Research Article ISSN: 2833-0390

International Journal of Research in Oncology

Elimination of Cancer Stem Cells is Essential to Save Cancer Patients

Ming C. Liau*, Christine L. Craig and Linda L. Baker

CDA Therapeutics, Inc., 3308 Sky Run Court, Missouri City, Texas, USA.

***Correspondence:**
Ming C. Liau, CDA Therapeutics, Inc., 3308 Sky Run Court, Missouri City, TX 77459, USA, Tel: 832-405-2660.

Received: 05 Apr 2024; **Accepted:** 12 Apr 2024; **Published:** 15 Apr 2024

Citation: Ming C. Liau, Christine L. Craig, Linda L Baker. Elimination of Cancer Stem Cells is Essential to Save Cancer Patients. Int J Res Oncol. 2024, 3(1): 1-9.

ABSTRACT

The objective of this study is to develop cancer drugs effective against cancer stem cells (CSCs) to save cancer patients. Cancer incidence and mortality keep on increasing during the past 50 years, which is an indication that the health profession is not handling cancer therapy right. Cancer therapy got to a bad start to rely on cytotoxic drugs to kill cancer cells (CCs) and to set up disappearance of tumor as a diagnostic criterion for the evaluation of cancer drugs. Cancer drugs developed by the health profession in the past can only benefit a minority of cancer patients in the early stage whose chemo-surveillance has not been fatally damaged, whereas these drugs cause the fatality of a majority of cancer patients in the advanced stage whose chemo-surveillance has been fatally damaged. Thus, cytotoxic drugs and radiation put up by the health profession are responsible for the ever- increasing cancer mortality.

Cancer evolves as a consequence of wound unhealing due to the collapse of chemo-surveillance. Chemo-surveillance is the nature's creation to ensure perfection of wound healing. Wound healing requires the proliferation and the terminal differentiation of progenitor stem cells (PSCs). Methylation enzymes (MEs) of PSCs are abnormal due to association with telomerase which is expressed in PSCs. MEs play a pivotal role on the regulation of cell replication and differentiation. The association of MEs with telomerase tilts the regulation in favor of cell growth. The nature creates chemo-surveillance as an allosteric regulation to prevent unnecessary build-up of cells with abnormal MEs. The collapse of chemo-surveillance disrupts wound healing and forces PSCs to evolve into cancer stem cells (CSCs) by silencing TET-1 enzyme to escape contact inhibition that limits the extent of PSCs to proliferate. The evolution of CSCs is the initial phase of cancer evolution closely related to wound unhealing. Subsequent cancer progression through chromosomal abnormalities such as translocations to activate oncogenes or deletions to inactivate suppressor genes is also due to wound unhealing, but is not as tightly related to wound unhealing as CSCs. Induction of terminal differentiation is the only option to solve the problem of CSCs which are needed to heal the wound. Elimination of CCs can be done by induction of differentiation or cell killing, which are not needed to heal the wound.

Myelodyspleastic syndromes (MDS) are typical diseases to illustrate cancer evolution due to wound unhealing. MDS are triggered by disorders such as chronic infections or wounds which prompts the patients to yield a high level of cytokines. Tumor necrosis factor (TNF) among such cytokines is most closely related to the development of MDS. It causes the apoptosis of bone marrow stem cells and cachexia symptoms to result in the collapse of chemo-surveillance and the evolution of CSCs. MDS are diseases attributable entirely to CSCs. CDA-2, Vidaza and Decitabine are the three drugs approved by the Chinese FDA for the therapy of MDS. Vidaza and Decitabine are also approved by the US FDA for the therapy of MDS. These drugs achieve MDS therapy by the induction of terminal differentiation of CSCs. MDS can be used to screen drugs effective against CSCs essential to save cancer patients.

CSCs are the dominant issue of metastatic, unresponsive and recurrent cancers. Induction of terminal differentiation is the only option for the solution of CSCs, and the solution of CSCs is essential to save cancer patients in desperate situation. CDA formulations are, therefore, the only drugs that can come to the rescue of such patients.

Keywords
Abnormal MEs, Chemo-surveillance, Cancer drugs, CDA, CSCs, DIs, DHIs, PSCs, Wound healing.

