1. Chapter 1: General Introduction

1.1 Introduction

Cancer is a generic term for a heterogeneous group of diseases characterized by uncontrolled growth of cells beyond their usual boundaries and can invade adjoining parts of the body and can spread to other organs (WHO). Other terms used are neoplasms and malignant tumors. The major driving force for the development of different cancer types is the unregulated proliferation of cancer cells that divide and grow in an uncontrolled manner, invading the surrounding normal tissues and eventually spreading to other organs; the latter process is referred to as metastasis. Such loss of control in cell growth and division results in accumulation of abnormalities in various regulatory systems, resulting in modifications of cell in terms of size, shape, structure and function that distinguish cancerous from normal cells. Development of cancer is a multistep process involving mutation and selection for cells with a gradually increasing capacity for proliferation, survival, invasion, and metastasis. Metastases is the primary cause of death from cancer. The change of pre-cancerous cell to a malignant tumor is the result of the interaction of a person's genetic factors with different external agents, including: chemical carcinogens, such as components of tobacco smoke, asbestos, arsenic (a drinking water contaminant) and aflatoxin (a food contaminant); physical carcinogens, such as ultraviolet and ionizing radiation; and biological carcinogens, such as infections from certain viruses, bacteria, or parasites. The incidence of cancer rises progressively with age, most likely due to accumulation of risks for specific cancers that increase with age. The overall risk build-up combines with the tendency of less effective cellular repair mechanisms as a person grows older.

An update on cancer includes essential highlights on the prevalence, pathophysiology, management, and pharmacological treatments. As a part of the literature review, PubMed, Google Scholar, and ScienceDirect databases were searched for English language peer-reviewed published studies with keywords relating to cancer. Additional references were identified using cross-references of clinical research on different cancer types, clinical trials and preclinical studies for the writing of this literature.

1.2 Prevalence

Cancer is the leading cause of death worldwide, and is responsible for nearly 10 million deaths in 2020 as per the GLOBOCAN 2020 estimates of cancer incidence and mortality produced by the International Agency for Research on Cancer (Sung et al., 2021). An estimated number of 19.3 million new cancer and nearly 10.0 million cancer

deaths occurred in 2020, worldwide. Lung cancer remained as the leading cause of death, with an estimated 1.8 million deaths (18%), followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and female breast (6.9%) cancers (Sung et al., 2021). Female breast cancer is the most commonly diagnosed cancer and surpassed the lung cancer with an estimated 2.3 million new cases (11.7%), followed by lung (11.4%), colorectal (10.0 %), prostate (7.3%), and stomach (5.6%) cancers (Sung et al., 2021). The global cancer burden is expected to be rise by 47% from 2020 with 28.4 million cases in 2040 (Sung et al., 2021). In Asia in 2020, where 59.5% of the global population resides, for both sexes combined, 58.3% of cancer deaths are estimated to occur (Sung et al., 2021). Europe accounts for 19.6% of the cancer deaths from 22.8% of the total cancer cases, although it represents 9.7% of the global population (Sung et al., 2021). America with 20.9% of incidence, accounts for 14.2% of the cancer deaths. The share of cancer deaths in Asia (58.3%) and Africa (7.2%) are higher than the share of incidence (49.3% and 5.7%, respectively) because of the different distribution of cancer types and higher case fatality rates in these regions (Sung et al., 2021).

Worldwide in 2020, the combined incidence rate for all cancers was 19% higher in men (222.0 per 100,000) than in women (186 per 100,000) (Sung et al., 2021). Overall cancer mortality worldwide for the gender gap is twice that for incidence, with 43% higher death rates in men than in women (120.8 and 84.2 per 100,000, respectively), partly because of differences in the distribution of the cancer types (Sung et al., 2021). The cumulative risk of dying among women in 2020 was higher in Eastern Africa (11.0%) than in Northern America (8.2%), Western Europe (8.8%), and Australia/New Zealand (7.4%) (Sung et al., 2021)

In India, the projected number of patients with cancer is nearly 1.4 million for the year 2020 with the majority of the patients were diagnosed at the locally advanced stage for head and neck (66.6%), cervix uteri (60.0%), breast (57.0%), and stomach (50.8%) cancer (Mathur et al., 2020).

1.3 Pathophysiology

The process of transformation of normal cells into cancer cells is called carcinogenesis or oncogenesis or tumorigenesis. The process of carcinogenesis involves changes at the genetic, epigenetic and cellular levels, and abnormal cell division (Anand et al., 2008). The majority of cancers upto 90–95% of cases are due to genetic mutations from environmental and lifestyle factors and the remaining 5–10% are due to genetic

inheritance (Anand et al., 2008). The common environmental factors that contribute to cancer death include diet and obesity (30–35%), tobacco use (25–30%), infections (15–20%), radiation by both ionizing and non-ionizing, up to 10%, lack of physical activity, and pollution (Anand et al., 2008; Islami et al., 2018).

1.4 Hallmarks of cancer

The biological capabilities of cancer that are acquired during their multistep development are called hallmarks of cancer. In the year 2000, Hanahan and Weinberg first described that the cancer cell genotypes is an indication of six essential alterations in cell physiology that collectively dictate malignant growth (Hanahan and Weinberg, 2000). Later in 2011, two emerging hallmarks and two enabling characteristics of cancer cells were added in their updated review (Hanahan and Weinberg, 2011). They include sustaining proliferative signaling, resisting cell death, evading growth suppressors, inducing angiogenesis, enabling replicative immortality, and activating invasion and metastasis. The hallmarks of cancer provides a foundation for understanding the cancer cell biology.

1.4.1 Sustaining proliferative signaling

Normal cells require growth-promoting signals before they can move from a quiescent state into an active proliferative state. These signals instruct the cell to entry into and progression through the growth-and-division cycle by transmembrane receptors that bind distinctive classes of signaling molecules like diffusible growth factors, extracellular matrix components, and cell-to-cell adhesion/interaction molecules, thereby ensuring a homeostasis of cell number and thus maintenance of normal tissue architecture and function.

Cancer cells, by deregulating these growth-promoting signals, become masters of their own destinies. Cancer cells acquires the ability to sustain proliferative signaling in a number of ways: (i) by autocrine proliferative stimulation in which they release growth factors by themselves and responds through the expression of cognate receptors, (ii) by sending signals to stimulate various growth factors to normal cells that are present within the supporting tumor-associated stroma (Cheng et al., 2008; Bhowmick et al., 2004) (iii) by deregulating receptor signaling through elevated levels of receptor proteins rendering the cancer cells to hyper responsive to limiting amounts of growth factor ligands.

1.4.2 Resisting cell death

Programmed cell death by apoptosis serves as a natural barrier to cancer development (Adams and Cory, 2007, Lowe et al., 2004). Apoptosis is triggered in response to various physiologic stresses that cancer cells experience during the course of tumorigenesis or as a result of anticancer therapy (Adams and Cory, 2007; Lowe et al., 2004). The "apoptotic trigger" that conveys signals between the regulators and effectors is controlled by counterbalancing pro- and antiapoptotic members of the Bcl-2 family of regulatory proteins (Adams and Cory, 2007). The archetype, Bcl-2, along with its closest members (Bcl-xL, Bcl-w, Mcl-1, A1) are inhibitors of apoptosis, acting in large part by binding to and thereby suppressing two proapoptotic triggering proteins (Bax and Bak) (Lowe et al., 2004). When relieved of inhibition by their antiapoptotic relatives, Bax and Bak disrupt the integrity of the outer mitochondrial membrane, causing the release of proapoptotic signaling proteins, the most important of which is cytochrome c (Adams and Cory, 2007; Willis and Adams, 2005). The released cytochrome c activates, in turn, a cascade of caspases that act via their proteolytic activities to induce the multiple cellular changes associated with the apoptotic program (Willis and Adams, 2005). Bax and Bak share protein-protein interaction domains, termed BH3 motifs, with the antiapoptotic Bcl-2-like proteins that mediate their various physical interactions (Willis and Adams, 2005). The activities of a subfamily of related proteins, each of which contains a single such BH3 motif, are coupled to a variety of sensors of cellular abnormality; these "BH3-only" proteins act either by interfering with antiapoptotic Bcl-2 proteins or by directly stimulating the proapoptotic members of this family (Adams and Cory, 2007; Willis and Adams, 2005).

Tumor cells evolve a variety of strategies to limit or circumvent apoptosis. Most common is the loss of TP53 tumor suppressor function, which eliminates this critical damage sensor from the apoptosis-inducing circuitry (Hanahan and Weinberg, 2011). Alternatively, tumors may achieve similar ends by increasing expression of antiapoptotic regulators (Bcl-2, Bcl-xL) or of survival signals (Igf1/2), by downregulating proapoptotic factors (Bax, Bim, Puma), or by short-circuiting the extrinsic ligand-induced death pathway (Hanahan and Weinberg, 2011). The multiplicity of apoptosis-avoiding mechanisms presumably reflects the diversity of apoptosis-inducing signals that cancer cell populations encounter during their evolution to the malignant state (Hanahan and Weinberg, 2011).

