Destabilization of Abnormal Methylation Enzymes as the Only Viable Option for the Elimination of Cancer Stem Cells to Save Cancer Patients

ABSTRACT

Cytotoxic chemotherapy was a tragic by-product of World War II. It was the major therapeutic modality employed but failed to win the War on Cancer during 1971-1976 declared by President Nixon. Despite the failure, killing of cancer cells (CCs) remained the commanding principle of cancer establishments to guide development of cancer therapies throughout. Apparently, this commanding principle is incorrect, since cancer mortality continues to increase.

Cancer evolves due to wound unhealing. Wound healing requires the proliferation and the terminal differentiation of progenitor stem cells (PSCs). PSCs are cells with abnormal methylation enzymes (MEs), which allow cells to gain a great advantage on cell growth. The nature creates chemo-surveillance made up by wound healing metabolites, differentiation inducers (DIs) and differentiation helper inducers (DHIs), as a protection mechanism to keep cells with abnormal MEs under control. Wound unhealing is due to the collapse of chemo-surveillance resulting in the failure to induce terminal differentiation of PSCs.The nature does not have a mechanism to detect the collapse of chemo-surveillance to make the correction. PSCs are forced to proliferate, which is limited by contact inhibition,another protection mechanism the nature createdto limit the buildup of normal stem cells. PSCs are then forced to evolve into cancer stem cells to escape contact inhibition.It takes a single hit to silence TET-1 enzyme, an enzyme responsible to direct lineage transitions, to turn PSCs into cancer stem cells (CSCs). The evolution of CSCs from PSCsis quite easy, since PSCs are equipped with very active abnormal MEs. The propagation of CSCs still cannot heal the wound, because the problem is the collapse of chemo-surveillance. The CSCs will be forced to progress to faster growing CCs by chromosomal abnormalities such as translocations to activate oncogenes, or deletions to inactivate suppressor genes. So, the correct solution of cancer is to restore chemo-surveillance by providing DIs and DHIs to destabilize abnormal MEs to achieve terminal differentiation of PSCs and CSCs which are closely linked to wound unhealing. Killing of CSCs and PSCs cannot heal the wound. Therefore, destabilization of abnormal MEs to achieve terminal differentiation of CSCs is the only viable option to take out CSCs by healing the wound to save cancer patients. The elimination of CSCs is essential to the success of cancer therapy. We have carried out extensive studies of natural and unnatural DIs and DHIs for the manufacturing of CDA formulations to save cancer patients.

Keywords: Cancer therapies; CCs; CSCs; DIs; DHIs; MEs; PSCs.

