against foreign invaders, much like T lymphocytes.

The specific cytokine in question is the transforming growth factor a beta (TGF-β), a multifunctional product produced by all white blood cells and is specific to growth and development, inflammation and repair, and host immunity response. TGF-β was chosen due to it being typically elevated during radiation-induced DNA damage and is in response to tissue injury. Since this form of cytokine does the opposite effect of T lymphocytes in producing/preventing inflammation and tissue scarring, it was rightly found that TGF-β had reduced activation during multiple studies of FLASH-RT (Bogaerts E et al., 2022). During a study by Buonanno et al., they saw a significant reduction in TGF-β expression when exposing 1000 Gy/s of UHDR to human lung fibroblasts. When compared to CONV-RT, there was a 1.8-fold increase in TGF-β expression with UDHR and a 6.5-fold increase with conventional. This indicated that FLASH-RT could have preventative measures that moderate the induction of TGF-β markers that code for the radiation-induced inflammatory response.

TGF-β also acts as a tumor suppressor during the early stages of cancer development by promoting apoptosis and *inhibiting* the cells' ability to pass through the cell cycle. However, TGF-β acts as a tumor *promoter* during late-stage cancer by promoting cell proliferation, invasion, metastasis, and immune suppression. This late-stage version allows malignant cells to avoid the growth-suppression effects and would ultimately give them a selective growth advantage, making them more radioresistant. If the whole TGF-β hypothesis were to be true, the reduced expression of this specific cytokine could make tumor cells more radiosensitive and would make it easier to treat and or control (Bogaerts E et al., 2022).

Interactions with the Cell's Mitochondria

A more recent hypothesis behind why FLASH-RT spares healthy tissues is the role of the mitochondria's mediated inflammation and apoptosis abilities. It is speculated that these responses for the cell's mitochondria are reduced under the use of FLASH-RT. For context, the mitochondria are considered the "powerhouse of the cell" due to their ATP production, and consumption but also play a role in calcium homeostasis, cell signaling, and apoptosis (Bogaerts E et al., 2022).

However, recent studies have shown that the mitochondria are actually major contributors to the body's innate immune system and inflammatory functionalities. These organelles contain mitochondrial DNA (mtDNA), and when their cells are exposed to ionizing radiation, an excess of intracellular ROS (mtROS), a reactive oxidative species, occurs. MtROS serve as signaling molecules for cell growth and survival, so having an excess amount of mtROS could lead to severe and irreversible damage to the cell's mitochondria given prolonged cell growth (Bogaerts E et al., 2022). This damage would eventually lead to a compromised external membrane, and would then allow molecules that would not normally be able to pass through to go inside the cell. Essential proteins within the mitochondria would then be allowed to escape and flow freely from the cell, this process is called MOMP (mitochondrial outer membrane permeabilization). One such protein would be cytochrome c (cyt c) to be released into the outer matrix/cytosol. The release of such proteins would target the cell for apoptosis. MOMP has also been noted to be important for pro-inflammatory responses. MtDNA is allowed to flow into the cytosol and trigger an inflammatory response (Bogaerts E et al., 2022).

Knowing this now about mitochondria, it has become another hypothesis to explain why FLASH-RT tends to spare healthy tissues surrounding the area of treatment. Since mitochondria are suspected to play a huge role in regulating apoptosis and inflammation, FLASH-RT could potentially reduce the amount of mitochondrial damage in the prevention of apoptosis of healthy

cells caused by the release of proteins, mtROS, and mtDNA, into the cytosol via MOMP, increasing the likelihood of the sparing of healthy tissue (Bogaerts E et al., 2022).