Introduction

During the past 50 years, the cancer incidence and mortality keep on increasing. According to NCI experts, the cancer incidence was 19 million and the cancer mortality was 10 million worldwide in 2019, which were 5% above the incidence and 5.3% above the mortality statistics of 2018 [1]. They predicted around 5% annual increment in the following years likewise. The statistics of USA look better. According to American Cancer Society, the cancer incidence was 1,958,310 and the cancer mortality was 609,820 in 2023, which were 2% above the incidence and 0.2% above the mortality statistics of 2022. It appears that the cancer mortality in the USA has reached the plateau. The ever-increasing cancer mortality is an indication of ineffective handling of cancer by the health profession. Cancer therapy got to a bad start to rely on cytotoxic chemicals to kill CCs. Cytotoxic chemotherapy was a tragic byproduct of World War II. During the war, sulfur mustard toxic gas bombs were used. Victims of toxic gas all displayed depletion of leukocytes in their blood specimens, which inspired oncologists to employ toxic chemicals to treat leukemia patients. Cytotoxic chemotherapy became the standard therapy of cancer, and the disappearance of cancer cells or the reduction of tumor size became the standard criteria to evaluate the effectiveness of cancer therapy. These were tragic mistakes made by cancer establishments at a time we did not have complete information of cancer. Perpetual proliferation of CCs was the most outstanding feature of cancer known at the early time. Toxic chemicals were apparently very effective to stop proliferation of CCs. When President Nixon declared War on Cancer during 1971 to 1976, cytotoxic agents and radiation were the major drugs employed to combat cancer, which were not successful to reduce cancer mortality [2]. When a treatment modality was drilled through as a presidential project with unlimited support of national resources and failed, it was fair to conclude that the treatment modality employed was not good for cancer therapy. Apparently, cancer establishments agreed to this conclusion, and shifted the emphasis immediately away from cytotoxic agents to gene and targeted therapies during 1976 to 1996, and then to anti-angiogenesis during 1996 to 2016, and now to immunotherapy from 2016 onward [3]. They did not develop new cancer drugs good enough to replace failed cancer drugs to win the war on cancer, and continue to rely on drugs most effective to kill CCs and to reduce tumor size for cancer therapy. The result is ever-increasing cancer mortality. We have to get to the very basic to find out the right solution.

Cancer is basically a problem of growth regulation going awry. MEs play a pivotal role on the regulation of cell replication and differentiation. Enzymes playing important regulatory roles are often subjected to delicate regulation. Allosteric regulation is a pervasive regulation to maintain biological optimum to avoid extremes often to create clinical symptoms. Because of important regulatory role on growth regulation, MEs are subjected to exceptional double allosteric regulations, one on the individual enzymes, and one on the enzyme complex [4]. MEs are ternary enzyme complex consisting of methionine adenosyltransferase (MAT)- methyltransferase (MT)-S-adenosylhomocysteine hydrolase (SAHH) [5]. SAHH is subjected to allosteric regulation by steroid hormones or related allosteric regulators which promote enzyme complex formation in favor of cell growth. In the absence of allosteric regulators, MEs dissociate to result in hypomethylations of nucleic acids to promote terminal differentiation and to terminate cell growth. In telomerase expressing cells, MEs become associated with telomerase [6]. The association changes kinetic properties of MAT-SAHH isozyme pair, and tilts the regulation in favor of cell growth. Thus, cells with abnormal MEs have a great advantage on cell growth. The nature creates chemo-surveillance to prevent the growth of cells with abnormal MEs to get out of control [7]. Actually, chemo-surveillance is the mechanism of allosteric regulation to switch off the very active state of abnormal MEs.