1. INTROCUCTION

Cancer therapy got to a bad start to rely on cytotoxic chemicals to kill CCs. It was a tragic by-productof World War II. During the war, toxic sulfur mustard gas bombs were used. Victims of toxic gas all displayed depletion of leukocytes in their blood specimens, which inspired oncologists to use toxic chemicals to treat leukemia patients. Cytotoxic drugs became the standard care of cancer therapy, and the disappearance of cancer cells or tumor became the standard criteria for the evaluation of the efficacy of cancer therapy. Cytotoxic drugs and radiation were the major therapeutic modalities employedduring the War on Cancer declared by President in 1971-1976, which was not successful [1].When therapeutic modalities were drilled through as a presidential project that received unlimited support from national resources but failed to achieve the goal, it was fair to conclude that the therapeutic modalities employed were not good for cancer therapy. Apparently cancer establishments agreed to this conclusion and tried to find replacements. They turned to gene and targeted therapies during 1976 to 1996, and then to anti-angiogenesis during 1996-2016 and now to immunotherapy from 2016 onward [2]. They waste 20 years to learn the difficult of gene therapy, and waste another 20 years on the unsuccessful attempt of developing anti-angiogenesis therapy. Can they succeed on immunotherapy? Immunotherapy definitely is a better version than cytotoxic chemotherapy and radiotherapy. The therapeutic target of cell surface antigens after all is a specific cancer target to reduce adverse effects.But immunotherapy has the same draw back as the cytotoxic chemotherapy to show ineffectiveness against CSCs and to cause damage to chemo-surveillance, which are the reason to contribute to the failure of winning the war on cancer. The effect of immunotherapy to cause the damage of chemo-surveillance is much worse than cytotoxic chemotherapy, because immunological response triggering the production of tumor necrosis factor (TNF) is the primary reason to cause the damage of chemo-surveillance. It is doubtful that immunotherapy can save cancer patients better than cytotoxic chemotherapy. Since cancer establishments have not found acceptable replacements, they stayed on to use failed drugs to treat cancer patients. The outcome is expectabe that cancer mortality keeps on increasing. It has reached 10 million annual deaths in 2019 worldwide with an annual increment of 5% as predicted by the NCI experts [3].The mistake of cytotoxic chemotherapy was made at a time when we did not have complete information of cancer. But now we have better knowledge of cancer, we should not make the same mistake in selecting drugs that can only kill CCs. A perfect cancer drug must be able to take out both CCs and CSCs, and to restore the functionality of chemo-surveillance [4].Cancer evolves from wound unhealing due to the collapse of chemo-surveillance [5-11]. Healthy people tend to produce enough metabolites active as DIs and DHIs to ensure destabilization of abnormal MEs of PSCs to achieve terminal differentiation [12, 13].DIs are chemicals capable of eliminating telomerase from abnormal MEs and DHIs are inhibitors of MEs capable of potentiating the activity of DIs. If chemo-surveillance is damaged due to pathological conditions that create cachexia symptoms, then wound cannot be healed to result in the evolution of CSCs from PSCs in order to escape contact inhibition which limits the extent of the proliferation of PSCs. It takes a single hit to silence TET-1 enzyme [14, 15], which is the enzyme responsible to direct lineage transitions, for the conversion of PSCs to become CSCs, a task that can be easily accomplished by PSCs since these cells are equipped with exceptionally active MEs. The propagation of CSCs is still unable to heal the wound, because the problem is the collapse of chemo-surveillance which the nature does not have a mechanism to correct.Chromosomal abnormalities then set in to activate

oncogenes by translocations or to inactivate suppressor genes by deletions to turn CSCs to faster growing CCs. Evidently, damages created by the woundare responsible for the evolution of cancer. Killing of CCs creates more wounds to aggravate the already bad situation and to promote the proliferation of CSCs to work on the damages cytotoxic drugs created [16]. Eventually, the proportion of CSCs will increase from less 2% in the primary cancer which respond well to cytotoxic chemotherapy to reach more than 10 % like that of the primary brain cancer which is unresponsive to cytotoxic chemotherapy [17, 18]. CSCs are protected by drug resistance and anti-apoptosis mechanisms, and, therefore, are unresponsive to cytotoxic chemotherapy and radiotherapy [19-22]. Solution of CSCs is very difficult with cytotoxic approaches. Yet, the solution of CSCs is essential to save cancer patients [23]. Since CSCs are critically linked to wound unhealing, destabilization of abnormal MEs is the only option to solve CSCs [24, 25]. The biological mission of CSCs is wound healing just like that of PSCs. Wound healing metabolites are the partners of their biological mission, and, therefore, can easily access CSCs to achieve induction of terminal differentiation. Cancer establishments are focused on the development of drugs to kill CCs, none of which can affect CSCs.Cancer establishments are not very nicetoput up toxic drugs that cause so many cancer deaths and to block the development of CDA formulations that can solve CSCs to reduce cancer deaths [26]. They should be removed to save cancer patients [27, 28]. We count on supreme authorities of King Charles of England and President Biden of USA to rectify cancer therapies to save cancer patients, King Charles to save himself and President Biden of to achieve his cancer moonshot initiative.