1.4.3 Evading growth suppressors

Within a normal tissue, multiple antiproliferative signals operate to maintain cellular quiescence and tissue homeostasis; these signals include both soluble growth inhibitors and immobilized inhibitors embedded in the extracellular matrix and on the surfaces of nearby cells (Hanahan and Weinberg, 2000). These growth-inhibitory signals, like their positively acting counterparts, are received by transmembrane cell surface receptors coupled to intracellular signaling circuits (Hanahan and Weinberg, 2000). Antigrowth signals can block proliferation by two distinct mechanisms. Cells may be forced out of the active proliferative cycle into the quiescent (G0) state from which they may reemerge on some future occasion when extracellular signals permit (Hanahan and Weinberg, 2000). Alternatively, cells may be induced to permanently relinquish their proliferative potential by being induced to enter into postmitotic states, usually associated with acquisition of specific differentiation-associated traits (Hanahan and Weinberg, 2000).

Cell proliferation depends on more than an avoidance of cytostatic antigrowth signals (Hanahan and Weinberg, 2000). One strategy for avoiding differentiation directly involves the c-myc oncogene (Hanahan and Weinberg, 2000). During normal development, the growth-stimulating action of Myc, in association with Max, can be displaced by alternative complexes of Max with Mad transcription factors; the Mad–Max complexes elicit differentiation-inducing signals (Foley and Eisenman, 1999). However, overexpression of the c-Myc oncoprotein, as is seen in many tumors, can reverse this process, shifting the balance back to favor Myc–Max complexes, thereby impairing differentiation and promoting growth (Foley and Eisenman, 1999).

1.4.4 Enabling replicative immortality

Cancer cells require unlimited replicative potential in order to generate macroscopic tumors (Hanahan and Weinberg, 2011). Multiple lines of evidence indicate that telomeres protecting the ends of chromosomes are centrally involved in the capability for unlimited proliferation (Blasco, 2005, Shay and Wright, 2000). Telomere maintenance is evident in virtually all types of malignant cells (Shay and Bacchetti, 1997); 85%–90% of them succeed in doing so by upregulating expression of the telomerase enzyme, which adds hexanucleotide repeats onto the ends of telomeric DNA (Bryan and Cech, 1999. By one or the other mechanism, telomeres are maintained at a length above a critical threshold,

and this in turn permits unlimited multiplication of descendant cells {Hanahan, 2000 14). Both mechanisms seem to be strongly suppressed in most normal human cells in order to deny them unlimited replicative potential (Hanahan and Weinberg, 2000).

1.4.5 Inducing angiogenesis

The oxygen and nutrients supplied by the vasculature are crucial for cell function and survival, obligating virtually all cells in a tissue to reside within 100 μm of a capillary blood vessel (Hanahan and Weinberg, 2000). Once a tissue is formed, the growth of new blood vessels, the process of angiogenesis, is transitory and carefully regulated (Hanahan and Weinberg, 2000). Counterbalancing positive and negative signals encourage or block angiogenesis. One class of these signals is conveyed by soluble factors and their receptors, the latter displayed on the surface of endothelial cells; integrins and adhesion molecules mediating cell–matrix and cell–cell association also play critical roles. The angiogenesis-initiating signals are exemplified by vascular endothelial growth factor (VEGF) and acidic and basic fibroblast growth factors (FGF1/2). Experimental evidence for the importance of inducing and sustaining angiogenesis in tumors is both extensive and compelling (Bouck et al., 1996; Hanahan and Folkman, 1996). The ability to induce and sustain angiogenesis seems to be acquired in a discrete step (or steps) during tumor development, via an “angiogenic switch” from vascular quiescence (Hanahan and Weinberg, 2000).

Tumors appear to activate the angiogenic switch by changing the balance of angiogenesis inducers and countervailing inhibitors (Hanahan and Folkman, 1996). One common strategy for shifting the balance involves altered gene transcription (Hanahan and Folkman, 1996). Many tumors evidence increased expression of VEGF and/or FGFs compared to their normal tissue counterparts (Singh et al., 1995; Volpert et al., 1997). In others, expression of endogenous inhibitors such as thrombospondin-1 or β-interferon is downregulated. Moreover, both transitions may occur, and indeed be linked, in some tumors (Singh et al., 1995; Volpert et al., 1997).

1.4.6 Tissue invasion and metastasis

The capability for invasion and metastasis enables cancer cells to escape the primary tumor mass and colonize new terrain in the body (Hanahan and Weinberg, 2000). Like the formation of the primary tumor mass, successful invasion and metastasis depend upon all of the other five acquired hallmark capabilities (Hanahan and Weinberg, 2000). Several classes of proteins involved in the tethering of cells to their surroundings in a tissue

are altered in cells possessing invasive or metastatic capabilities (Hanahan and Weinberg, 2000). The affected proteins include cell–cell adhesion molecules (CAMs)—notably members of the immunoglobulin and calcium-dependent cadherin families, both of which mediate cell-to-cell interactions—and integrins, which link cells to extracellular matrix substrates (Hanahan and Weinberg, 2000). The most widely observed alteration in cell-to-environment interactions in cancer involves E-cadherin, a homotypic cell-to-cell interaction molecule ubiquitously expressed on epithelial cells (Hanahan and Weinberg, 2000). Coupling between adjacent cells by E-cadherin bridges results in the transmission of antigrowth and other signals via cytoplasmic contacts with β-catenin to intracellular signaling circuits that include the Lef/Tcf transcription factor (Christofori and Semb, 1999). E-cadherin function is apparently lost in a majority of epithelial cancers, by mechanisms that include mutational inactivation of the E-cadherin or β-catenin genes, transcriptional repression, or proteolysis of the extracellular cadherin domain (Christofori and Semb, 1999). Forced expression of E-cadherin in cultured cancer cells and in a transgenic mouse model of carcinogenesis impairs invasive and metastatic phenotypes, whereas interference with E-cadherin function enhances both capabilities (Christofori and Semb, 1999). The frequently observed downregulation and occasional mutational inactivation of E-cadherin in human carcinomas provided strong support for its role as a key suppressor of this hallmark capability (Hanahan and Weinberg, 2000).

Changes in expression of CAMs in the immunoglobulin superfamily also appear to play critical roles in the processes of invasion and metastasis (Johnson, 1991). Changes in integrin expression are also evident in invasive and metastatic cells. The second general parameter of the invasive and metastatic capability involves extracellular proteases (Coussens and Werb, 1996; Chambers and Matrisian, 1997). In many types of carcinomas, matrix-degrading proteases are produced not by the epithelial cancer cells but rather by conscripted stromal and inflammatory cells; once released by these cells, they may be wielded by the carcinoma cells (Werb, 1997). The activation of extracellular proteases and the altered binding specificities of cadherins, CAMs, and integrins are clearly central to the acquisition of invasiveness and metastatic ability.

1.4.7 Emerging hallmark: Reprogramming energy metabolism

The chronic and often uncontrolled cell proliferation that represents the essence of neoplastic disease involves not only deregulated control of cell proliferation but also corresponding adjustments of energy metabolism in order to fuel cell growth and division

(Hanahan and Weinberg, 2011). Under aerobic conditions, normal cells process glucose, first to pyruvate via glycolysis in the cytosol and thereafter to carbon dioxide in the mitochondria; under anaerobic conditions, glycolysis is favored and relatively little pyruvate is dispatched to the oxygen-consuming mitochondria (Hanahan and Weinberg, 2011). Otto Warburg first observed an anomalous characteristic of cancer cell energy metabolism: even in the presence of oxygen, cancer cells can reprogram their glucose metabolism, and thus their energy production, by limiting their energy metabolism largely to glycolysis, leading to a state that has been termed "aerobic glycolysis" (Warburg, 1930, Warburg, 1956; House et al., 1956). Markedly increased uptake and utilization of glucose have been documented in many human tumor types, most readily by noninvasively visualizing glucose uptake using positron emission tomography (PET) with a radiolabeled analog of glucose (18F-fluorodeoxyglucose, FDG) as a reporter (Hanahan and Weinberg, 2011). Glycolytic fueling has been shown to be associated with activated oncogenes (e.g., RAS, MYC) and mutant tumor suppressors (e.g., TP53) (DeBerardinis et al., 2008; Jones and Thompson, 2009), whose alterations in tumor cells have been selected primarily for their benefits in conferring the hallmark capabilities of cell proliferation, avoidance of cytostatic controls, and attenuation of apoptosis. This reliance on glycolysis can be further accentuated under the hypoxic conditions that operate within many tumors: the hypoxia response system acts pleiotropically to upregulate glucose transporters and multiple enzymes of the glycolytic pathway (Semenza, 2010; DeBerardinis et al., 2008). Thus, both the Ras oncoprotein and hypoxia can independently increase the levels of the HIF1α and HIF2α transcription factors, which in turn upregulate glycolysis (Semenza, 2010). Altered energy metabolism is proving to be as widespread in cancer cells as many of the other cancer-associated traits that have been accepted as hallmarks of cancer (Hanahan and Weinberg, 2011). This realization raises the question of whether deregulating cellular energy metabolism is therefore a core hallmark capability of cancer cells that is as fundamental as the six well-established core hallmarks (Hanahan and Weinberg, 2011). In fact, the redirection of energy metabolism is largely orchestrated by proteins that are involved in one way or another in programming the core hallmarks of cancer (Hanahan and Weinberg, 2011). When viewed in this way, aerobic glycolysis is simply another phenotype that is programmed by proliferation-inducing oncogenes (Hanahan and Weinberg, 2011).