Cancer and wound healing are closely related to involve PSCs as common entities [8-11]. Wound healing requires the proliferation and the terminal differentiation of PSCs. Wound triggers biological and immunological responses. The biological response involves the release of arachidonic acid (AA) from membrane bound phosphatidylinositol through phospholipase A2 for the synthesis of prostaglandins (PGs) by cyclooxygenases and PG synthases [12,13]. Although AA and PGs are active differentiation inducers (DIs) [14], the induction of terminal differentiation of PSCs at the initial stage of the wound is not the primary objective of PGs. Rather, the localized inflammation caused by PGs [15] is responsible for the increase of membrane permeability to facilitate the extravasation of plasma proteins and regulatory factors into the wound resulting in edema response which is the primary objective of PGs to orchestrate the healing process. Chemo-surveillance mediated through DIs and differentiation helper inducers (DHIs) normally functions as a brake to prevent the build-up of PSCs. This brake must be released in order for PSCs to proliferate to produce enough cells to heal the wound. PGs are metabolically unstable [12]. Their biological effects are most likely brief and confined to the wound area. Thus, the promotion of the proliferation of PSCs is the primary objective of PGs on wound healing, whereas the induction of terminal differentiation of PSCs at the final stage of wound healing is accomplished by DIs and DHIs of chemo-surveillance. The stable end products of PGs are also active as DIs, although not as active as PGs [14], which may get involved in the promotion of terminal differentiation of PSCs at the final stage of wound healing.

The biological response of the wound that generates PGs is good for wound healing. The immunological response of the wound that generate cytokines is not good for wound healing. Tumor necrosis factor (TNF) among cytokines triggered to produce by immunological response is particularly bad to wound healing. On one hand, it causes the apoptosis of bone marrow stem cells, and on the other hand, it causes cachexia symptoms to result in the collapse of chemo-surveillance. The apoptosis of stem cells invites the proliferation of PSCs, and the collapse of chemo-surveillance

removes the brake for PSCs to build-up. PSCs are normal stem cells. The expansion of normal stem cells is limited by contact inhibition. PSCs will be forced to evolve into CSCs in order to escape contact inhibition. TET-1 enzyme provides another safety mechanism of normal stem cells to prevent the build-up of PSCs by carrying out lineage transition through oxidative demethylation process [16]. When this enzyme is silenced, PSCs will be converted to become CSCs. By a single hit through de novo methylation on the promoter of TET-1, this enzyme can be silenced. It is within the reach of PSCs since these cells are equipped with exceptionally active abnormal MEs. Wound unhealing in most instances is due to the collapse of chemo-surveillance. The evolution of PSCs to CSCs to expand stem cell population still cannot get the wound healed, because these cells are unable to undergo terminal differentiation to become functional cells. Pressure is then set in to force chromosomal abnormalities such as translocations to activate oncogenes or deletions to inactivate suppressor genes to speed up replication of CSCs, eventually pushing CSCs to progress to faster growing CCs. The production of CSCs is needed for the wound healing. Therefore, the only option for the solution of CSCs is by induction of terminal differentiation. The production of CCs is not needed for the wound healing, which can be eliminated by the induction of differentiation or cell killing. A perfect cancer drug must be able to take out both CSCs and CCs, and to restore chemo-surveillance [17,18]. Elimination of CSCs and restoration of chemo-surveillance must be done by CDA formulations, and elimination of CCs can be done by therapies aimed to kill CCs or to direct terminal differentiation. Cancer therapy by cell killing and cell differentiation are two diversely different approaches. Cell killing is the choice of cancer establishments to solve cancer, which can only put away CCs to solve a fraction of cancer problems, clearly an imperfect solution of cancer. We choose cell differentiation to put away CSCs, CCs and to restore chemo-surveillance, clearly a perfect solution of cancer.

Commentaries and Discussion

Abnormal MEs as the Most Critical Issue of Cancer

A right approach is essential to solve any problem. Cancer is caused by multiple incidences. Wounds inflicted by toxic chemicals including carcinogens, radiation, infectious agents, or physical means, collapse of protection mechanisms such as chemo-surveillance and immune-surveillance, and breakdown of regulatory mechanisms. A perfect solution of cancer must be able to eliminate all causes contributing to the evolution of cancer. A stroke to kill cells capable of replication is the most simple approach. We have tried that, but the cancer mortality keeps on increasing. We need to get to the basic to find out what are the most critical issues of cancer, and try to solve cancer by putting away critical issues confronting cancer.