2. COMMENTARIES AND DISCUSSION

2-1. Cancer Evolves as A Consequence of Wound Unhealing

The concept of cancer evolve as a consequence of wound unhealing was first introduced by the great German scientist Virchow in the 19th century [5]. It was again brought up by Dvorak in 1986 [6]. The close relationship between cancer and wound healing was noticed by MacCarthy-Morrough and Martin [7]. We provided the most important details on this subject that included abnormal MEs to promote perpetual proliferation of CCs [29-31]; chemo-surveillance as the nature's creation of allosteric regulation on abnormal MEs to ensure perfection of wound healing to avoid disastrous consequence of wound unhealing [12, 13, 32-34]; DIs and DHIs as wound healing metabolites and also as active players of chemo-surveillance [12, 13, 32-34]; hypomethylation of nucleic acids as a critical mechanism on the induction of terminal differentiation of cells with abnormal MEs [35]; mechanism of wound healing to involve the proliferation and the terminal differentiation of PSCs [8-11]; and the evolution of CSCs from PSCs through a single hit to silence TET-1 enzyme [14, 15, 36]. These studies strongly support the concept that cancer evolves as a consequence of wound unhealing. Our carcinogenesis studies also confirm the validity of this concept. During the challenges with hepatocarcinogens, we noticed the appearance of numerous tiny preneoplastic hyperplastic nodules before the appearance of large size carcinomas, which displayed abnormal MEs [37]. These tiny

hyperplastic nodules must represent the proliferation of PSCs in the process of active wound healing. Most of these tiny nodules disappeared shortly afterward, indicating completion of wound healing, and only those which did not disappear later developed to become large size carcinomas. During the challenges with hepatocarcinogens, if the animals were provided Antineoplaston 10, which was phenylacetylglutamine effective as anti-cachexia chemical [12], the development of hepatocarcinomas could be prevented [38]. Therefore, cancer evolves as a consequence of wound unhealing due to the collapse of chemo-surveillance is a valid concept. Cancer therapy based on pro-wound healing approach we advocate is, therefore, the most appropriate modality of cancer therapy [8, 13, 16, 24, 25, 34, 39, 40]. We have attributed the success of extremely difficult moon shot project of President Kennedy to the assumption of right approach. A right approach is the magic code to the success [41]. The commanding principle of killing CCs preferred by cancer establishments is an anti-wound healing approach, clearly a wrong approach for cancer therapy. No wonder it has not been successful even supported as a presidential project.

2-2. CDA-2 as A Persuasive Good Cancer Drug for the Elimination of Cancer Stem Cells

Myelodyaplastic syndromes (MDS) are a classic caseof cancerwith a clear connection to wound unhealing, and CDA-2 is a preparation of wound healing metaboliteas the drug of choice for the therapy of MDS.MDS often start with a display of an immunological disorder [42],which prompts the production of inflammatory cytokines. Among cytokines produced TNF is a critical factor related to the development of MDS[43]. 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 due to its effect to causeblood vessel hyperpermeability [44, 45], resulting in the excessive urinary excretion of low molecular weight metabolites.Wound healing metabolites are among low molecular weight metabolite excreted to result in the collapse of chemo-surveillance, which the nature does not have a mechanism to rectify. The evolution of cancer starts from the collapse of chemo-surveillance. The high level of telomerase expression in the peripheral and bone marrow leukocytes in MDS patients is an indication of the wildspread multiplication of CSCs evolving from PSCs[46, 47]. The expansion of PSCs is limited by contact inhibition.MDS are diseases at the stage of CSCs evolving from PSCs. The propagating pathological cells of MDShave been identified as human CSCs [48].So, MDS are diseases attributable entirely to the propagation of CSCs. These diseases are ideal for the development of drugs effective against CSCs. So far, Vidaza, Decitabine and CDA-2 are the three drugs approved for MDS therapy by the Chinese FDA. Vidaza and Decitabine are also approved for the therapy of MDS by the US FDA.Professor Jun Ma, the Director of Harbin Institute of Hematology and Oncology, is instrumental in conducting clinical trials of these three MDS drugs for the approval by the Chinese FDA. According to his assessmentsbased on two cycles of treatment protocols, CDA-2 had a noticeable better therapeutic efficacy, although slower to achieve complete remission, based on cytological evaluation, and marked better therapeutic efficacy based on hematological improvement evaluation, which was based on the dependence of blood transfusion to stay healthy as shown in Figure 1, which is reproduced from the reference [49].All three drugs achieve MDS therapy by the inactivation of abnormal MEs,