1.4.8 Emerging Hallmark: Evading immune destruction

The long-standing theory of immune surveillance proposes that cells and tissues are constantly monitored by an ever-alert immune system, and that such immune surveillance is responsible for recognizing and eliminating the vast majority of incipient cancer cells and thus nascent tumors (Hanahan and Weinberg, 2011). According to this logic, solid tumors that do appear have somehow managed to avoid detection by the various arms of the immune system or have been able to limit the extent of immunological killing, thereby evading eradication (Hanahan and Weinberg, 2011). The role of defective immunological monitoring of tumors would seem to be validated by the striking increases of certain cancers in immunocompromised individuals (Vajdic and van Leeuwen, 2009). An increasing body of evidence, both from genetically engineered mice and from clinical epidemiology, suggests that the immune system operates as a significant barrier to tumor formation and progression, at least in some forms of non-virus-induced cancer (Hanahan and Weinberg, 2011). When mice genetically engineered to be deficient for various components of the immune system were assessed for the development of carcinogen-induced tumors, it was observed that tumors arose more frequently and/or grew more rapidly in the immune deficient mice relative to immunocompetent controls (Hanahan and Weinberg, 2011). In particular, deficiencies in the development or function of CD8+ cytotoxic T lymphocytes (CTLs), CD4+ Th1 helper T cells, or natural killer (NK) cells each led to demonstrable increases in tumor incidence; moreover, mice with combined immune deficiencies in both T cells and NK cells were even more susceptible to cancer development (Hanahan and Weinberg, 2011). The results indicated that, at least in certain experimental models, both the innate and adaptive cellular arms of the immune system are able to contribute significantly to immune surveillance and thus tumor eradication (Teng et al., 2008; Kim, 2007). Highly immunogenic cancer cell clones are routinely eliminated in immunocompetent hosts, a process that has been referred to as “immunoediting”, leaving behind only weakly immunogenic variants to grow and generate solid tumors; such weakly immunogenic cells can thereafter colonize both immune deficient and immunocompetent hosts (Hanahan and Weinberg, 2011). Clinical epidemiology also increasingly supports the existence of antitumoral immune responses in some forms of human cancer (Bindea et al., 2010) (Ferrone and Dranoff, 2010; Nelson, 2008). For example, patients with colon and ovarian tumors that are heavily infiltrated with CTLs and NK cells have a better prognosis than those that lack such abundant killer lymphocytes (Pages et al., 2010; Nelson, 2008). In truth, the cancer immunology simplify tumor-host

immunological interactions, as highly immunogenic cancer cells may well evade immune destruction by disabling components of the immune system that have been dispatched to eliminate them (Hanahan and Weinberg, 2011). For example, cancer cells may paralyze infiltrating CTLs and NK cells, by secreting TGF-β or other immunosuppressive factors (Yang et al., 2010) (Shields et al., 2010). More subtle mechanisms operate through the recruitment of inflammatory cells that are actively immunosuppressive, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). Both can suppress the actions of cytotoxic lymphocytes (Mougiakakos et al., 2010; Ostrand-Rosenberg and Sinha, 2009).

1.4.8.1 Factors that tumors exploit to avoid immune responses

Regulatory cells:

Immune suppression in the tumor microenvironment, mediated by CD4+CD25+ FoxP3+ regulatory T cells (Tregs), or other types of suppressive cells, seems to be a major mechanism of tumor immune escape and can be a crucial hurdle for tumor immunotherapy {Jacobs, 2012 }. A number of studies have shown that tumor-derived Tregs have comparatively higher suppressive activity than naturally occurring Tregs (Yokokawa et al., 2008). Tregs are drawn into the tumor microenvironment via tumor cell-mediated chemokine production (Curiel et al., 2004). Evidence also suggests that transforming growth factor (TGF)-β, produced by tumor cells among other cells, aids conversion of CD4+ T cells into suppressive Tregs in situ (Zou, 2006). Thus, elimination of Tregs by anti-CD25 monoclonal antibodies (mAbs) or by other means may promote tumor rejection.

Defective antigen presentation:

It is well established that another fundamental mechanism by which tumors evade immune surveillance is by down-modulating antigen processing machinery affecting the major histocompatibility complex (MHC) I pathway, proteosome subunits latent membrane protein (LMP)2 and LMP7, transporter associated with antigen processing (TAP) protein, and tapasin (Vinay et al., 2015). Thus, expression of tumor antigen is downregulated, which can lead to enhanced tumor incidence and metastasis because cytotoxic T lymphocyte (CTL) can no longer recognize target antigens on the tumor cells (Maeurer et al., 1996).

Immunosuppressive mediators:

Tumors can evade immune surveillance by crippling CTL functionality via production of several immune suppressive cytokines, either by the cancer cells or by the non-cancerous cells present in the tumor microenvironment, especially including immune cells and epithelial cells. TGF-β is a chief mediator of this activity (Pasche, 2001). In addition, TNF-α, IL-1, IL-6, colony stimulating factor (CSF)-1, IL-8, IL-10, and type I IFNs can also significantly contribute to cancer growth (Lind et al., 2004). In addition to immune suppressive cytokines, other factors such as VEGF produced by tumors, inhibit the differentiation of progenitors into DCs, thus affecting efficient uptake and antigen presentation (Laxmanan et al., 2005). VEGF and IL-10 and TGF-β are also known inhibit maturation of DCs. DCs retaining the immature phenotype are tolerogenic as they do not present antigen in the proper context with appropriate costimulation to T cells (Gabrilovich, 2004). Immunosuppressive enzymes such as IDO, arginase, and inhibitor of nuclear factor kappa-B kinase (IKK)2 may also contribute significantly to tumor progression via direct actions on tumor cell proliferation or through induction of T cell tolerance/suppression (Vinay et al., 2015).

1.5 Management and treatment

The four pillars of cancer treatment includes: surgery, chemotherapy, radiation therapy and a more recently added immunotherapy. The choice for the type of treatment will depend on the type of cancer and how advanced it is.

1.5.1 Surgery

The primary method of treatment for most localized, solid cancers is the surgery and it plays a role in palliation and prolongation of survival. It typically attempts to remove the entire mass along with, in certain cases, the lymph nodes in the area (Siamof et al., 2020). Surgery is used to diagnose, stage and treat cancer, and certain cancer-related symptoms (Yildizhan et al., 2018). Whether a patient is a candidate for surgery depends on factors such as the type, size, location, grade and stage of the tumor, as well as general health factors such as age, physical fitness and whether the patient has other illness or conditions (Yildizhan et al., 2018).

1.5.2 Chemotherapy

Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs known as chemotherapeutic agents as part of a standardized regimen (Yildizhan et al., 2018). Traditional chemotherapeutic agents act by killing cells that divide rapidly, a

critical property of most cancer cells (Yildizhan et al., 2018). Conventional chemotherapeutic agents are classified generally according to their mechanisms of action: (1) DNA-intercalating agents (e.g., anthracyclines and bleomycin); (2) Alkylating agents that cross links inter- and intra-strand DNA and destabilize during replication (e.g., oxaliplatin and cyclophosphamide); (3) Antimetabolites that disrupt RNA and/or DNA synthesis (e.g., gemcitabine and 5-fluorouracil); (4) Microtubule disruptors (e.g., paclitaxel); (5) topoisomerase inhibitors (e.g., irinotecan) (Yildizhan et al., 2018). The efficacy of chemotherapy depends on the type of cancer and the stage (Yildizhan et al., 2018). In combination with surgery, chemotherapy has proven useful in cancer types including breast cancer, colorectal cancer, pancreatic cancer, osteogenic sarcoma, testicular cancer, ovarian cancer and certain lung cancers. Chemotherapy is curative for some cancers, such as some leukemia's (Nastoupil et al., 2012), ineffective in some brain tumors (Rampling et al., 2004), and needless in others, such as most non-melanoma skin cancers (Madan et al., 2010). The effectiveness of chemotherapy is often limited by its toxicity to other tissues in the body. Even when chemotherapy does not provide a permanent cure, it may be useful to reduce symptoms such as pain or to reduce the size of an inoperable tumor.