Cancer is basically a problem of growth regulation going awry. Since MEs play a pivotal role on the regulation of cell growth, these enzymes must be closely involved in cancer. Cancer establishments were very close to solve cancer when aberrant tRNA methylation was hotly pursued in a few years span around 1966 and aberrant DNA methylation was hotly pursued in a few years span around 1985 [3]. Unfortunately, cancer establishments missed the critical target of MEs to let the solution of cancer to slip away. 2'O-Ribose ME of pre-rRNA which controls the production of ribosome [19] and DNA ME which controls the expression of genes involved in specific differentiation functions [20] are particularly important on the issue related to growth and differentiation. The critical issue is MEs become abnormal due to association with telomerase [6]. Cells with abnormal MEs have a great advantage on cell growth. K_m values of telomerase associated MAT-SAHH isozymes are 7-fold higher than that of normal isozymes pair [21]. A higher K_m is an indication that the enzyme has the capacity to bind more substrate. If a protein binds more S-adenosylmethionine (AdoMet) that protein becomes more stable as Prudova et al.'s study showed that binding of AdoMet could protect protein against protease digestion [22]. Obviously, abnormal MEs are much more stable than normal MEs. A higher K_m is also an indication that cells with abnormal MEs have larger pool sizes of enzyme products. Larger pool sizes of AdoMet and S-adenosylhomocysteine (AdoHcy) are needed to maintain growth of malignant cells as Chiba et al.'s study showed that when HL-60 cells were induced to undergo terminal differentiation, pool sizes of AdoMet and AdoHcy were greatly diminished [23].

Embryonic stem cells express telomerase. Evidently build-up of cells with abnormal MEs are necessary for the normal development of the fetus. Premature disruption of abnormal MEs by thalidomide is detrimental for normal development of the fetus, resulting in the malformation of body parts, notably limbs. PSCs are embryonic cells to initiate the development of organs or tissues. A small fraction, usually less than 2% of the organ or tissue mass, is reserved in the organ or tissue for future expansion or repair. MEs of PSCs are also abnormal. Embryonic stem cells including PSCs display specific features of drug resistance and anti-apoptosis capability. These cells are resistance to toxic chemicals and radiation. The other specific feature is that these cells express chemokine receptors easily attracted by chemokine signals. Peptides are strong chemokine signals. The injured body part usually produces a high level of protein degradation products to recruit PSCs to work on the repair. MEs become abnormal do not seem to cause problems for normal stem cells, as there are safety mechanisms such as contact inhibition, TET-1 enzyme, and chemo-surveillance to prevent these normal stem cells from getting out of control. When safety mechanisms are dysfunctional or damaged, e.g., contact inhibition is ineffective on CSCs and CCs, TET-1 can be eliminated by silencing and chemo-surveillance can be damaged by agents causing cachexia symptoms to trigger excessive urinary excretion of low molecular weight metabolites, then abnormal MEs become a critical issue of cancer. We considered it to be the most important issue of cancer [24], because these enzymes are involved in growth regulation which is the most fundamental issue of cancer. Abnormal MEs are shared by all human cancers [25]. Chemo-surveillance is damaged in cancer patients [7]. Silencing of TET-1 enzyme constitutes a requirement of malignant transformation [16]. These happenings provide a convincing argument that abnormal MEs and damaged chemo-surveillance and silenced TET-1 enzyme are responsible for the perpetual

proliferation of cancer cells, which is the most outstanding feature of cancer. Consequently, abnormal MEs are an ideal target for cancer therapy. They are indeed the bullseye of cancer target [26]. Once abnormal MEs are eliminated by wound healing metabolites or chemicals active as DIs and DHIs, both CSCs and CCs are induced to undergo terminal differentiation to become terminally differentiated cells no longer capable of replication, and chemo-surveillance is restored to the functioning status of healthy people. Of course, chromosomal abnormalities to activate oncogenes or to inactivate suppressor genes are important issues of cancer. As a matter of fact, cancer establishments put up a great effort to solve problems related to oncogenes and suppressor genes during 1976-1996. All efforts at that time were put on to develop gene and targeted therapies. Entire human chromosomal DNA sequences were elucidated in a preparation to develop gene therapy. They gave up, because it was simply too difficult and too expensive to develop gene therapy. Besides, it was not feasible to develop gene therapy. There are multiple chromosomal abnormalities that can influence cancer development. One chromosomal abnormality is solved. There may soon pop up another chromosomal abnormality to negate the previous effort. It is an endless struggle trying to solve chromosomal abnormalities. Gene therapy is a right approach. But it is too difficult and too expensive to achieve. Targeted therapy against oncogene products, namely anti-signal transduction, is not as difficult, which produces many excellent cancer drugs. Signal transduction inhibitors are excellent DHIs [27]. Therapeutic endpoint of targeted therapy is the terminal differentiation, which will not cause the tumor to shrink. Such drugs are not the favor of cancer establishments. These drugs are primarily used in the therapy of hematological cancers. Cancer establishments gave up on the development of gene and targeted therapies, and then turned to anti-angiogenesis in 1996 [3]. Cancer establishments should turn to CDA formulations, because once CSCs and CCs were induced to undergo terminal differentiation, the problems of chromosomal abnormalities could also be put to rest. After all, oncogenes and suppressor genes are cell cycle regulatory genes, these genes have important roles to play when cells are in cell cycle replicating. But when cells exit cell cycle to undergo terminal differentiation, they have no roles to play. So, CDA formulations provide an easy way to solve chromosomal abnormalities which are otherwise very difficult to achieve. Killing replicating cells is another easy way that has been tried but failed.