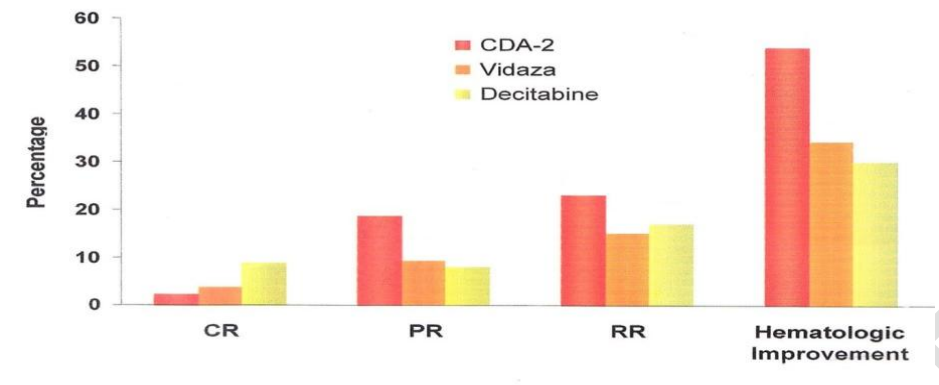

Fig. 1. Relative effectiveness of MDS drugs

Vidaza and Decitabine by the covalent bond formation of methyltransferase and the 5'-aza-cytosine baseincorporated into DNA [50], whereas CDA-2 by the elimination of telomerase of abnormal MEs [51]. The action of CDA-2 is selective on the tumor factor of abnormal MEs, whereas the action of Vidaza and Decitabine is non-selective to affect MEs of all stem cells. Thus, CDA-2 is without adverse effects, whereas Vidaza and Decitabine are known carcinogens [52, 53], and very toxic to DNA [54-56]. Obviously, CDA-2 is the drug of choice for the therapy of MDS with a better therapeutic efficacy and devoid of adverse effects.Induction of terminal differentiation of CSCs like the induction of terminal differentiation of PSCs in wound healing is obviously the only optionto solve CSCs.Killing of CSCs cannot heal MDS. So that destabilization of abnormal MEs is the only viable option for the elimination of CSCs to save cancer patients. The elimination of CSCs is essential to save cancer patient [23]. Cancer establishments blocked Antineoplastons, which were preparationssimilar to CDA-2.Apparently, these preparationsarethe only viable option for the elimination of CSCs to save cancer patients.Killing of CSCs and CCs favored by cancer establishments cannotsolve the issue related to CSCs.

2-3. Abnormal MEs as the Most Critical Issue of Cancer

Perpetual cell proliferation is the most outstanding feature of cancer. Obviously,cancer is basically a problem of growth regulation going awry. MEs play a pivotal role on the regulation of cell replication and differentiation, and, therefore, are closely related to the issue of cancer [50, 56, 57].Since MEs play such an important role on the regulation of cell growth, they are subjected to exceptional double allosteric regulations: one on the individual enzymes and the other on the enzyme complex [32]. Enzymes involve in important biological regulation are often subjected to delicate regulations. Allosteric regulation is the most pervasive regulation to maintain biological equilibrium to avoid extreme often to result in creating clinical symptoms.MEs are a ternary enzyme complex consisting of methionine adenosyltransferase (MAT)-methyltransferase (MT)-S-adenosylhomocysteine hydrolase (SAHH) [58].In steroid