1.5.3 Radiotherapy

The use of radiation such as high-energy beams, X-rays, gamma rays, electron beams, or protons, to cure diseases is called radiotherapy (Yildizhan et al., 2018). Radiotherapy will be chosen when the location of cancer cells is only in one particular part of the body (Yildizhan et al., 2018). Radiotherapy destroys unwanted cancer cells in the treatment site by damaging DNA (Yildizhan et al., 2018). The goal of this treatment is to apply the highest doses to cancer cells, while administering the lowest doses to the adjacent healthy cells (Yildizhan et al., 2018). Radiation can be given alone or combine with other treatments, such as surgery or chemotherapy (Yildizhan et al., 2018). This combined procedure may help primary treatments be more effective (Yildizhan et al., 2018). Radiation applications can be used for cancer treatment, reducing the size of the tumor, controlling the tumor growth, and also for decreasing the pain (Yildizhan et al., 2018). Radiotherapy is used before (neoadjuvant) or after (adjuvant) other treatments. The aim is to make the main treatment more effective (Yildizhan et al., 2018).

1.5.4 Immunotherapy

Immunotherapy has become an established pillar of cancer treatment improving the prognosis of many patients with a broad variety of hematological and solid malignancies (Kruger et al., 2019). Unlike standard cancer treatments such as chemotherapy and radiotherapy, immunotherapy modulates the immune system by regulating the tumor microenvironment, so that immune cells can attack in multiple targets and directions and clear tumor cells at several important nodes (Chevolet et al., 2015). Types of immunotherapy includes: (i) Immune checkpoint therapy which blocks the ability of immune checkpoint proteins to limit the strength and duration of immune responses (Ex: ipilimumab (Yervoy), nivolumab (Opdivo), pembrolizumab (Keytruda), (ii) adoptive cellular therapy which generates a robust immune mediate antitumor response through ex vivo manipulation of T cells called chimeric antigen receptor (CAR) T cells (Ex: Tisagenlecleucel (Kymriah), (iii) cancer treatment vaccines like sipuleucel-T (Provenge) (McCune, 2016). Despite different approaches, main drivers behind the success of immunotherapy are checkpoint inhibitors (CPIs) and CAR T cells (Kruger et al., 2019). Despite the unprecedented durable response rates observed with cancer immunotherapies, the majority of patients do not benefit from the treatment (primary resistance), and some responders relapse after a period of response (acquired resistance), only a subset (20-40%) of patients respond to immunotherapy (Sharma et al., 2017). Several common cancer types have shown very low frequency of response (breast, prostate, and colon cancers), and heterogeneous responses have been seen even between distinct tumors within the same patient. Several mechanisms of resistance to immune checkpoint blockade have been investigated, such as lack of tumor neoantigens, defects in class I antigen presentation, and defects in interferon signaling (LaFleur et al., 2018).

1.5.4.1 Safety and toxicology of immune checkpoint inhibitors

In general, immunotherapy agents demonstrate unique safety profiles that may differ considerably from the majority of conventional oncology drugs. For example, treatment with checkpoint inhibitors, including monoclonal antibodies that target CTLA-4, PD-1, or PD-L1 have been associated with a variety of autoimmune-like inflammatory phenomena that appear to be driven by disruption of self-tolerance to various normal tissues including thyroid, pituitary, liver, lung, colon, eye, and skin among others (Bertrand et al., 2015; Naidoo et al., 2015). The combination of ipilimumab and nivolumab has demonstrated superior progression-free survival (PFS) compared to ipilimumab alone in patients with

melanoma (Larkin et al., 2015; Postow et al., 2015), and this combination has now been approved by the FDA for treatment-naive patients with melanoma. The initial phase I study of ipilimumab/nivolumab demonstrated grade 3/4 drug-related adverse events (AEs) in 53% of patients across the range of doses tested, while rates of grade 3/4 AEs in the subsequent randomized phase III were 55% in patients treated with the combination versus 27.3% or 16.3% among patients treated with either ipilimumab or nivolumab alone, respectively (Larkin et al., 2015). Notably, although standard doses of ipilimumab (3 mg/kg) could be combined safely with doses of nivolumab up to 1 mg/kg, and standard doses of nivolumab (3 mg/kg) could be combined safely with doses of ipilimumab up to 1 mg/kg, combined administration of standard doses of both ipilimumab (3 mg/kg) and nivolumab (3 mg/kg) was poorly tolerated and exceeded the maximum tolerated dose (MTD) for the combination (Wolchok et al., 2013).

These studies clearly highlight the clinical development challenges and risks in combining immuno-oncology agents at standard doses and schedules. In order to increase the efficacy of these immune checkpoint inhibitors, novel combinations that can overcome the resistance associated with these agents with tolerated safety profile are further warranted.

1.6 Caffeine

Caffeine (1,3,7-trimethylxanthine) is one of the most commonly consumed food ingredient throughout the world for thousands of years. It is a frequently consumed dietary constituent, which is an added ingredient or is naturally present in varying amounts in both beverages and solid foods (Drewnowski and Rehm, 2016). It was reported that each cup of coffee contains 115 mg of caffeine and the global consumption of caffeine per day corresponds to a single serving of one caffeine beverage per individual (Ran et al., 2021). The well-known effects of caffeine are enhancement of mood and alertness (Zabelina and Silvia, 2020), improvement of performance during exercise, and improvement of cognitive functions such as awareness, reaction time, problem-solving, and decision-making (McLellan et al., 2016). Caffeine has been shown to reduce the motor and non-motor symptoms associated with Parkinson's disease and play a preventive role in disease onset and progression (Prediger, 2010). Several epidemiological studies suggested that consumption of coffee reduces the risk of cancers of brain (Holick et al., 2010), endometrium (Friberg et al., 2009), colon (Oba et al., 2006), skin (Song et al., 2012), breast (Nkondjock, 2009), liver (Nkondjock, 2009), and kidney (Nkondjock, 2009). Even though

caffeine was investigated in a variety of disease areas, the focus of this review will be on its ability as an anticancer agent with primary focus on the molecular mechanisms when used alone or in combination with other anticancer agents.

1.6.1 Effect of caffeine on cell cycle progression

The cell cycle of an actively dividing eukaryotic cell consists of four distinct phases such as S phase (DNA synthesis), M phase (DNA separation and cell division), and two gap phases (G1 and G2) whereas, in the absence of mitogenic stimulus, cells stop dividing and enter into a resting state called as G0 phase (Nurse, 1994). G1 is the first gap phase between S phase and G0 phase and the period between S phase and M phase is the G2 phase (Hartwell and Kastan, 1994). The G1/S checkpoint restricts entry to S phase whereas G2/M checkpoint delays the entry into mitotic phase. Dividing cells have the capability to repair DNA damage by pausing or arresting the cell cycle progression at G1/S and G2/M checkpoints (Figure 1.1). If the damaged DNA is repairable, the cells are subjected to apoptosis (Tej and Nayak, 2018).

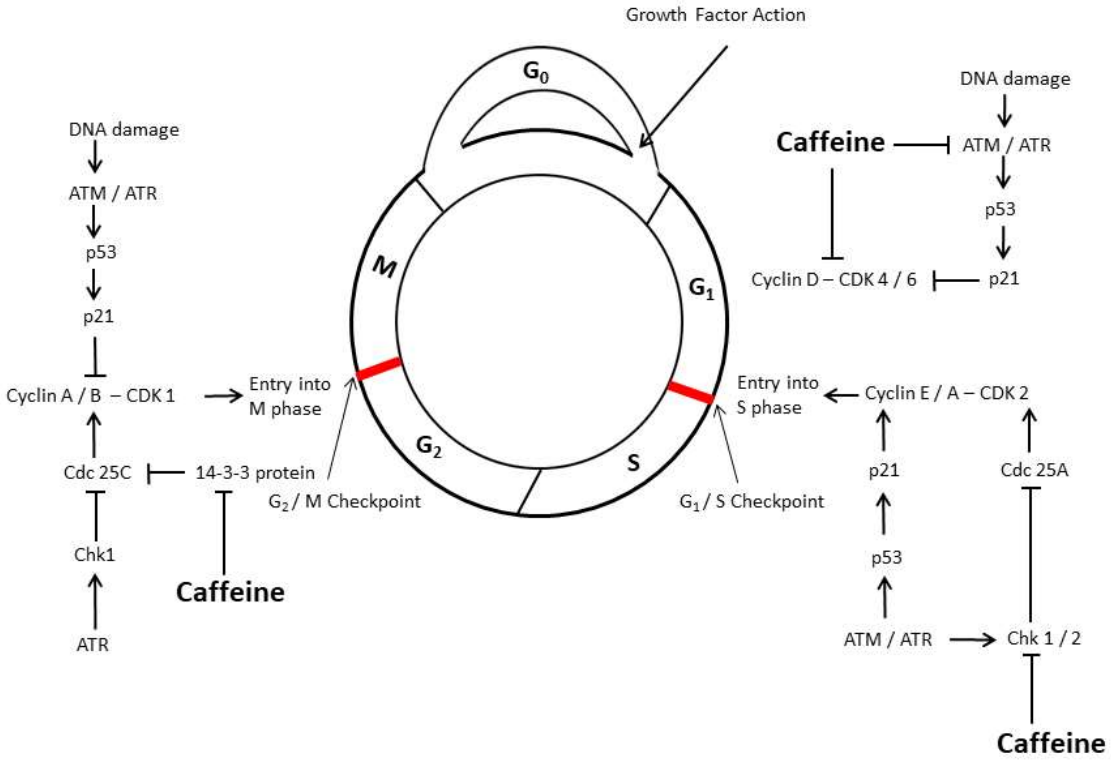

Figure was generated from the previously published report by Geoffrey et.al. (Shapiro and Harper, 1999)

