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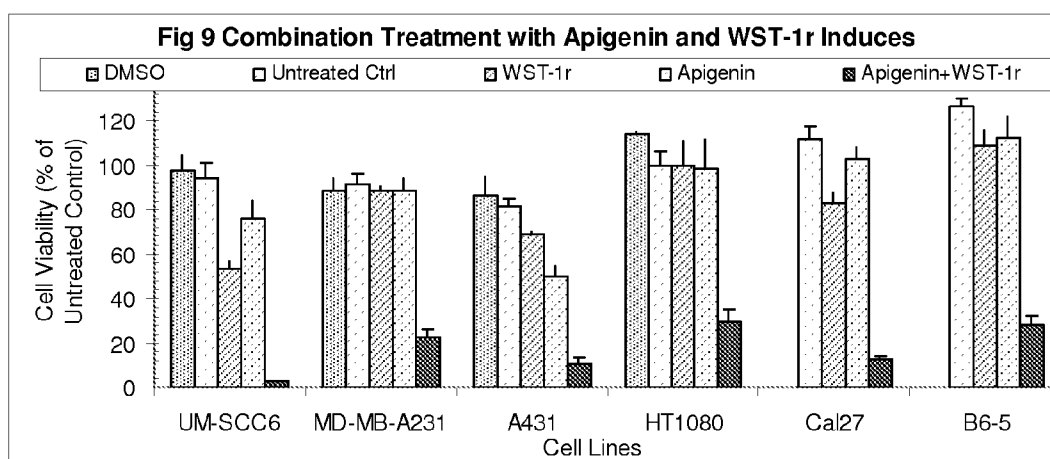
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(54) Title: METHODS, COMPOSITION, TARGETS FOR COMBINATIONAL CANCER TREATMENTS



(57) Abstract: This invention describes methods and pharmaceutical compositions for combinational cancer treatments that are capable of inducing JNK phosphorylation and induce programmed cell death. It also identified genes as target for anti-cancer drug development and enhancement of the chemotherapeutic drug effect for the treatment of cancer. This invention points to a novel method and principle for a new avenue of developing more efficient and low or non cytotoxic cancer treatment.

METHODS, COMPOSITION, TARGETS FOR COMBINATIONAL CANCER TREATMENTS

Related Application

This application claims the benefit of U.S. Provisional Application No. 60/929,535, filed Jul. 2, 2007, which are herein incorporated by reference in their entirety.

Government Interests

The research carried out in the present application was supported in part by NIH. The government may have certain rights in the invention of the present application.

DESCRIPTION OF THE INVENTION

TECHNICAL FIELD OF THE INVENTION

This invention relates to the fields of oncology and chemotherapy. Specifically, the invention provides novel methods, pharmaceutical composition and targets for more efficient and less or non cytotoxic treatments of cancer.

BACKGROUND ART OF THE INVENTION

Up to date, chemotherapy and radiation therapy are still the mainstream for cancer treatment. These treatments were based on targeting proliferating cells rather than cancer cells only. All these treatments are cytotoxic to bone marrow cells and mucosa and the side effects of these treatments can be lethal and at extremes usually are lethal. Efforts for improving these treatments by synergizing the drug effect to lower the drug dose and for alternative treatments have been actively undergoing development. There remains a need in the art for improved methods of enhancing the efficacy of cancer treatment while limiting the cytotoxic side effects. The present invention is directed to a method of treating cancer by combination of non-cytotoxic agents, which leads to synergistic selective killing of cancer cells.

Targeted therapy, a new generation of cancer treatment, is aimed to target cancer specific changes of molecules and signaling pathways to induce cancer cell death, but limit such effects on normal cells. Enormous efforts have been made in finding the targets and the ways of targeting the targets inside the cells as a treatment. However, up to date, the success rate of this new generation is limited. One major challenge comes from the complexity of cellular

regulation mechanisms and overlapping pathways inside the cells. Combination treatment is the key for overcoming these overlapping pathways and redundant regulation mechanisms.