hormone target organs, SAHH is the steroid hormone receptor. SAHH requires a steroid hormone to assume a stable configuration in order to form a dimeric complex with MT. SAHH-MT dimer has a molecular size similar to MAT. These three enzymes are then form a ternary enzyme complex, which is the stable and active functional unit of MEs. In the absence of steroid hormone, the ternary enzyme complex dissociates into individual enzymes to lose activity. MT in the monomeric form has a tendency to be modified to become nuclease which can cause damages to trigger apoptosis, resulting in the involution of the steroid hormone target organs. In telomerase expressing cells such as embryonic stem cells(ESCs) and PSCs which are also ESCs, MEs are associated with telomerase. The association changes kinetic properties of MEs and the regulation greatly in favor of cell growth. $K_m$values of telomerase associated MAT-SAHH isozyme pair are 7-fold higher than those of normal isozymes [28-30].The increased K_m values indicate that abnormal MEs hold a higher level of S-adenosylmethionine (AdoMet) and S-adenosylhomocysteine (AdoHcy), which are important for the stability of MEs to promote growth of cells with abnormal MEs as Prudova et al. foundAdoMet could protect protein from protease digestion [59], and Chiba et al. found when HL-60 cells were induced to undergo terminal differentiation the pool sizes of AdoMet and AdoHcy shrunk greatly [60].Obviously, abnormal MEs play an important role to promote the growth of cells with abnormal MEs. Normal stem cells with abnormal MEs do not seem to cause problems, because normal stem cells are protected by safety mechanisms such as contact inhibition, TET-1 enzyme to direct lineage transitions and chemo-surveillance as a brake to prevent the buildup of cells with abnormal MEs. Evidently, abnormal MEs are important for the normal functions of cells with abnormal MEs. Premature interruption of the normal functioning of abnormal MEs, e.g. the application of thalidomide, is detrimental, resulting in malformation of body parts notably limbs.It is a horror balance, any misstep will result in disastrous consequences. There is no room for mistake. We have to solve cancer very precisely!

Both abnormal MEs and chromosomal abnormalities for the activation of oncogenes or inactivation of suppressor genes are very critical issues of cancer. Abnormal MEs are responsible for the blockade of differentiation for the proliferation to carry on, and chromosomal abnormalities are responsible to promote proliferation. Which is more important, the blockade of differentiation or the activation of proliferation? Most people bet on the activation of proliferation, including cancer establishments. They are wrong. Activation of proliferation happens quite late in the carcinogenesis process. There are multiple ways to activate proliferation. One way is solved. There may soon pop up another way to negate the previous effort. It is an endless struggle to put out activation of proliferation. The cancer establishments tried to put out activation of proliferation during 1976-1996. They learned the difficult of gene therapy, and gave up. Destabilization of abnormal MEs is the bestsolution. Abnormal MEs happen on PSCs, the precursors of CSCs, and pass on to CSCs and CCs. Abnormal MEs are universal to all human cancers [36]. A stroke to solve abnormal MEs, can also put to rest chromosomal abnormalities to activate proliferation. After all, oncogenes and suppressor genes are cell cycle regulatory genes. They have important roles to play when cells are in cell cycle replicating. But if replicating cells exit cell cycle to undergo terminal differentiation, they have no roles to play. So, obviously, abnormal MEs are the bullseye of cancer therapy. Destabilization of abnormal MEs is the only option to solve CSCs, and the solution of CSCs is

essential to save cancer patients.Cancer establishments must approve CDA formulations for the sake of saving cancer patients, which is also their goal.

2-4. Development of CDA Formulationsfor the Solution of CSCs to Save Cancer Patients

We have carried out extensive studies of natural and unnatural DIs and DHIs for the manufacture of CDA formulations [2, 8, 23-25, 34, 36, 39, 51, 46, 58, 63-72]. Our findings of effective DIs and DHIs are summarized in Table 1 and 2.ATRA is the standard care of acute

Table 1. Effective DIs

DIs	ED_{25} (μM)	ED_{50} (μM)	ED_{75}(μM)
	ATRA	0.180.36	0.75
	PGJ27.9	13.8	20.5
PGE2	20.6	32.0	46.5
	DicycloPGE221.0	43.5-	
AA	21.0	42.0	-
BIBR1532	32.3	43.7	55.1
	Boline	60.1	78.8 94.2