Relevant Studies and Experiments

Rat Studies

Author, year	System	Dose, Gy	Dose rate, Gy/sec	Assay	(Refs.)
Hornsey and Bewley, 1971	Mouse intestine	11.9	17-83	LD50/5	(10)
Field and Bewley, 1974	Mouse foot skin	24	56-83	Early and late reactions	(8)
Hendry *et al*, 1982	Mouse tail skin	50	17-170	Necrosis ND50	(95)
Favaudon *et al*, 2014	Mouse lung	15-17	40-60	Lung fibrosis	(11)
Montay-Gruel *et al*, 2017	Mouse brain	10	100-10^6	Memory tests	(12)
Vozenin *et al*, 2019	Mouse intestine	14.7	70-210	LD50/5 (survival)	(56)
Montay-Gruel *et al*, 2018	Mouse brain	10	37	Neurocognitive tests	(13)
Simmons *et al*, 2019	Mouse brain	30	200/300	Neurocognitive tests	(71)
Montay-Gruel *et al*, 2019	Mouse brain	10	>100	Neurocognitive tests	(14)
Abel *et al*, 2019	Mouse lung	15/17.5/20	40	Survival, dermatitis, breathing function	(96)
Girdhani *et al*, 2019	Mouse lung	15/17.5/20	40	Lung fibrosis, skin dermatitis	(35)
Vozenin *et al*, 2019	Mini-pig skin	22-34	300	Skin toxicity/injury	(5)
Montay-Gruel *et al*, 2019	Zebrafish embryo	8	>100	Morphology	(14)
Alaghband *et al*, 2020	Mouse brain	8	4.4x10^6	Neurocognitive tests	(15)
Fouillade *et al*, 2020	Mouse lung	17	40-60	Cellular proliferation, inflammation	(76)
Levy *et al*, 2020	Mouse abdomen	12-16	216	Crypt cells, stool production, survival, regeneration	(57)
Diffenderfer *et al*, 2020	Mouse abdomen	15	78	Intestinal crypt cell proliferation	(38)
Diffenderfer *et al*, 2020	Mouse intestine	18	78	Fibrosis	(38)
Cao *et al*, 2021	Mouse mammary gland	20	300	Oxygen depletion test	(60)
Liew *et al*, 2021	Mouse skin	30	125	Survival	(32)
Cunningham *et al*, 2021	Mouse skin	15,35	57,115	Plasma and skin levels of TGF-β1 and skin	(97)
Velalopoulou *et al*, 2021	Mouse skin, muscle, bone	30,45	69-124	Survival, histology, pathology	(98)
Montay-Gruel *et al*, 2021	Mouse brain	10-30	1.8x10^6	Survival, neurocognitive tests	(99)

Table 1 | Studies Examining the Effects of Different Modes on FLASH Irradiation on Normal Tissue (Yinghao LV et al., 2022)

As said in the introduction, the FLASH Effect was first documented in 1959, and since then a bunch of rat studies has been conducted to further investigate the phenomenon. The first of these studies occurred in 1971 with 2 others following soon right after in 1974 and 1978. There was then a break in between these initial studies with the next round of rat studies starting back up again in 2014 with additional studies occurring about every other year to more than a few times per year (**Table 1**). Each of these studies examined various dose rates, the location of the tumor, and the effect of the FLASH effect on the mice's physical and mental states.

Studies 1971-1982

The first three documented studies surveying the effects of UHDR occurred between the years 1971 and 1982. This laid the foundation for future research and the hypothesis behind the FLASH Effect that we know today. The Hornsey and Bewley study from 1971 was some of the first documented cases of UHDR sparing healthy tissue while also killing the tumor. Those observations were then seconded by the Field and Bewley study three years later in 1974, but

this time, they gave a hypothesis as to why this phenomenon might be occurring. It was this study that first brought up the idea of tumor hypoxia and the reduction of the radiosensitivity of the various tissues. After these discoveries, research on the FLASH effect came to a halt. This was due in part to the ill success in translating the research to actual clinical applications.

Studies 2014-2021

The research into the FLASH Effect did not pick back up again until 2014 when Favaudon *et al*. started comparing CONV-RT to FLASH-RT in the lung tissue of mice. It was noted in this study that the mice treated with CONV-RT developed severe cases of pneumonia and fibrosis (tissue scarring) while all of the mice treated with FLASH-RT had developed no case of either pneumonia or fibrosis. This, however, was only the case when the authors of the studies treated the UHDR mice with ~17 Gy/s. When they increased it to 30 Gy/s, they noticed that this dose rate started to induce both pneumonia and fibrosis within the mice. The result of that treatment indicates that the dose rate can be heavily dependent on the size of the organism being treated as well as the size of the tumor, so too much can still have negative effects on the body. With that, the Favaudon *et al.* studies also hypothesized that the ~17 Gy/s dose rate might have produced those bodily effects due to the activation of TGF-β. All of the studies following the research done in 2014 have further backed all of the observations seen thus far. This excited researchers with the amount of potential this form of treatment could have on the world of radiology, so much so that they started moving on to human trials. The first study was recorded in 2019 and the second being in 2022.