Nuclear factor-kappa B (NF- κ B) is a family of Rel proteins functioning as a transcription factor. Its activities have been involved in stress induced cellular responses and multiple pathogenic processes, such as infection and inflammation, oxidative stress, injury and programmed cell death. Aberrant NF- κ B activation has been detected in a variety of tumors and cancer cells have been associated with oncogenesis, regulation of cell proliferation, control of apoptosis, angiogenesis, tumor invasion and metastasis as well as cancer cell resistance to chemotherapy and radiation therapy Treatment (Kim HJ, Hawke N, and Baldwin AS, NF- κ B and IKK as therapeutic targets in cancer, *Cell Death and Differentiation*, (2006) 13:738-47; Karin M, Nuclear factor- κ B in cancer development and progression, (2006) *Nature* 441:431-6). Efforts have been made to Inhibit NF- κ B activity to facilitate cancer cell death and sensitize cancer cells to chemotherapy drugs and radiation therapy (Kim HJ, Hawke N, and Baldwin AS, NF- κ B and IKK as therapeutic targets in cancer, *Cell Death and Differentiation*, (2006) 13:738-47; Karin M, Nuclear factor- κ B in cancer development and progression, (2006) *Nature* 441:431-6; Chikashi Nakanishi and Masakazu Toi, Nuclear Factor- κ B Inhibitors As Sensitizers To Anticancer Drugs, *NATURE REVIEWS CANCER* (2005) 5:297-309). However, these efforts have yielded results far from spectacular.

Normally, in quiescent cells NF- κ B stays in cytoplasm in inactive form by binding to Inhibitor κ B (I κ B) and is activated by external stimuli through signaling processes. Inhibitor κ B Kinases (IKK) are the direct upstream NF- κ B activator of the signaling chain, which phosphorylate I κ B to release NF- κ B to translocation into the nucleus, to regulate transcription in the cell. There are two similar, but different IKKs that are capable of activating NF- κ B. Each may respond to different signaling pathways and activate different members of the NF- κ B family. In addition, alternative NF- κ B activation pathways, such as protein kinase CK2 (CK2), also exist. In most cancer cells, NF- κ B is constitutively activated. In addition to the IKK classic pathway, these alternative NF- κ B activation pathways may also contribute to the aberrant NF- κ B activity in cancer cells (Ming Yu, Jason Yeh, and Carter Van Waes Protein Kinase CK2 Mediates Inhibitor-Kappa B Kinase and Aberrant Nuclear Factor- κ B Activation by Serum Factor(s) in Head and Neck Squamous Carcinoma Cells *Cancer Research*, 2006 July 1; 66(13): 6722–6731. and other for NF κ B activation). Dozens of IKK inhibitors have been produced and are in trials for treating anti-inflammatory diseases, but not so effective to cancer therapy. These alternative NF- κ B activation pathways may be at least in part the cause of the disappointing

efficacy outcomes of IKK inhibitors in cancer treatment (Chikashi Nakanishi and Masakazu Toi, Nuclear Factor- κ B Inhibitors As Sensitizers To Anticancer Drugs, NATURE REVIEWS CANCER (2005) 5:297-309).

NF- κ B is known to inhibit programmed cell death, including apoptosis and necrosis, by inducing transcription of antiapoptotic genes, interfering caspases activation and inhibition of prolonged activation of c-JUN N-terminal kinase (JNK) (Chikashi Nakanishi and Masakazu Toi, Nuclear Factor- κ B Inhibitors As Sensitizers To Anticancer Drugs, NATURE REVIEWS CANCER (2005) 5:297-309). Inhibition of NF- κ B and IKK leads to tumor cell death, growth inhibition and/or sensitizing cancer cells to chemotherapy treatment (Kim HJ, Hawke N, and Baldwin AS, NF- κ B and IKK as therapeutic targets in cancer, Cell Death and Differentiation, (2006) 13:738-47; Chikashi Nakanishi and Masakazu Toi, Nuclear Factor- κ B Inhibitors As Sensitizers To Anticancer Drugs, NATURE REVIEWS CANCER (2005) 5:297-309). Proteasome inhibitor, Bortezomib/Velcade (PS341) that can block I κ B α degradation has been approved for the treatment of multiple myeloma, a disease dependent on NF- κ B activation. IKK is an upstream regulator of NF- κ B activation and NF- κ B activity. More than a dozen of IKK2 inhibitors have been developed for inhibiting NF- κ B activation (Michael Karin, Yumi Yamamoto and Q. May Wang, The IKK NF-KAPPAB System: A Treasure Stove for Drug Development, Nature Reviews|Drug Discovery 3:17-26, January, 2004). Most of them are targeting the treatment of inflammatory diseases. Several IKK inhibitors have show efficacy in hematological malignancies¹. On the other hand, clinical efficacy of these IKK inhibitors for treatment of solid tumor is missing. Some of these inhibitors have shown partial inhibitory effects on NF- κ B activity at functional levels and inhibitory effect on cell survival and proliferation in vitro. A few reports showed inhibitory effects of IKK inhibitors on tumor growth in vivo (Hu MC, and Hung MC, Role of IkappaB kinase in tumorigenesis, (2005) Future Oncol. 1:67-78; Luo JL, Kamata H, and Karin M, IKK/NF-kappaB signaling: balancing life and death-a new approach to cancer therapy, (2005) J. Clin Invest 115:2625-32). However, these successes are very limited. Most efforts using IKK inhibitors for an anti-tumor drug were not so successful and far from practical. This may be because of the complexity of the regulatory mechanisms and other effects from these inhibitors. To identify key target processes leading to cancer cell death and combinational targeting at multiple points of the process is crucial for achieving a successful breakthrough.