Anatomy of Cancer Evolution

Induction of cancer by carcinogens is the most straight forward demonstration of how cancer is evolved. Our carcinogenesis studies showed that when animals were challenged with hepatocarcinogens, we observed numerous tiny hyperplastic nodules appeared in the liver before the appearance of large size carcinomas, which were later disappeared [28]. These preneoplastic hyperplastic nodules displayed abnormal MEs. We were puzzled at that time. Now we have good explanation of the appearance and disappearance of tiny preneoplastic hyperplastic nodules. These tiny preneoplastic hyperplastic nodules must represent the active proliferation of PSCs in the process of wound healing, and the disappearance of tiny hyperplastic nodules was the result of completion of wound healing. Only the unhealed tiny preneoplastic hyperplastic nodules later developed to become large size carcinomas. During the challenge with hepatocarcinogens, if the animals were given Antineoplaston A10, which was phenylacetylglutamine effective as anti-cachexia agent [7], hepatocarcinogenesis could be effectively prevented as shown in Fig. 1, which is reproduced from [29]. These studies convincingly show that carcinogenesis proceeds from wounds triggered by carcinogens. There are active wound healing processes going on trying to heal the wounds created by carcinogens.

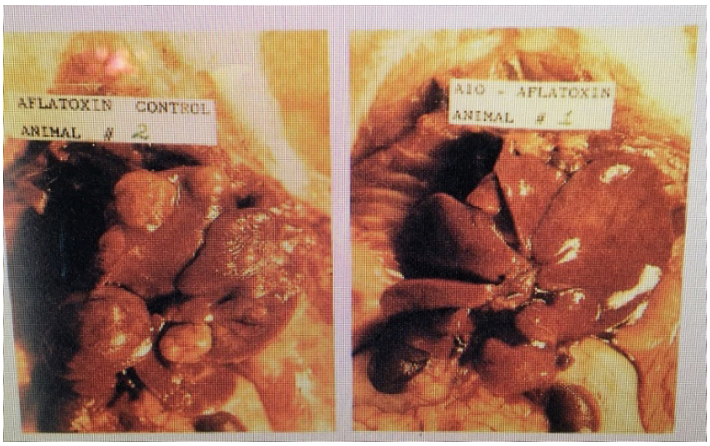

Figure 1: Prevention of hepatocarcinogenesis by the protection of chemo-surveillance.

If wounds are healed by the wound healing mechanism naturally or through the employment of Antineoplaston A10, carcinogenesis can be prevented. If not, PSCs involved in the wound healing will be forced to evolve into CSCs, and then to progress to faster growing CCs.

The studies above described clearly show that cancer is triggered by the infliction of wounds with toxic carcinogens. The host recruits PSCs to engage in active wound healing. It is the battle between the toxic carcinogens and chemo-surveillance capability to decide the outcome. If the toxic carcinogens prevail, cancer becomes established, and if chemo-surveillance prevails, cancer can be prevented. Chemo-surveillance is the nature's creation to ensure perfection of wound healing. Wound if healed perfectly can avoid disastrous consequences of wound unhealing, that include tissue fibrosis, dementia, organ failure and cancer [3,9-11,30]. It was our belief that the protection of the functionality of chemo-surveillance was very crucial to avoid cancer [31,32]. We strongly advocated that the restoration of the functionality of chemo-surveillance was a top priority to save cancer patients [33].