Human Trials

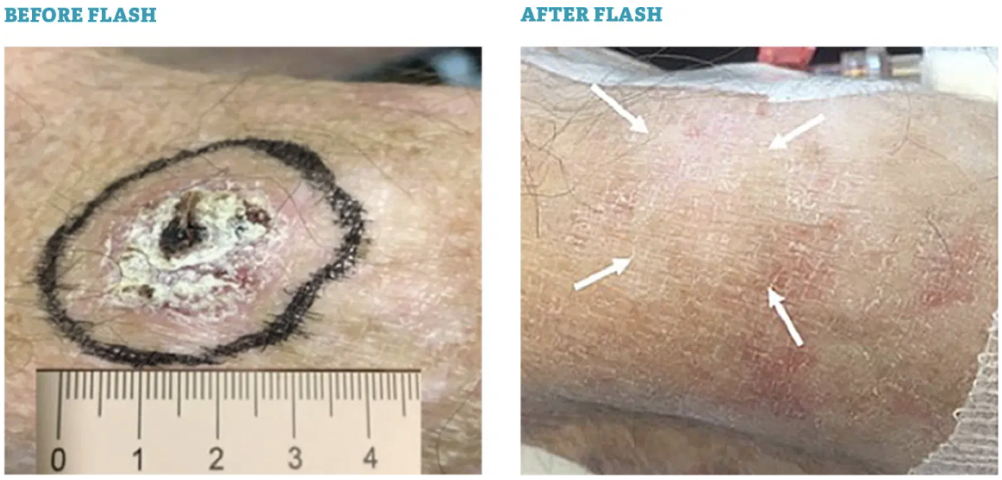

Figure 5 | The first human trial involving a 75-year-old man with multiresistant CD30+ T-Cell cutaneous lymphoma on the surface of the skin (Bouthis et al. Radiation Therapy and Oncology, 2019).

At the time of this paper, there have been two instances of human experimentation using FLASH-RT. The first study occurred in 2019 where only one human patient was experimented on while the second study occurred in 2022 using ten patients. To view whether or not these two instances had successful results, we shall dive into each of them individually.

First Human Trial, 2019

Starting with the first-ever human trial in October of 2019, this study involved a 75-year-old man with a surface-level tumor (**Figure 5**). Scientists opted for a more surface-level tumor as it makes it easier to deliver such high doses of radiation without compromising the patient. Since all of this is purely experimental given this is the first instance of UHDR used on a human, some specific parameters such as age, stage of cancer, position of the tumor, and willingness of the patient were considered when trying to conduct this trial. Another detail taken into account when choosing this patient, was that he responded poorly to clinical radiation therapy. He was treated over 110 times with localized skin radiation treatment and saw continuous tumor growth despite systematic treatment (Bourhis J et al., 2019).

These parameters made this patient an ideal candidate to test whether or not something like UHDR can be effective in humans compared to how effective it has been using rat studies. The method used during this study was to isolate the 3.5 cm diameter tumor and treat it using a linac specifically designed for UHDR. This machine was able to use 5.6 MeV to produce 15 Gy in 90ms. The patient was then treated over the course of three (3) weeks, and the radiologist was able to see a vast improvement, like the one seen in **Figure 5**. These results were ultimately phenomenal! Given how resistant the tumor originally was to clinical radiation therapy, having been treated over 110 times, to see a massive tumor reduction in three (3) weeks is a step in the right direction for potentially advancing the possibilities of care using FLASH-RT. The patient also experienced reduced radiation sickness and increased sparing of healthy tissue (Bourhis J et al., 2019). All of these results were also consistent with the results found using the rat studies. However, despite all of these great implications, this study was only done using a single person. Using a patient requiring such specific parameters while only using one patient makes it difficult to apply such results to the general public. Even so, this is still a step in approving more advanced and broader versions of human trials, such as the second-ever human trial.