It has been reported that TNF α , which is known to induce NF- κ B activation, induces cell death under certain circumstances (Ref). More recent studies pointed to the balance between NF- κ B activity and JNK activity, which regulates cell death or proliferation (reviews). In this

theory, NF- κ B and JNK cross talk through reactive oxygen species (ROS). TNF α induces activation of both NF- κ B and JNK. Both JNK and NF- κ B activity leads to Cell proliferation. However, ROS induces JNK activation and prolonged JNK activation will induce programmed cell death. Conversely, activated NF- κ B suppresses ROS and, hence, ROS induced JNK activation. Therefore, by inhibiting NF- κ B while activating JNK switches the balance to programmed cell death. To date, no such treatment method has been reported. Most importantly, although this specific theory of relationships has been proposed, none have prior succeeded in demonstrating this effect. Herein, I will disclose the means to demonstrate and thus validate this specific series of relationships.

I have observed that combined inhibition of IKK1 and IKK2 by dominant negative IKK1+2 DNA vectors led to synergistic inhibition of expression of NF- κ B inducible genes. However, this combination showed little effect on improving the efficacy on enhancing chemotherapy drugs. At the same time, I observed that when measuring combined effect of dominant negative IKK1 DNA vector in combination with IKK2 inhibitors on human cancer cells and using WST-1 reagent for monitoring cell viability, this combination caused excess dramatic cell death after changing back to normal growth medium followed by overnight incubation. The parallel treatment during the same experiment with no DNA transfection did not cause any cell death (Fig 1, 2). The three components required for this treatment include Dominant negative IKK1 DNA transfection, IKK inhibitor and WST-1 treatment. This process included activation of JNK and induction of ROS (Fig 5), which fits in the proposed JNK-ROS-NF- κ B theory.

Further characterization showed that this effect was not related to the dominant negative IKK1 DNA sequence. Instead, the cotransfected Puc19 DNA vector backbone played a crucial role in this triple combination effect (Fig 3). The transfection of Puc19 vector in this combination can be partially substituted by interferon beta and alphas (IFN) when it is used as the initial treatment (Fig 4). The Puc19 DNA sequence was found to play an important role in this triple combination treatment. Blast analysis of the Puc19 DNA sequence against human transcripts and human genome revealed several short matches to human transcripts and to flanking regions of multiple genes. The siRNAs of some of these sequences and the siRNAs against some of these genes were capable of acting as substitutes for the Puc19 for the triple combination treatment suggesting that interfering function of other genes may also be required for this triple combination treatment.

The Cell Proliferation WST-1 reagent is a research reagent used for measuring cellular metabolism, which is used as an indicator for live cells. The Cell Proliferation WST-1 is

composed of WST-1, a tetrazolium salt, and 1-mPMS, an electron coupling reagent. WST-1 is a cell plasma membrane impermeable tetrazolium salt that can be reduced to form water-soluble formazan. WST-1 is reduced by trans-plasma membrane electron transport via the electron carrier, 1-methoxyPMS (mPMS), in which case the cellular reductant is NADH (Berridge, Herst, and Tan, *Biotechnology Annual Review* Vol.2:127-52). This process involves NOX and generates ROS.