Figure 1.1: Effect of caffeine on cell cycle progression: Caffeine inhibits the DNA damage induced ATM / ATR kinases and Chk1/2 phosphatases and progresses the cell cycle through the

G1/S checkpoint without the repair of damaged DNA which ultimately causes apoptosis. Caffeine directly acts on cyclin D – CDK 4/6 complex and arrests the cell in G1 phase. Caffeine prevents the inhibitory binding of 14-3-3 protein with Cdc25c thereby enhancing Cdc25c which in turn activates Cdc2 and progresses the cell cycle through the G2/M checkpoint. ATM, ataxia telangiectasia mutated; ATR, ATM- and Rad3-related kinase; CDK, cyclin dependent kinase; Chk, checkpoint kinase

1.6.2 Effect of caffeine on apoptosis

Efferth et al. reported that caffeine (1 mM) synergistically potentiates the irradiation- or carboplatin-induced apoptosis in human leukemic cells whereas, caffeine alone was able to induce apoptosis at 8 mM. They further suggested that caffeine modulates apoptosis through glutathione depletion and formation of reactive oxygen species. The suggested mechanism was supported by later findings that glutathione prevents apoptosis induced by reactive oxygen species. Efferth et al. further identified that caffeine modulates the apoptotic activity by increasing the expression of apoptotic inducers (c-Fos, c-Myc) and decreasing the expression of apoptotic repressors (Bcl-2, c-N-Ras) (Figure. 1.2). Thereafter, several researchers reported that caffeine enhances apoptosis and mitochondrial damage in different cancer cell lines such as human U937 and HL-60 cancer cells (2 mM), human pancreatic adenocarcinoma cells (4 mM), human neuroblastoma cells (10 mM), and human A549 lung adenocarcinoma cells (5 mM) (Tej and Nayak, 2018).

In a recent study, Saiki et al. reported that caffeine (25 mM) induces apoptosis of Hela cells, rat adrenal gland pheochromocytoma cancer cells (PC12D), and human neuroblastoma cells (SH-SY5Y) by enhancing autophagy through PI3K/Akt/mTOR/p70S6K inhibition in a dose-dependent manner. While using mouse epidermal JB6 Cl41 cells, He et al. showed that caffeine alone induces apoptosis even at lower concentration (450 μM). They further explored the mechanism involved and found that caffeine increases the phosphorylation of p53 in JB6 Cl41 cells and p53 proficient fibroblast cells but not in p53 deficient fibroblast cells. There are several pathways by which p53 mediates apoptosis, one of these involves Bax protein which is a proapoptotic member of Bcl-2 family of proteins. Bax promotes apoptosis by the cytosolic release of cytochrome c, which further activates key executioner apoptotic protein caspase 3. In the same experiment, He et al. observed that caffeine treatment enhances accumulation of Bax and caspase 3 leading to apoptosis of JB6 Cl41 cells. In agreement with the results of He et al., recently it has been reported that caffeine induces the apoptosis of human gastric

cell lines through the activation of the caspase 9/caspase 3 signaling pathway (Figure 1.2) (Tej and Nayak, 2018).

Overall, caffeine alone seems to induce apoptosis through different mechanisms, which depends on the type of cell, experimental concentrations, and experimental conditions. It is also reported that caffeine inhibits apoptosis by upregulation of antiapoptotic mediators, which suggests further experiments are required to ascertain the mechanisms involved.

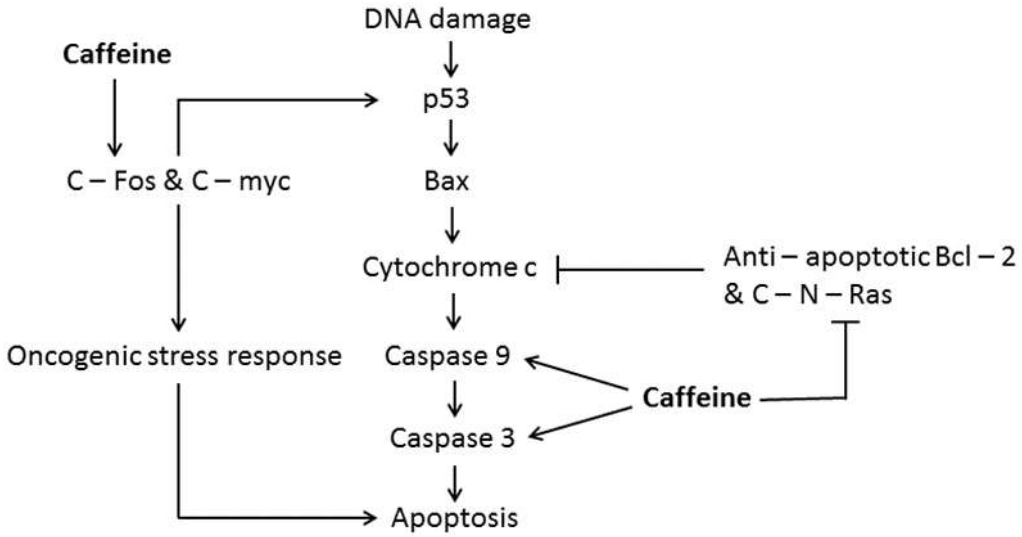

Figure was generated from the previously published reports (Efferth et al., 1995, Liu, 2017).

Figure 1.2: Effect of caffeine on apoptosis. Caffeine causes apoptosis by increasing the expression of apoptotic inducers (c-Fos, c-Myc) and decreasing the expression of apoptotic repressors (Bcl-2, c-N-Ras) and by activating caspase 9/ caspase 3 signaling pathway. Bax, Bcl-2-associated X protein**.**

1.6.3 Effect of caffeine on drug efflux

Drug efflux is a process through which cells remove different types of drugs from cytoplasm using efflux pumps leading to multidrug resistance. It is well known that free form of the drug is effluxes readily than the bound form of the drug. Sadzuka et al. reported that caffeine inhibits efflux of antitumor drugs from tumor cells by forming drug-caffeine stacking complexes (Figure 1.3). Formation of drug-caffeine stacking complexes increases the accumulation of intracellular drug concentration and thereby enhancing anti-tumor effect in both in vitro and animal tumor models. Most of the anti-tumor drugs used clinically are DNA intercalator drugs and often possess polyaromatic core that can interact

with DNA. Spectroscopic and molecular modeling studies revealed that caffeine complexes with planar aromatic molecules of DNA intercalating drugs via Π-Π type of interaction. The predominant force for the formation of complexes arrives from vander Waals interactions that results in maximum ring overlap between two molecules of the complex. This mechanism was further supported by earlier studies by Kapuscinski and Kimmel who proposed a thermodynamic model and confirmed the complex forming ability of caffeine experimentally using acridine orange against caffeine. Similarly, several studies reported that caffeine forms complexes with anthracyclines (daunorubicin, doxorubicin, nogalamycin), actinomycin D, and mitoxantrone (Tej and Nayak, 2018).

Sadzuka et al., further reported that caffeine increases the intracellular concentration of doxorubicin by acting on P-glycoprotein (P-gp)-1 (Figure. 1.3). P-gp is an ATP-dependent efflux pump with broad substrate specificity and largely responsible for eliminating the chemotherapeutic drugs from the tumor cells. Later, it was clarified that the inhibitory effect of caffeine on drug efflux is through recovery of cAMP levels in the tumor cells. Kakuyamanee Iwazaki and group observed doxorubicin-induced reduction in cAMP levels in tumor cells that was restored by caffeine. Moreover, addition of cAMP decreased the efflux of doxorubicin from tumor cells in vitro. Recently, Ding et al. reported that caffeine also inhibits ABCG2 transporter and reverses the multidrug resistance (MDR) associated with this transporter. ABCG2 is an ATP-binding-cassette (ABC) transporter responsible for extruding a wide variety of chemotherapeutic agents. However, caffeine sensitized the MDR cells to the chemotherapeutic agents by reducing ABCG2 protein through rapid internalization and lysosomal degradation (Figure. 1.3) (Tej and Nayak, 2018).