Second Human Trial, 2022

This second trial was published in October 2022, nearly exactly three years after the first human trial occurred, and was titled FLASH-01. While the first trial strictly used one patient with defined parameters, this second trial was broader and consisted of ten patients, ranging in age from 27-81 years old. The requirements for this trial consisted of the patients being at least 18 years of age, having up to three (3) painful metastases in their extremities (excluding their hands, wrists, and feet), having no prior radiotherapy to the tumor, and having a life expectancy of more than two (2) months, and then had to have no fractures and/or metal implants in the treatment field. Despite all of these strict parameters, this human trial is still rather broad when compared to the first human trial. This one consists of more people with a broader age range while also having tumors in other areas of the body (Mascia A et al., 2022).

The methods then used to treat these ten patients consisted of ≥40 Gy/s over the course of one day. In total, there were, again, ten patients, but there were a total of twelve metastatic sites provided that qualification for this study stated that a patient could have *up to* three (3) metastases. The average time each patient spent on the treatment couch was 11-33 minutes while the average time per treatment site was 11-22 minutes. Following up on this treatment afterward, researchers contacted these patients for an average of 2.3-13 months (Mascia A et al., 2022). At the conclusion of this study, it was found that eight (8) out of the twelve (12) or 67% of the treatment sites had patient reports of pain relief while six (6) of those eight (8) reported complete response (no pain) (Mascia A et al., 2022).

Future Research

The goal for research regarding the future of FLASH-RT is to start applying those findings to more clinical practices. Again, there have been only two human trials so far, but the goal is to do much more considering the outcome of both studies. Alongside moving into more clinical practices, it is still essential to focus on *in vivo* studies. There is no concrete reason for why UHDR spares healthy tissue and reduces the effects of chemotherapy in patients. Some of the theories provided earlier in this paper are all still speculation, so future studies will still dive into oxygen depletion, peroxides, the role of the immune system, and the role of the mitochondria until they are either disproven, proven, or another hypothesis takes its place.

Conclusion

UHDR is a promising future for the field of radiology. It has the potential to not only decrease treatment time for patients, but it could also reduce chemotherapy symptoms and spare the surrounding tissue from things such as scarring. The work that has been done so far is a big step in the right direction to help lower the number of people dying from cancer all over the globe while also diminishing the harmful effects of such treatment. However, there is still work to be done as there have been only two (2) human trials regarding the FLASH Effect with both of them having rather limiting parameters that make it difficult to apply such results to a broader spectrum of illnesses. As FLASH-modified linacs become more accessible and the knowledge behind the mechanism of FLASH becomes more pronounced, perhaps then such research may be done.

References

Abolfath, R., Grosshans, D. and Mohan, R. (2020), Oxygen depletion in FLASH ultra-high-dose-rate radiotherapy: A molecular dynamics simulation. Med. Phys., 47: 6551-6561. https://doi.org/10.1002/mp.14548

ACS. (2014, June 12). *History of cancer treatments: Radiation therapy*. American Cancer Society. Retrieved from https://www.cancer.org/treatment/understanding-your-diagnosis/history-of-cancer-treatment-radiation.html

Biomedical Engineers TV. (2022). *Linear Accelerators (LINAC) | Biomedical Engineers TV |*. Retrieved from https://www.youtube.com/watch?v=DP70cIFiPEo.

Bogaerts E, Macaeva E, Isebaert S, Haustermans K. Potential Molecular Mechanisms behind the Ultra-High Dose Rate "FLASH" Effect. Int J Mol Sci. 2022 Oct 11;23(20):12109. Doi: 10.3390/ijms232012109. PMID: 36292961; PMCID: PMC9602825.

Boscolo D, Scifoni E, Durante M, Krämer M, Fuss MC. May oxygen depletion explain the FLASH effect? A chemical track structure analysis. Radiother Oncol. 2021 Sep;162:68-75. doi: 10.1016/j.radonc.2021.06.031. Epub 2021 Jun 29. PMID: 34214612.

Bourhis J, Sozzi WJ, Jorge PG, Gaide O, Bailat C, Duclos F, Patin D, Ozsahin M, Bochud F, Germond JF, Moeckli R, Vozenin MC. Treatment of a first patient with FLASH-radiotherapy. Radiother Oncol. 2019 Oct;139:18-22. Doi: 10.1016/j.radonc.2019.06.019. Epub 2019 Jul 11. PMID: 31303340.