ROS are potentially harmful by-products of normal cellular metabolism that directly affect cellular functions. ROS is generated by all aerobic organisms and it seems to be indispensable for signal transduction pathways that regulate cell growth and reduction-oxidation (redox) status. However, overproduction of these highly reactive oxygen metabolites can initiate lethal chain reactions, which involve oxidation and damage to structures that are crucial for cellular integrity and survival. In fact, many antitumor agents, such as vinblastine, cisplatin, mitomycin C, doxorubicin, camptothecin, inotamycin, neocarzinostatin and many others exhibit antitumor activity via ROS-dependent activation of apoptotic cell death, suggesting potential use of ROS as a fundamental antitumor principle. The "oxidation therapy" a unique anticancer strategy by inducing the generation of ROS directly to solid tumors as cytotoxic oxystress for cancer treatment has been developed. However no successful and practical results were obtained probably because of the lack of tumor selective ROS delivery and hence resulting in subsequent induction of severe side effects (Fang, Nakamura, and Iyer, *J Drug Target* Vol15: 475-86).

The majority of cancer cells are defective in mitochondrial (mitochondria) and hence, depend more on glycolysis for energy production, which accumulates NADH in cytoplasm. Lacking in mitochondrial for metabolism makes these cancer cells more dependent on trans-plasma membrane electron transport (Herst et al. *Biochim Biophys Acta* 1656:79-87; Tan and Berridge *Redox Rep* 9:302-06). This feature of cancer cells allows this combination treatment to be specific in order to target this metabolic pathway in cancer cells.

The WST-1 and the mPMS, each represent a class of chemicals that can be used for this goal. The concentration of WST-1 and mPMS as well as the ratio between the two, could be adjusted and optimized to maintain synergistic induction of cancer cell death while avoiding triggering the direct toxicity by the ROS. The finding of this combination treatment protocol provided a method of inducing low level ROS generation to function as a messenger for signaling pathways of the cells. The cell death was induced by the combinational treatment, but not by the WST-1 reagent alone. This specific finding, used as a treatment, is able to induce the production of ROS for signaling while avoiding massive cytotoxicity.

Apigenin is a naturally occurring plant flavone (4', 5, 7,-trihydroxyflavone) abundantly present in common fruits and vegetables including apple, parsley, onions, oranges, tea, chamomile, wheat sprouts and some seasonings. Apigenin has been shown to possess remarkable anti-inflammatory, antioxidant and anti-carcinogenic properties and is under active study. Studies on the biological effects of apigenin at cellular and molecular levels have found that apigenin interferes with a wide range of critical molecules and signaling and regulatory pathway of the cells, including depleting the HER2 protein and suppressing the Her2/Her3-phosphatidylinositide 3-kinase/AKT pathway (Way and Lin *Future Oncol* 1:841-49), inhibit HIF, PKC, CDK, VEGF NF- κ B, CK2, AKT, MAPK, AR and ER pathways, activate wild type p53, modulate the deregulated cell cycle checkpoint and induce apoptosis (Induction of caspase-dependent, p53-mediated apoptosis by apigenin in human neuroblastoma -- Torkin et al. 4 (1): 1 -- ...;Apigenin Inhibits Expression of Vascular Endothelial Growth Factor and Angiogenesis in Human Lung Cancer Cells: Implication of ...;Apigenin inhibits VEGF and HIF-1 expression via PI3K/AKT/p70S6K1 and HDM2/p53 pathways -- Fang et al. *The FASEB* 19 (3): 342 -- ...;Balasubramanian and Eckert *Toxicol Appl Pharmacol* 224:214-19;Birt et al. *Carcinogenesis* 7:959-63;Patel, Shukla, and Gupta *Int J Oncol* 30:233-45;Sato et al. *Biochem Biophys Res Commun* 204:578-84). In addition, apigenin has also been reported to generate ROS, which disrupt mitochondrial membranes. Current research trials indicate that it may reduce DNA oxidative damage; inhibit the growth of human leukemia cells and induced these cells to differentiate; inhibit cancer cell signal transduction and induce apoptosis; act as an anti-inflammatory; and as an anti-spasmodic or spasmolytic. More than 100 patents applications related to apigenin have been filed. Among those, apigenin was claimed to be used as a drug for treating inflammatory and autoimmune diseases. In addition, apigenin was also claimed for the use as a cancer chemoprevention drug and for synergizing chemotherapy drugs for cancer treatment at 10 μ M concentration (US Patent Application 20060189680). Research to use apigenin as cancer chemoprevention drug are actively going on by multiple research groups. However, for using apigenin as an enhancement for chemotherapy treatment, my in vitro data showed different results. By the data from three human cancer cell lines (HT1080, a human soft tissue sarcoma cell line and Cal27 and UM-SCC6 cells, human head and neck squamous carcinoma cell lines) apigenin at up to 30 μ M in combination with three chemotherapy drugs, Taxel, Cis-platinum, and Doxorubicin, showed no combined effect in vitro. Also from my data, apigenin by itself at up to 100 μ M showed little effect on cell death. To be a cytotoxic drug for treating cancer, apigenin has to be combined with other treatments.