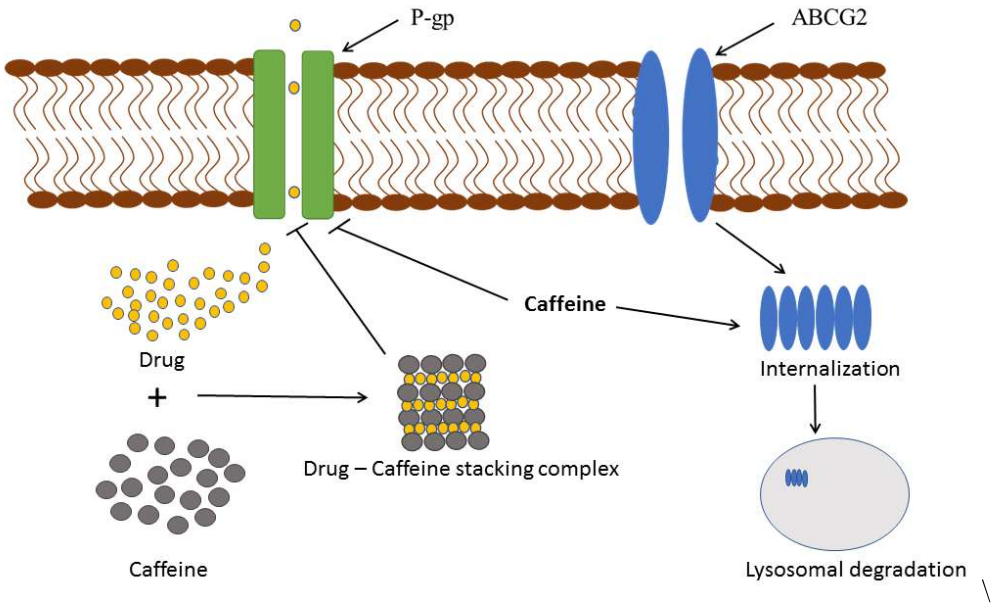

Figure was generated from the previously published reports (Sadzuka et al., 1995b, SADZUKA et al., 1995a).

Figure 1.3: Effect of caffeine on drug uptake. Caffeine by forming intracellular stacking complexes with drugs increases the accumulation of intracellular drug concentration by inhibiting their efflux. Caffeine inhibits anti-tumor drugs efflux by acting directly on P-gp 1 and by causing internalization and subsequent lysosomal degradation of ABCG2 efflux pumps. P-gp, P-glycoprotein; ABCG2, ATP-binding cassette sub-family G member 2.

1.6.4 Effect of caffeine on anti-tumor immunity

The immune system can recognize and destroy nascent tumor cells in a process called anti-tumor immune response or cancer immunosurveillance. A low degree of anti-tumor immunity has been implicated in the pathogenesis of cancer. Anti-tumor immune responses are mediated by both innate and adaptive immunity, and the main cells involved are cytotoxic CD8+ T cells, natural killer (NK) cells, and natural killer T (NKT) cells. In addition, dendritic cells are involved in presenting tumor antigens to naive T lymphocytes and thus induce a cytotoxic T-cell response. It was reported that the anti-tumor activity of these immune cells is strongly affected by adenosine. Adenosine is a crucial regulatory paracrine and autocrine factor that accumulates in tumor microenvironment. It is constitutively present in lower concentrations at extracellular spaces, but its extracellular levels drastically increase in stressful conditions, such as hypoxia, ischemia, inflammation, or trauma. In hypoxic microenvironment of solid tumors, the extracellular adenosine concentration is 10- to 20-fold higher than adjacent normal tissues. After being released from cells or derived extracellularly from related molecules, adenosine diffuses to cell membranes of target cells where it can bind to specific adenosine receptors. The adenosine

receptors are G-protein coupled receptors consisting of four subtypes namely A1, A2A, A2B, and A3. Of these four subtypes, A2A is predominantly present on T cells. Upon activation, the A2A receptors inhibit T cell proliferation and cytokine production through the induction of immunosuppressive intracellular cAMP. Inhibition of cytokine production, particularly interferon (IFN)-γ, in turn decreases the cytotoxicity of T cells, which corresponds to the low degree of anti-tumor immunity. In addition, activation of A2A receptor has been shown to suppress NK cell activity and cytokine production by NKT cells (Tej and Nayak, 2018).

Caffeine is a non-selective adenosine receptor blocker with relatively high affinity (KD of 8.1 μM and 2.4 μM against rat and human A2A receptor, respectively) towards A2A receptors. It is now clear that anti-tumor T cells are inhibited by elevated levels of cAMP through activation of A2A receptors by extracellular adenosine (Figure. 1.4). An increased intracellular cAMP level induces the protein kinase-A mediated phosphorylation, and subsequent activation of COOH-terminal Src kinase (Csk). Activated Csk then phosphorylates and inhibits lymphocyte-specific protein tyrosine kinase (Lck), which ultimately diminishes the T-cell receptor (TCR) signaling and IFN-γ production. The role of A2A receptor and the positive effects of caffeine on anti-tumor immunity were previously determined in mice inoculated with tumor cells. In a study conducted by Ohata et al., 60% of A2A−/− mice showed complete tumor rejection and survival whereas, tumor inoculated wild-type (WT) mice, which expressed A2A receptor on anti-tumor CD8+ T cells, did not show tumor rejection and survival. The mice treated with caffeine and ZM241385 (a specific A2A receptor antagonist) showed increased tumor cell destruction by anti-tumor CD8+ T cells. In a separate study, the blockade of adenosinergic pathway with caffeine shown to increase the efficacy of adoptive T cell immunotherapy and improve the survival of tumor bearing mice. In a recent study, Eini et al. showed that caffeine treatment lowers the rate of development of tumors in 3-methylcholanthrene (3-MCA) injected mice. The same study concluded that caffeine treatment promotes anti-tumor immune response (production of INF-γ and tumor-specific memory T cells) during initiation and promotion of tumor development, partly through antagonism of A2A receptor

(Figure. 1.4) (Tej and Nayak, 2018). The ability of caffeine to promote anti-tumor immune response suggests that a combination of caffeine and immune checkpoint (PD-1 or CTLA-4) blockers could represent a novel synergistic approach to treat cancer.

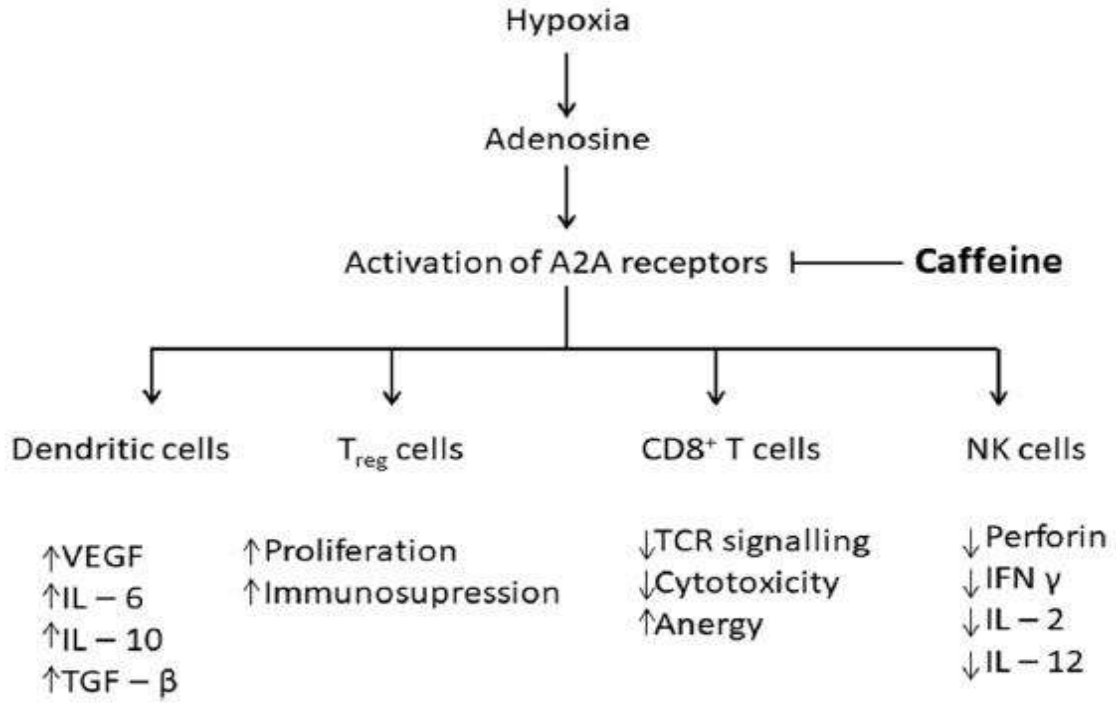

Figure was generated from the previously published reports (Ohta et al., 2006, Jin et al., 2010, Eini et al., 2015).