C. Crockett, C. Lorimer, OncoFlash – Research updates in a flash! (March edition), Clinical Oncology,Volume 35, Issue 3,2023,Pages 150-152,ISSN 0936-6555, https://doi.org/10.1016/j.clon.2023.01.009.

CERN. (2021). *FLASH An innovative electron radiotherapy technology*. Retrieved from https://www.youtube.com/watch?v=87JLhcsuIao.

Chen, X., Zhang, T., Su, W. *et al.* Mutant p53 in cancer: from molecular mechanism to therapeutic modulation. *Cell Death Dis* 13, 974 (2022). https://doi.org/10.1038/s41419-022-05408-1

El Naqa, I., Pogue, B. W., Zhang, R., Oraiqat, I., & Parodi, K. (2022). Image guidance for FLASH radiotherapy. *Medical Physics*, *49*(6), 4109–4122 .https://doi.org/10.1002/mp.15662

Farr, J., Grilj, V., Malka, V., Sudharsan, S., & Schippers, M. (2022). Ultra‐high dose rate radiation production and delivery systems intended for FLASH. *Medical Physics*, *49*(7), 4875–4911. https://doi.org/10.1002/mp.15659

FlipItPhysics. *Physics in Biomedicine*. MacMillian Learning, 2014.

Friedl, AA, Prise, KM, Butterworth, KT, Montay-Gruel, P, Favaudon, V. Radiobiology of the FLASH effect. *Med Phys*. 2022; 49: 1993– 2013. https://doi.org/10.1002/mp.15184

Gray Cancer Institute. (1996). *The novice's Guide to Electron Linear Accelerators - University of Oxford*. Gray Cancer Institute. https://users.ox.ac.uk/~atdgroup/referencematerial/The%20novices%20guide%20to%20electron%20linear%20accelerators.pdf

Institut Curie. (2021, May 25). *Flash radiotherapy: The excitement of a promising breakthrough*. Institut Curie. Retrieved from https://institut-curie.org/actualite/radiotherapy/flash-radiotherapy-excitement-promising-breakthrough

IntraOp. "Flash Radiotherapy - Ultra-High Dose Rates (UHDR) w/ Electrons." *IntraOp*, 8 May 2023, intraop.com/flash-radiotherapy-electrons/.

IntraOp Medical Corporations . (2021). *FLASH Radiotherapy: The Clinical Translation of Ultra-High Dose Rate Electron Therapy**. Retrieved from https://www.youtube.com/watch?v=d82zm7A2dP0.

Kacem, H., Almeida, A., Cherbuin, N., & Vozenin, M.-C. (2022). Understanding the FLASH effect to unravel the potential of ultra-high dose rate irradiation. *International Journal of Radiation Biology*, *98*(3), 506–516. https://doi.org/10.1080/09553002.2021.2004328.

Karzmark CJ, Morton rJ. *A Primer on Theory and Operation of Linear Accelerators in Radiation Therapy.* Rockville, MD: U.S. Department of Health and Human Services, BRH

Khan, Faiz M. "Chapter 3 - Production of X-Rays." *Khan's the Physics of Radiation Therapy (5th Edition)*, LWW (PE), Philadelphia , PA, 2014, pp. 28–38.

Kit Betts-Masters. (2017). *How does the electron gun work? Thermionic Emission and Particle Acceleration - A Level Physics*. Retrieved from https://www.youtube.com/watch?v=tYCET6vYdYk.

Lin B, Huang D, Gao F, Yang Y, Wu D, Zhang Y, Feng G, Dai T, Du X. Mechanisms of FLASH effect. Front Oncol. 2022 Sep 23;12:995612. doi: 10.3389/fonc.2022.995612. PMID: 36212435; PMCID: PMC9537695.

Lin B, Gao F, Yang Y, Wu D, Zhang Y, Feng G, Dai T, Du X. FLASH Radiotherapy: History and Future. Front Oncol. 2021 May 25;11:644400. doi: 10.3389/fonc.2021.644400. PMID: 34113566; PMCID: PMC8185194.