promyelocytic leukemia [73].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 [74].The therapeutic effect of ATRA on promyelocytic leukemia is excellent, achieving 90% complete remission by a single agent, almost reachingthe goal of 100% complete remission by a single agent demandedby cancer establishments.DI alone cannot achieve 100% remission. DI alone creates damages, e.g. nuclease attack on the replicating DNA, to interrupt the completion of differentiation.The damages can be repaired to resume malignant growth. Therefore, the complete remission achieved by ATRA alone cannot last very long. But if a DHI is applied with DI, that can prevent the dissociation of MT-SAHH dimer, then the possibility of MT monomer to become nuclease to cause the problem of recurrence can be greatly reduced. Therefore, it is a good policy to use DI and DHI to achieve good therapy of cancer. Multiple agents are good for the completion of cancer therapy.Arsenic acid is a DHI, which can prevent recurrence of ATRA alone.Arsenic acid is very toxic. We have found excellent DHIs presented in Table 2which are nontoxic to function as DHI to prevent the recurrence of ATRA alone. The rest of DIs presented in Table 1 work directly on abnormal MEs. AA and its metabolites PG derivatives are natural DIs involved in chemo-surveillance. PG derivatives are approved drugs for the delivery. BIBR1532 and boldine are approved cancer drugs as telomerase inhibitors. Application of change of indication of the approved drugs should not take as long as the application for the approval of new indication.
Table 2. Effective DHIs

SAHH Inhibitors	$RI_{0.5}$ (μM)	Inhibitors	$RI_{0.5}$ (μM)
Pyrivinium 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		
Pregnenolone	7.16		

MT Inhibitors	$RI_{0.5}$ (μM)
Uroerythrin	1.9
Hycanthone	2.1
Riboflavin	2.9

MAT Inhibitors	$RI_{0.5}$ (μM)	Silibinin	3.80
Indol Acetic Acid	220	Caffeic Acid	3.87
Phenylacetylvaline	500	Ellagic Acid	4.45
Phenylacetylleucine	780	Gallic Acid	5.35
Butyric Acid	850	Ferulic Acid	7.41
Phenylbutyric Acid	970	Phloroglucinol	38.82

Polyphenols	$RI_{0.5}$ (μM)
Tannic Acid	0.37
EGCG	0.62
Resveratrol	1.16
Curcumin	1.24
Kuromanine	1.43
Coumestrol	1.95
Genisteine	2.19
Pyrogallol	3.18

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 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 around 50 mg at 20-25 years old [75]. The youngest and the oldest people produce relatively smaller amounts, and these are the two age groups most vulnerable to develop cancer. Pregnenolone is a single metabolite to have a great influence on the evolution of cancer. It is our top choice of natural DHI. The finding of inhibitors of signal transduction as excellent DHIs is not a surprise, since signal transductions produce factors to promote cell growth. Gleevec is the standard care of chronic myeloid leukemia and GIST [76]. Thus, DIs and DHIs can be very effective cancer drugs. The finding of polyphenols as excellent DHIs is a surprise, but is a good surprise since polyphenols are considered good for health, and greatly promoted as health food. The finding of polyphenols as excellent DHIs increases their credibility as health food.

Effective CDA formulations can be made by DIs and DHIs with different formulations as 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 [69]. We have provided these data in Table 1 and 2. $RI_{0.5}$ of a DHI is equivalent to ED_{25} of a DI, which can

be determined by the procedure previously described [67]. 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.It is very important too that we have to come up easy procedures for the evaluation of therapeutic endpoint of CDA formulations. A lot of work remains to be done.

3. CONCLUSION

Cancer evolves as a consequence of wound unhealing. Wound healing process is the most appropriate modality of cancer therapy. The commanding principle of killing CCs opposing wound healing process the cancer establishments assumed for cancer therapy in the past is incorrect. That is why that approach failed to win the war on cancer declared by President Nixon in 1971-1976, and continued to cause the increase of cancer mortality. Destabilization of abnormal MEs is a critical mechanism of wound healing, which is also the only viable option for the elimination of CSCs.The elimination of CSCs by induction of terminal differentiationis critically linked to the completion of wound healing, and the elimination of CSCs is essential for the success of cancer therapy to save cancer patients.CDA formulations are the best drugs for the induction of terminal differentiation of CSCs.

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