Figure 1.4: Effect of caffeine on anti – tumor immunity. Increased accumulation of adenosine in tumor microenvironment by hypoxic conditions causes decreased anti-tumor immunity by acting on A2 A receptors of different immune cells. Caffeine by antagonizing A2 A receptors on these immune cells and increases the cytokine production, T cell proliferation which ultimately increases the antitumor immunity. VEGF, Vascular endothelial growth factor; IL, Interleukin; TGF-β, Tumor growth factor beta; TCR, T cell receptor; IFN-γ, Interferon gamma.

1.6.5 Effect of caffeine on tumor angiogenesis

Tumor angiogenesis is the process of formation of new blood vessels by the tumor cells. Tumor growth and metastasis depends on angiogenesis triggered by the chemical signals from tumor cells in a period of rapid growth. As the tumor expands, the diffusion distance from the existing blood vessels increases which results in hypoxia. It is well established that hypoxia leads to an increased expression and induction of hypoxia-inducible factor-1 (HIF-1). HIF-1 is one of the key transcriptional factors that coordinate the cellular responses to hypoxia. Up regulation of HIF-1 increases the transcription of

vascular endothelial growth factor (VEGF) gene which ultimately contributes to tumor angiogenesis and metastasis. Furthermore, hypoxic condition increases the extracellular concentration of adenosine. It has been reported that increased adenosine concentration in hypoxic condition up-regulates the expression of HIF-1 in a dose- and time-dependent manner through the activation of A3 receptors (Figure. 1.5). The molecular mechanism involves the activation of A3 receptors by adenosine which leads to activation of p44/p42 extracellular signal-regulated kinases (ERK) and p38 mitogen-activated protein kinases (MAPK), which in turn, increase the expression of HIF-1 and another angiogenic factor, angiopoietin-2. Overall, there is cooperation between hypoxia and adenosine pathway that ultimately leads to increased HIF-1 mediated angiogenesis (Tej and Nayak, 2018).

Interestingly, caffeine has been shown to inhibit adenosine (through A3 receptor)-induced accumulation of HIF-1α and VEGF in hypoxic human colon cancer cells through the inhibition of ERK1/2, p38, and Akt. In the same study, caffeine inhibited the expression of interleukin (IL)-8 through the inhibition of A2B receptors (Figure. 1.5). IL-8 is a chemotactic factor for leukocytes but also contributes to cancer progression through its potential as a mitogenic, angiogenic, and motogenic factor. Overall, caffeine decreases the migration and angiogenesis of tumor cells through its antagonizing effect on adenosine (A3 and A2B) receptors (Tej and Nayak, 2018).

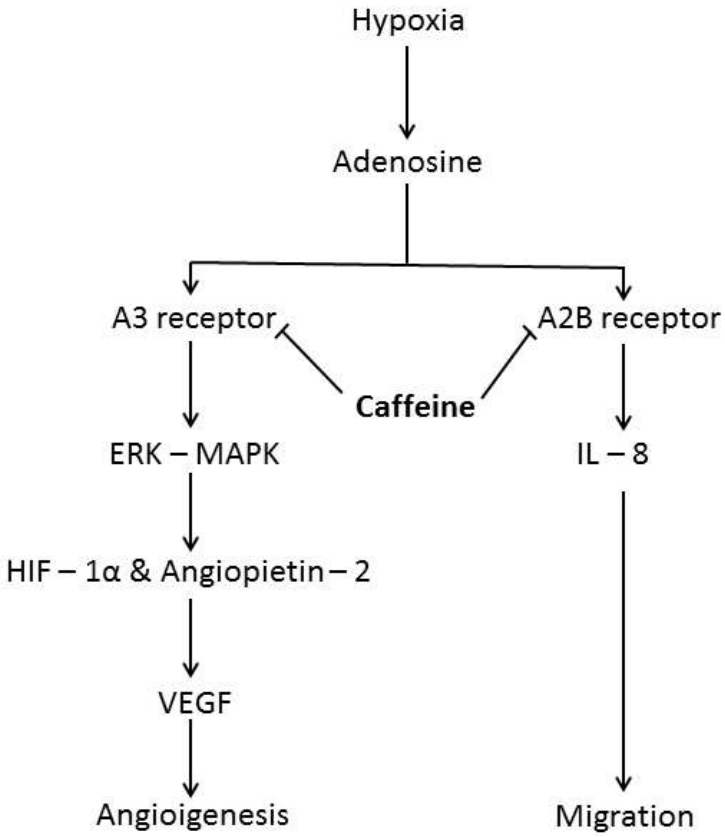

Figure was generated from the previously published reports (Merighi et al., 2007, Xie, 2001).

Figure 1.5: Effect of caffeine on tumor angiogenesis. Caffeine by antagonizing A3 receptors on immune cells decreases the ERK-MAPK induced VEGF production and ultimately angiogenesis. Caffeine by antagonizing A2 A receptors inhibits the IL-8 mediated migration of tumor cells. VEGF, Vascular endothelial growth factor; ERK, Extracellular signal-regulated kinases; MAPK, Mitogen-activated protein kinases; HIF-1α, Hypoxia-inducible factor-1; IL, Interleukin.

1.7 Oxaliplatin

Oxaliplatin ({[oxalate (2-)-O, O′][1R,2R-cyclohexanediamine-N, N’] platinum-(II)}, belongs to the third generation of platinum-based antitumor agents, in which 1, 2-diaminocyclohexane (DACH) ligand substitutes for the amine groups of cisplatin (Zhou et al., 2020). It was reported that oxaliplatin could produce fewer DNA adducts but caused higher cytotoxicity than cisplatin, and it also shows stronger activity in colorectal and other gastrointestinal cancers, while cisplatin and carboplatin show no efficacy (Zhou et al., 2020). Various mechanisms of action are ascribed to oxaliplatin. Like other platinum-based compounds, oxaliplatin exerts its cytotoxic effect mostly through DNA damage. Apoptosis of cancer cells can be caused by formation of DNA lesions, arrest of DNA synthesis, inhibition of RNA synthesis, and triggering of immunologic reactions.

Oxaliplatin also exhibits synergism with other cytotoxic drugs, but the underlying mechanisms of those effects are less well understood (Zhou et al., 2020).

1.7.1 Immunologic Mechanisms: Immunogenic Cell Death (ICD)

ICD is defined by chronic exposure of damage-associated molecular patterns (DAMPs) in the tumor microenvironment (TME), which stimulates the dysfunctional antitumor immune system (Zhou et al., 2019). The induction of ICD contributes to long-lasting protective antitumor immunity. During the cell death process of ICD, immunogenic dead cells expose different hallmarks on the cell surface and release different substances to interact with APCs or other immune cells (Zhou et al., 2019). These molecules that mediate immunogenicity are considered to be DAMPs, including calreticulin (CRT), ATP and high-mobility group box 1 (HMGB1), which are responsible for the 'anticancer vaccine effect' of ICD (Zhou et al., 2019).

In the pre-apoptotic stage, immunogenic dead cells translocate calreticulin (CRT), from the perinuclear ER to the cellular periphery and similarly relocalize ERp57 (Menger et al., 2012). Once the CRT/ERp57 complex is exposed on the cell surface, it provides an 'eat me' signal to promote phagocytosis by DCs (Obeid et al., 2007a). Moreover, the exposure of CRT on the surface of cancer cells succumbing to ICD also induces tumor antigen presentation and tumor-specific CTL responses (Obeid et al., 2007b).

HMGB1 is a non-histone chromatin-binding protein. In the late stages of apoptosis, cells are damaged and disrupted and HMGB1 is released from the nucleus (Bell et al., 2006). Accumulation of extracellular HMGB1 also occurs at this stage. It has been widely reported that the binding of HMGB1 (released from immunogenic dying cells) to Toll-like receptor 4 (TLR4, mainly expressed on DCs) is vital for activating dendritic cells and facilitating antigen presentation by DCs to T cells (Bell et al., 2006). Moreover, the recognition of HMGB1 by TLR4 subsequently triggers MyD88 (the primary myeloid differentiation response gene), the adapter for TLR4 (Apetoh et al., 2007). The TLR4/ MyD88 pathway enhances tumor antigen processing by inhibiting fusion between phagosomes and lysosomes, which promotes the processing of phagocytic cargo in DCs and accelerates the engulfment of antigenic components by DCs (Shiratsuchi et al., 2004). As one of the characteristics of ICD, the expression of ecto-HSP70 and ecto-HSP90 on dying cell membranes has immunostimulatory properties, which lead to specific CD8+ T cell responses by driving the cross-presentation of tumor-derived antigenic peptides on

major histocompatibility complex (MHC) class I molecules (Shiratsuchi et al., 2004). Accordingly, CRT exposure, ATP secretion and HMGB1 release by human cancer cells appear to be the gold-standard for accurately predicting the ICD-inducing capacity of chemotherapeutic agents (Shiratsuchi et al., 2004).