My data showed that 10-30 μ M apigenin is capable of substituting for the puc19 transfection and IKK inhibitor, in combination with WST-1 reagent to reach the synergetic induction of cancer cell death. This effect is Apigenin and WST-1, dose and time dependent, and is highly reproducible for multiple different human cancer cell lines.

Apigenin inhibits CK2, through which it also inhibits IKK and NF- κ B. WST-1 treatment generated ROS. This combination treatment did show induction of ROS generation and JNK activation, which provide supporting evidences to the ROS-JNK-NF- κ B theory and the JNK-NF- κ B balance in determining cell life or death. On the other hand, two more tested CK2 inhibitor did not show this inhibitory effect. siRNA against CK2 only showed weak inhibitory effect. IKK inhibitor did not show this effect except for one human melanoma cell line. As described above, apigenin may affect even more critical molecules and signaling pathways. The function of apigenin in this combination treatment is unique. The underlying mechanism must be much more complicated than we now recognize.

Normally, the electron transfer occurs on the mitochondrial membrane. The WST-1 reagent induced the generation of ROS through electron transfer across the plasma membrane to convert the NADH to NAD⁺ in cytoplasm. As the majority of cancer cells are defective in their mitochondrial energy metabolism and more depend on glycolysis in the cytoplasm, which will generate NADH in the cytoplasm, this combination treatment provides a novel way to potentially target cancer specifically.

WST-1 reagent is composed of WST-1 and mPMS that may represent a class of tetrazolium salts and a class of electron coupling reagents, respectively. This reagent is capable of conducting trans-plasma membrane electron transfer, the process that generates ROS. One of the significant features of this treatment is that the level of ROS that is generated by this treatment is controllable through optimizing the concentration and the ratio of the two components. It provides a way for the induction of controlled level of ROS from the cells that can act as signaling molecules to induce the specific signal that will lead to programmed cell death rather than to destroy the cells directly.

High level of ROS causes damages to all kinds of macro molecules in the cells and could be catastrophic to normal cells, as well. Other's previous efforts with ROS induction agents as a cancer treatment failed because of this high toxicity. On the other hand, low level ROS may function as a messenger involved in signaling pathways of the cells. This specific finding, used selectively as a treatment, may be able to induce the production of ROS for signaling while avoiding massive cytotoxicity. The WST-1 reagent, the concentration of WST-1 and mPMS as well as the ratio between the two, has been adjusted and optimized to maintain synergistic

induction of cancer cell death while avoiding the direct toxicity from the ROS. The cell death was induced by the combinational treatment, but not by the WST-1 reagent alone.

In addition, this treatment includes use as pulsed treatment, which may further limit any potential toxicity from long term treatment.

The significance of this invention includes:

- This treatment represents a way for targeting a metabolic pathway that is mainly relied on by cancer cells.
- This treatment indicates a way of selectively controlled induction of ROS in cancer cells for signaling as a prerequisite yielding a subsequent method of treatment.
- This treatment is an inducer of prolonged induction of JNK phosphorylation and activation and induction of cell necrosis.
- Each of the inhibitors or reagent chemicals, such as WST-1, that are used for this combination treatment represent classes of chemical(s) as potential candidates for each of the components of this treatment. Some other members in these classes have also been examined as to their potential use for this treatment. The whole therapy is new and given the high cancer cell death outcome and potential capability of selectively targeting cancer cells, each stage and each product becomes a new "cancer" target avenue. For example, WST-1 was formerly a reagent. Now, it will be recognized as a cancer therapeutic. Prior to this discovery and disclosure, WST-1 has never been explored as a cancer treatment.
- This invention provides new avenues for anti-cancer drug development