Cancer Arising as a Consequence of Wound Failed to Heal

The concept of cancer arising as a consequence of wound failed to heal was first introduced by the great German scientist Virchow in the 19th century [34]. It was again brought up by Dvorak in 1986 [35]. The close relationship of cancer and wound healing was noticed by MacCarthy-Morrough and Martin [8]. We provided the most important details on this subject that included abnormal

MEs to promote perpetual growth of cancer cells [6,21,24-26], chemo-surveillance as the nature's creation of allosteric regulation on abnormal MEs to ensure perfection of wound healing to avoid disastrous consequences of wound unhealing [4,7,31-33]; DIs and DHIs as wound healing metabolites and active players of chemo-surveillance [4,7,31-33]; hypomethylation of nucleic acids as a critical mechanism on the induction of terminal differentiation [36]; mechanism of wound healing to involve the proliferation and the terminal differentiation of PSCs [3,9-11]; and the evolution of CSCs from PSCs through a single hit to silence TET-1 enzyme [16,37]. These studies strongly support the concept that cancer arises as a consequence of wound failed to heal. Wound failed to heal is because of the collapse of chemo-surveillance as above described. Therefore, restoration of chemo-surveillance for the perfection of wound healing obviously is the most appropriate approach of cancer therapy [33,38-42].

Wound healing is a simple matter. It comes naturally without having to put up any effort. Take surgical wound for instance, suture and antibiotics are subsidiary to speed up and to prevent infection. Likewise, cancer therapy should also be a simple matter, if the therapy follows the process of wound healing. Obviously, the functionality of chemo-surveillance is critical to dictate the success of wound healing [31-33]. Chemo-surveillance has to be damaged for cancer to set in. The progress of cancer contributes to the damage of chemo-surveillance. The progress of cancer invites immunological response that yields TNF to cause cachexia symptoms leading to the damage of chemo-surveillance. Cytotoxic agents cause the acceleration of the damage to chemo-surveillance. Ineffectiveness against CSCs and the contribution to cause the damage of chemo-surveillance are the reason cytotoxic chemotherapy failed to win the war on cancer.

In final analysis, cancer therapy mediated through CDA formulations displays the feature as pro-wound healing, which is the right indication of cancer therapy, because cancer arises due to wound unhealing. Cancer therapy mediated through cytotoxic agents including immunotherapeutic agents displays the feature as anti-wound healing, clearly the contra-indication of cancer therapy. A right approach is the magic code to the success [43], and a wrong approach cannot achieve the solution of a simple matter even supported by a presidential project [2].

Elimination of CSCs is Essential to Save Cancer Patients

MDS are unique diseases to illustrate the evolution of cancer due to wound unhealing, and CDA-2 is a preparation of wound healing metabolites to show excellent therapeutic effect on MDS. MDS often start with a display of immunological disorders [44], which prompts the production of inflammatory cytokines. Among such cytokines, TNF is a critical factor related to the development of MDS [45]. It causes excessive apoptosis of bone marrow stem cells, thus severely affect the ability of the patient to produce hematopoietic cells such as erythrocytes, platelets or neutrophils. TNF is also responsible for the collapse of chemo-surveillance as above described. As a consequence, chemo-surveillance normally operating in health people to keep PSCs in check becomes dysfunctional, allowing PSCs to build up and to evolve into CSCs in order to replenish unipotent stem cells wiped out by TNF. The high level of telomerase expression in the peripheral and bone marrow leukocytes in MDS patients is an indication of the widespread multiplication of CSCs evolving from PSCs [46,47]. The propagating pathological cells have been identified as human CSCs [48]. So, MDS are diseases attributable entirely to the propagation of CSCs. Therapy of MDS requires the induction of differentiation of CSCs to become functional erythrocytes, platelets or neutrophils just like the terminal differentiation of PSCs to complete wound healing. Killing of CSCs cannot cure MDS. So far, Vidaza, Decitabine and CDA-2 are the three drugs approved for the therapy of MDS by the Chinese FDA. CDA-2 is our creation, which was a preparation of wound healing metabolites purified from freshly collected urine [49]. Vidaza and Decitabine are also approved by the US FDA for the therapy of MDS. Professor Jun Ma, Director of Harbin Institute of Hematology and Oncology, was instrumental to conduct clinical trials of all three MDS drugs in China. According to his assessments based on two cycles of treatment protocols, each cycle 14 days as shown in Figures 2,