Lansonneur, P., Favaudon, V., Heinrich, S., Fouillade, C., Verrelle, P., & De Marzi, L. (2019). Simulation and experimental validation of a prototype electron beam linear accelerator for preclinical studies. *Physica Medica*, *60*, 50–57. https://doi.org/10.1016/j.ejmp.2019.03.016

Laurent, Pierre-Antoine, Daphne Morel, Lydia Meziani, Stephane Depil, and Eric Deutsch. "Radiotherapy as a Means to Increase the Efficacy of T-Cell Therapy in Solid Tumors." *OncoImmunology* 12, no. 1 (December 31, 2023): 2158013. https://doi.org/10.1080/2162402X.2022.2158013.

Lempart, Michael, Börje Blad, Gabriel Adrian, Sven Bäck, Tommy Knöös, Crister Ceberg, and Kristoffer Petersson. "Modifying a Clinical Linear Accelerator for Delivery of Ultra-High Dose Rate Irradiation." *Radiotherapy and Oncology* 139 (October 2019): 40–45. https://doi.org/10.1016/j.radonc.2019.01.031.

Lv, Y., Lv, Y., Wang, Z., Lan, T., Feng, X., Chen, H., Zhu, J., Ma, X., Du, J., Hou, G., Liao, W., Yuan, K., & Wu, H. (2022). FLASH radiotherapy: A promising new method for radiotherapy (Review). *Oncology Letters*, *24*(6), 419. https://doi.org/10.3892/ol.2022.13539

Mascia, Anthony E., Emily C. Daugherty, Yongbin Zhang, Eunsin Lee, Zhiyan Xiao, Mathieu Sertorio, Jennifer Woo, et al. "Proton FLASH Radiotherapy for the Treatment of Symptomatic Bone Metastases: The FAST-01 Nonrandomized Trial." *JAMA Oncology*, October 23, 2022. https://doi.org/10.1001/jamaoncol.2022.5843.

Mayo Foundation for Medical Education and Research. (2022, December 7). *Cancer*. Mayo Clinic. Retrieved from https://www.mayoclinic.org/disease-conditions/cancer/symptoms-causes/syc-20370588

Miller, K. D., Nogueira, L., Devasia, T., Mariotto, A. B., Yabroff, K. R., Jemal, A., Kramer, J., & Siegel, R. L. (2022). Cancer treatment and survivorship statistics, 2022. *CA: A Cancer Journal for Clinicians*, *72*(5), 409–436. https://doi.org/10.3322/caac.21731

Oncology medical physics. Oncology Medical Physics. (n.d.-a). https://oncologymedicalphysics.com/linac-subsystem-design/

Oncology medical physics. Oncology Medical Physics. (n.d.). https://oncologymedicalphysics.com/introduction-to-clinical-linear-accelerators/

Pipman, Y. (2019, April 5). School of Medical Physics for Radiation Therapy: Dosimetry and Treatment Planning and Delivery for Basic and Advanced Applications | (smr 3278) (25 March 2019 - 5 April 2019). https://indico.ictp.it/event/8651/session/0/contribution/14/material/slides/

Radiological Society of North America (RSNA) and American College of Radiology (ACR). (n.d.). *Linear accelerator*. Radiologyinfo.org. https://www.radiologyinfo.org/en/info/linac#:~:text=The%20linear%20accelerator%20uses%20microwave%20technology%20%28similar%20to,a%20heavy%20metal%20target%20to%20produce%20high-energy%20x-rays.

Schüler E, Acharya M, Montay-Gruel P, Loo BW Jr, Vozenin MC, Maxim PG. Ultra-high dose rate electron beams and the FLASH effect: From preclinical evidence to a new radiotherapy paradigm. Med Phys. 2022 Mar;49(3):2082-2095. doi: 10.1002/mp.15442. Epub 2022 Jan 19. PMID: 34997969; PMCID: PMC9032195.

Simões, R., and K.T. Jayaprakash. "OncoFlash – Research Updates in a Flash! (February 2023)." *Clinical Oncology* 35, no. 2 (February 2023): 70–71. https://doi.org/10.1016/j.clon.2022.12.011.

Zhu, H., Xie, D., Wang, Y., Huang, R., Chen, X., Yang, Y., Wang, B., Peng, Y., Wang, J., Xiao, D., Wu, D., Qian, C.-N., & Deng, X. (2022). Comparison of intratumor and local immune response between MV X-ray FLASH and conventional radiotherapies. *Clinical and Translational Radiation Oncology*, *38*, 138–146. https://doi.org/10.1016/j.ctro.2022.11.005.