In this present invention, evidence of combinational treatment by the inhibition of both IKK1 and IKK2 leading to synergistic inhibition of NF-KAPPAB constitutive activity in tumor cells and that treatment of cancer cells with tetrazolium salts, WST-1, following the inhibition of NF-κB activity synergistically facilitate cancer cell death are described. The combination of the two findings and the implication for WST-1 will have profound effect delivering a new avenue of pharmaceutical development for a novel, efficient cancer therapies.

Combined Treatment of Apigenin and Stattic Synergistic Inhibition of Cal27 Cell Survival And Induced Cell Death

In addition to NF-κB, Signal transducer and activator of transcription (Stat) is another family of transcription factors. They mediate extra cellular signals stimulated by cytokines and growth factors, translocation to the cell nucleus where they act as transcription activators. These proteins mediate the expression of a variety of genes in response to cell stimuli, and thus play a key role in many cellular processes such as cell growth and apoptosis. Stat, such as STAT3, play an important role in cancer cells survival and proliferation. However, Stat Inhibitors or IKK

inhibitors alone showed little inhibiting effect on cancer cell survival. Evidence showed that these two transcription factors interact with each other and to functionally cooperate with each other. In addition, NF- κ B and STAT binding sites linked together to form promoter modules. Combination of Stattic, a Stat inhibitor with either IKK inhibitor or apigenin results in synergetic induction of cell death. This combination provides a method of treating cancer. The finding of some DNA and siRNA sequences and their corresponding genes as potential targets for cancer treatment.

As described above, the Puc19 DNA vector transfection is necessary for reaching the efficacy effect for the triple combination, combination of Puc19 transfection with chemotherapy drugs, could reduce the IC₅₀ of all four tested chemotherapy drugs (paclitaxel, Doxorubicin, Cis-platinum, and 5-FU) by a factor of 3-30 fold. siRNAs with the sequences that derived from these short matches and siRNA against the corresponding genes of some of these matches substitute the Puc19 DNA vector in combination with chemotherapy drugs. The sequences, thus identified, represent some rarely studied genes. Some of those are still hypothetical genes, meaning they have not been studied yet. The finding of these genes may provide potential targets for anti-cancer drug development.

The basic findings of this invention include the use of WST-1r as a drug for combinational treatments of cancer and the biological functions coded in the DNA sequence of Puc19, which led to the identification of some genes as target for cancer therapy and for enhancing efficacy of chemotherapy drug effects. Based on these findings, several methods for cancer treatment by combination with other chemicals, biological molecules and chemotherapy drugs have been described in this application. The subjects of this application include a composition of the tetrazolium salts, intermediate electron acceptor and the combination of both of them as a drug for combinational cancer treatment, methods of using this drug in combination with other chemical compounds, extracts, inhibitors and biological molecules as cancer treatments. A diagram of the relationship among these findings is provided in Supplement Fig for easy understanding the subjects of this application.

SUMMARY OF THE INVENTION

The present invention provides pharmaceutical composition and combinational composition and methods of combinational treatments of cancer and exploration of cellular targets to enable the treatment of cancer in a mammal to synergize efficacy of the treatment with less or no cytotoxic side effects.

In one embodiment of the invention, the present invention provided the pharmaceutical compositions of WST-1r and any of the valid substitutes of WST-1 for the said combination treatment that are capable of conducting trans-plasma membrane electron transfer and induces ROS. The WST-1r and any of the valid substitutes of WST-1 is a mixture of tetrazolium salt and an electron coupling reagent (IEA), or at least one of the tetrazolium salt or at least one of the IEA in optimized concentration. The compounds may be administered in a pharmaceutically acceptable carrier medium.