It has recently been discovered that oxaliplatin can cause the immunogenic death of colon cancer cells in murine and human cell lines (Tesniere et al., 2010). After exposure to oxaliplatin, colon cancer cells emit several immunogenic signals on their surface before undergoing apoptosis (Tesniere et al., 2010). These signals trigger the production of interferon γ by T cells and also interact with the toll like receptor 4 of dendritic cells, the whole process resulting in a sort of tumor vaccine (Tesniere et al., 2010). A particularly convincing argument of the importance of this mechanism is that humans carrying a mutant allele of the TLR4 gene resulting in loss of function were found to experience a lesser benefit from oxaliplatin chemotherapy in the metastatic setting, with a statistically significant shorter progression-free and overall survival (Tesniere et al., 2010).

1.8 Aims

The overall aims of the research conducted in this thesis were to evaluate the combination strategies for enhancing anti-tumor activity of anti-PD1 monoclonal antibody. Specifically, the aims were to:

1) Evaluate the antitumor immune response efficacy of caffeine against 3-MCA induced tumors in mice (Chapter 2)

2) Evaluate the antitumor immune response of caffeine and anti-PD1 monoclonal antibody combination against 3-MCA and B16F10 melanoma induced tumors in mice (Chapter 3)

3) Evaluate the antitumor immune response of oxaliplatin and anti-PD1 monoclonal antibody combination against 3-MCA and B16F10 melanoma induced tumors in mice (Chapter 4)

1.9 Hypothesis

Immunotherapy has become an established pillar of cancer treatment, improving the prognosis of many patients with a broad variety of hematological and solid malignancies. Unlike standard cancer treatments such as chemotherapy and radiotherapy,

immunotherapy modulates the immune system by regulating the tumor microenvironment, so that immune cells can attack in multiple targets and directions and clear tumor cells at several important nodes. Immunotherapy represented by immune checkpoint blockers (ICBs) such as anti–CTLA4 and anti–PD1 monoclonal antibodies (mAbs) has emerged as a promising treatment option for cancer patients in recent years. ICBs have shown durable clinical responses in multiple cancers of both early and advanced stages. However, the clinical response rate of ICBs is limited to a subset of patient population (15-30%), while majority of patients are primarily resistant to PD1 blockade (Topalian et al., 2012, Brahmer et al., 2012). The tumor biopsies of patients treated with anti–PD1 mAb revealed that patients who did not respond to the therapy lacked CD8+ T cells inside tumor lesions. There is an accumulating evidence indicating that resistant to anti–PD1 therapy is largely dependent on tumor microenvironment where, tumor cells utilize multiple and non-overlapping immunosuppressive mechanisms to facilitate immune escape.

One of the immunosuppressive pathways involved in tumor immune escape is adenosine-A2A receptor pathway (Allard et al., 2012; Bours et al., 2006). During hypoxic conditions of solid tumors, adenosine concentration is increased locally (Sitkovsky et al., 2004). Following its release, adenosine acts on A2A receptors expressed on stromal cells and downregulates adhesion proteins such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), or P-selectin leading to decreased infiltration of tumor-infiltrating lymphocytes (TILs) (Allard et al., 2012). In addition, adenosine inhibits the proliferation of cytotoxic T cells and increases the proliferation of T regulatory cells (Tregs) (Allard et al., 2012). Expression of inhibitory receptors like programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on TILs is the other major contributing immunosuppressive pathway. Thus, it is hypothesized that combination immunotherapy designed to attract CD8+ T cells into tumor microenvironment and to block non-overlapping immunosuppressive mechanisms like adenosine pathway by caffeine may improve the antitumor activity of anti–PD1 mAb in resistant patients (Figure 1.6).

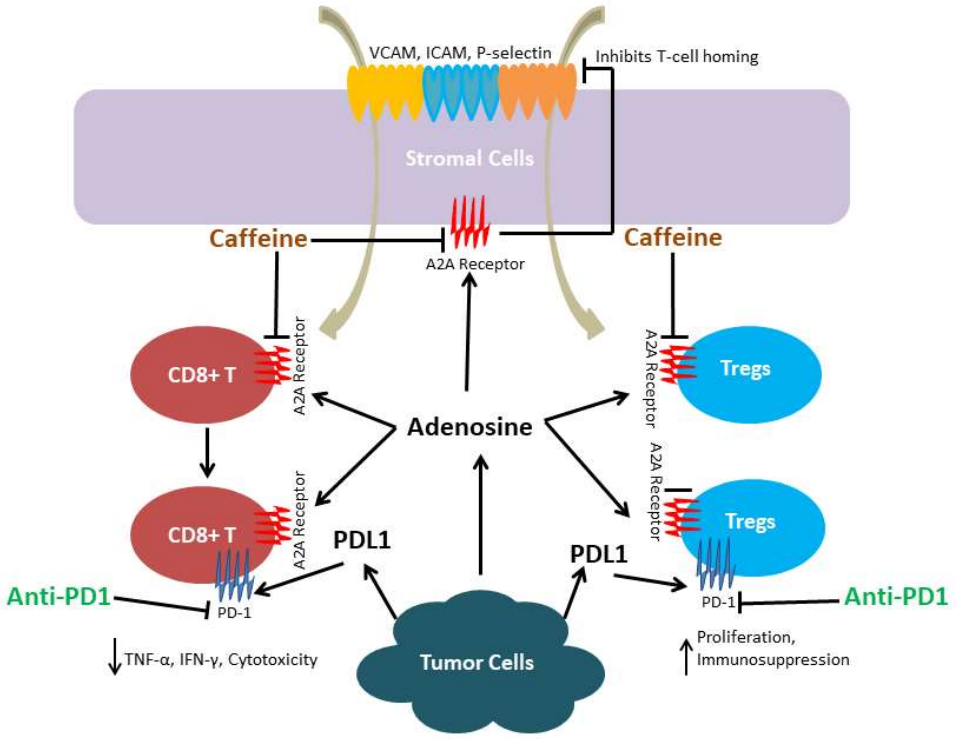

Figure 1.6: Mechanism of caffeine and anti-PD1 induced antitumor immune response

In order to broaden the number of responding patients to immune checkpoint inhibitors, extend the duration of response, and to overcome the resistance of single agent therapy, it appears important to employ the strategies that convert tumors lacking T cell infiltration to ones displaying antitumor T cells and then to determine whether this approach sensitizes tumors to checkpoint therapy. One approach to attain this goal involves the induction of immunogenic conditions in the tumor microenvironment. Majority of chemotherapeutic agents can elicit antitumor immunity and modulate the composition, density, function, and distribution of tumor TILs, to influence differential therapeutic responses and prognosis in cancer patients. Oxaliplatin is one of the platinum based chemotherapeutic agent that can effectively augment antitumor immune response by inducing immunogenic cell death (ICD) and also facilitates the activation of dendritic cells, which inturn causes the activation, generation and proliferation of cytotoxic T cells. With this concept in mind, we hypothesized that simultaneous blocking of PD-1 with anti-PD1 and induction of ICD with oxaliplatin may improve the anti-tumor immune response of anti-PD1 antibody (Figure 1.7) .

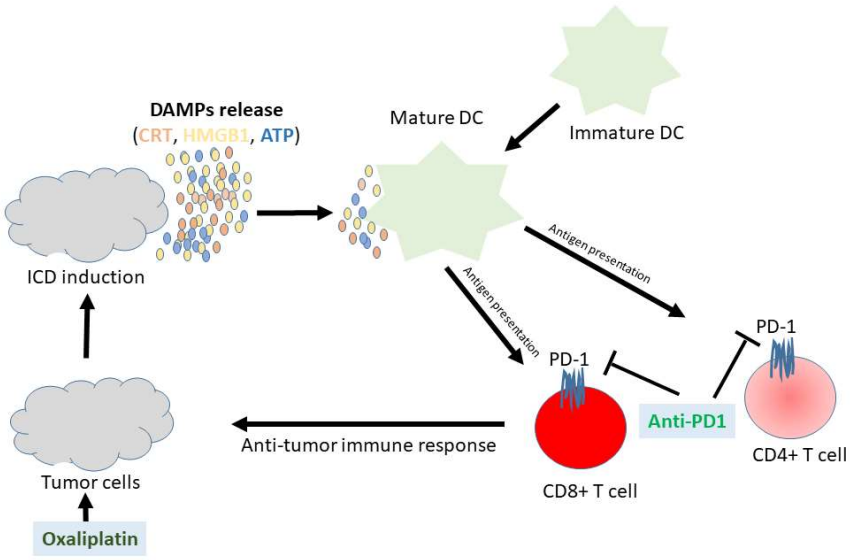

Figure 1.7: Mechanism of oxaliplatin and anti-PD1 induced antitumor immune response