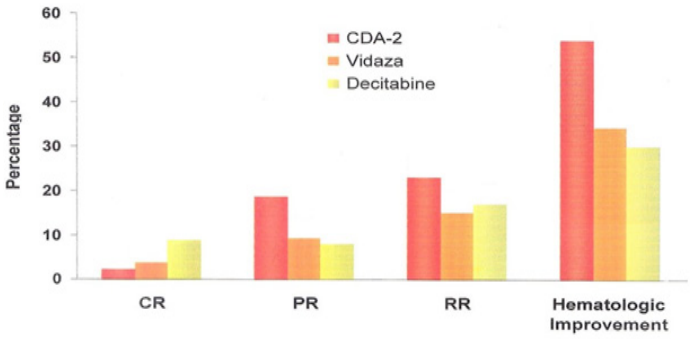

Figure 2: Relative Effectiveness of MDS Drugs.

CDA-2 has a noticeably better therapeutic efficacy based on cytological evaluation, although slower to achieve complete remission, and markedly better therapeutic efficacy based on hematological improvement evaluation, which is an evaluation based on the dependence of blood transfusion. All these drugs achieve MDS therapy by inactivation of MEs, Vidaza and Decitabine by the covalent bond formation between DNA methyltransferase and 5-azacytosine base incorporated into DNA to eliminate MEs [50], whereas CDA-2 destabilizes MEs by the elimination of telomerase [49]. CDA-2 achieves MDS therapy by targeting telomerase of abnormal MEs which is a selective cancer target to constitute abnormal MEs as the most critical issue of cancer [24], a very important cancer target. The action of CDA-2 is devoid of adverse effect, whereas Vidaza and Decitabine eliminate methyltransferase without specificity to affect all normal stem cells, which are known carcinogens [51,52] and very toxic to DNA [53-55]. CDA-2 is obviously a drug of choice for the therapy of MDS with better therapeutic efficacy and devoid of adverse effects. Vidaza and Decitabine should also be commended as rare drugs effective against CSCs. MDS are diseases attributable entirely to CSCs which are ideal for the screen of drugs effective against CSCs. Evidently, induction of terminal differentiation of CSCs is

the only option to cure MDS. Killing of CSCs cannot cure MDS. Most fatal effects of cancer such as metastasis, recurrence, drug resistance, and angiogenesis are the making of CSCs. Elimination of CSCs is extremely important to the success of cancer therapy. It appears that CDA formulations are the best to handle CSCs, since CDA formulations are the natural partners of CSCs on their mission to heal the wound [37,56].

Development of CDA Formulations to Fulfill Cancer Moonshot and to Win the War on Cancer

We have carried out extensive studies of natural and unnatural DIs and DHIs for the formulation of CDA formulations for cancer therapy [14,27,57-62]. Our findings of effective DIs and DHIs are summarized in Table 1 and 2. ATRA is the standard therapeutic drug of acute promyelocytic leukemia [63]. It requires the expression of the receptor of ATRA to activate oligoisoadenylate synthetase to achieve the therapeutic effect. The product of this enzyme oligoisoadenylate is the actual DI [64]. The rest of DIs work directly on abnormal MEs. AA and its metabolites PG derivatives are natural DIs involved in chemo-surveillance. BIBR1532 and boldine are approved cancer drugs as telomerase inhibitors. PGs are approved drugs for the delivery. Drugs requested to change indication do not require clinical trial as long as drugs requested for new indication.

Table 1: Effective Dis.

Dis	ED_{25} (μM)	ED_{50} (μM)	ED_{75} (μM)
ATRA	0.18	0.36	0.75
PGJ2	7.9	13.8	20.5
PGE2	20.6	32	46.5
DicycloPGE2	21	43.5	-
AA	21	42	-
BIBR1532	32.3	43.7	55.1
Boldine	60.1	78.8	94.2

As shown in Table 2, SAHH and MT inhibitors are much better DHIs than MAT inhibitors. MAT is the most stable enzyme of the three MEs. The association with telomerase further increases its stability. Therefore, it is not easy to shake loose of this enzyme. Pregnenolone is a major DHI

Table 2: Effective DHIs.

SAHH Inhibitors	$RI_{0.5}$ (μM)	Signal Transduction Inhibitors	$RI_{0.5}$ (μM)
Pyrvinium Pamoate	0.012	Sutent	0.28
Vitamin D_3	0.61	Berberine	1.62
Dexamethasone	0.75	Vorient	10.1
Beta-Sitosterol	1.72	Gleevec	11.9
Dihydroepiandrosterone	1.79	Selenite	19.7
Prenisolone	2.22		
Hydrocortisone	4.59	Polyphenols	$RI_{0.5}$ (μM)
Pregnenolone	7.16		
MT Inhibitors	$RI_{0.5}$ (μM)	Tannic Acid	0.37
-	-	EGCG	0.62
		Resveratrol	1.16
Uroerythrine	1.9	Curcumin	1.24
Hycanthone	2.1	Kuromanin	1.43
Riboflavin	2.9	Coumestrol	1.95
-	-	Genisteine	2.19
MAT Inhibitors	$RI_{0.5}$ (μM)	Pyrogallol	3.18
-	-	Silibinin	3.8
-	-	Caffeic Acid	3.87
Indol Acetic Acid	220	Ellagic Acid	4.45
Phenylacetylvaline	500	Gallic Acid	5.35
Phenylacetylleucine	780	Ferulic Acid	7.41
Butyric Acid	850	Phloroglucinol	38.82
Phenylbutyric Acid	970	-	-

of CDA-2. Apparently, pregnenolone is an important player of chemo-surveillance. It is the master substrate of steroid metabolites to have a great influence on growth regulation. The production of pregnenolone is bell shape in relation to age with a peak daily production of 50 mg at 20-25 years old [65]. The younger and the older people produce relatively little amounts of pregnenolone, and these are two age groups most vulnerable to develop cancer. Pregnenolone is a single metabolite to greatly influence the evolution of cancer. It is our top choice of DHI to make CDA formulations. The finding of inhibitors of signal transduction as excellent DHIs is not a surprise, since signal transductions produce factors to activate MEs. The finding of polyphenols as effective DHIs is a surprise, but is a good surprise since polyphenols are considered good for health, and greatly promoted as health food.

Effective CDA formulations are made up by DIs and DHIs [14, 27, 57-62]. Effective CDA formulations can be ED_{25} of a DI + 3x $RI_{0.5}$ of a DHI, or ED_{50} of a DI + 2x $RI_{0.5}$ of a DHI, or ED_{75} of a DI + $RI_{0.5}$ of a DHI [57]. We have provided ED of DIs and $RI_{0.5}$ of DHIs for quick design of CDA formulations. $RI_{0.5}$ of a DHI is equivalent to ED_{25} of a DI, which is determined according to the procedure we provided [61]. In the design of CDA formulations, we must take into considerations of non cancer issues such as blood brain barrier of brain cancer, collagen envelop of pancreatic cancer, and hypoxia of melanoma to select DIs and DHIs to overcome non cancer issues. A lot of work remains to be done.

Conclusion

Cancer mortality is primarily caused by CSCs, which are responsible for metastasis, recurrence, drug resistance, and angiogenesis. Solution of CSCs is essential to save cancer patients. Cytotoxic agents are ineffective against CSCs to contribute to cancer mortality of advanced cancer patients. To solve CSCs, induction of terminal differentiation is the only option. Thus, CDA formulations are the best to come to the rescue of metastatic, unresponsive, and recurrent cancer patients, which constitute the major cancer fatalities.

Acknowledgements

We are grateful for the support of the studies on abnormal MEs by Professor Robert B. Hurlbert of MD Anderson Tumor Institute and Professor George C.Y. Chiou of Texas A&M Health Center, the establishment of chemo-surveillance by Dr. Stanislaw R. Burzynski of Burzynski Research Institute, the development of CDA-2 by Mr. Ringo M. L. Chang of Ever Life Pharmaceutical Company, the development of CDA formulations by Professor John P. Fruehauf of the University of California Irvine Medical

Center. We appreciated very much the encouragement on the development of CDA formulations from President Joe Biden through personal communications, who has expressed a desire of collaboration to fulfill cancer moonshot he brought up in 2022.

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