According to one aspect of the present invention, genes, molecules, and polynucleotide sequences and polypeptide sequences are provided as target for designing drugs for the treatment of a cancer in a patient in need. These targets are the human transcripts, and their corresponding protein/peptide molecules and/or the genomic DNA sequences that are selected from the blast analysis of the DNA sequence of pUC19 DNA vector against human genome and transcripts, the DNA sequences of which mapped to the human transcripts and/or genomic sequences in short pieces. The transcripts and their corresponding coding molecules may become a target for enhancing the efficacy of the treatments of cancer. Other sequences that, thus, mapped to human genomic sequences may be used as targets as well as being used for targeting these corresponding genes. The potential drugs that can be designed to targeting these targets include, but not limited to, siRNA, small molecule inhibitors, peptides inhibitors, anti-sense RNA, anti-sense Oligo, antibodies, antibody fragments, proteins, dominant negative DNA vectors and Interferon (IFN). In a particular embodiment of this invention, these targets are, but not limited to, polynucleotide sequences of TRPC6 (SEQ ID NO: 2), MAGI-3 (SEQ ID NO: 4), TMEM182 (SEQ ID NO: 5), SH3PXD2B (SEQ ID NO: 3), or c6orf108 (SEQ ID NO: 14), and the polypeptide sequences of TRPC6 (SEQ ID NO: 6), MAGI-3 (SEQ ID NO: 8), TMEM182 (SEQ ID NO: 9), SH3PXD2B (SEQ ID NO: 7), or c6orf108 (SEQ ID NO: 15). The sequence to target human genomic sequence and or transcripts are, but not limited to, puc19 DNA vector (SEQ ID NO: 1), siRNA2 (SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12). Synthetic siRNA that against these target genes (SEQ ID NO: 2 to 5 and SEQ ID NO: 14) were selected for demonstrating the potential use of these genes as a target for the combination treatment for cancer.

In one embodiment of the invention, a method is provided for treating a cancer in a patient in need thereof comprising administering to the patient, concurrently or sequentially, a therapeutically effective amount of (1) at least one of the transfection of puc19 DNA vector or administering at least one of the substitutes of puc19 DNA transfection and (2) at least one IKK inhibitor and (3) an additional third agent, WST-1r or at least one of the valid substitutes of

WST-1r, in a pharmaceutically acceptable carrier medium. Wherein said combination enhances the induction of cancer cell death while otherwise any of these agents separately are demonstrated not to be toxic.

The valid substitutes for Puc19 DNA transfection are selected from the group consisting of (1) type I IFN, (2) Synthetic small interfering RNAs (siRNA) the nucleotide sequence SEQ ID NO10-12 of which mapped to both the DNA sequence of the pUC19 DNA vector and human transcripts and genome DNA sequences, (3) the biological compounds selected from the group consisting of biological and non-biological organic or non-organic compounds. The said method of screening compounds, wherein said biological chemicals are further selected from the group of polypeptides, proteins, peptides, antibodies, antibody fragments, nucleic acids, and polynucleotide the products of which interact and interfere said selected targets of the polynucleotide sequences of TRPC6 (SEQ ID NO: 2), MAGI-3 (SEQ ID NO: 4), TMEM182 (SEQ ID NO: 5), SH3PXD2B (SEQ ID NO: 3), or c60rf108 (SEQ ID NO: 14), and the polypeptide sequences of TRPC6 (SEQ ID NO: 6), MAGI-3 (SEQ ID NO: 8), TMEM182 (SEQ ID NO: 9), SH3PXD2B (SEQ ID NO: 7), or c60rf108 (SEQ ID NO: 15). Synthetic siRNA that against these target genes (SEQ ID NO: 2 to 5 and SEQ ID NO: 14) were selected for demonstrating the potential use of these genes as a target for the combination treatment for cancer.

One aspect of the present invention provides a method of inducing programmed cell death of cancer cells in a malignant cell population, and treating a patient with cancer comprising the use of a combination therapy. The combination therapy of the present invention comprises administering an effective dose of WST-1r or any valid substitutes, that is capable of conducting trans-plasma membrane electron transfer and induces ROS in a cell and apigenin, a multi-function inhibitor that inhibits NF- κ B activity and other molecules and/or signaling pathways, or at least one of the IKK inhibitor. The said combination treatment enhances apigenin anti-neoplasm effect and synergizes the induced cancer cell death.

In yet another embodiment of the invention, a method is provided for treating a cancer in a patient in need thereof by administering to the patient, concurrently or sequentially, a therapeutically effective amount of at least one GSK3 β inhibitor and protein kinase CK2 (CK2) inhibitor and addition of a third agent, WST-1r. In a particular embodiment of the invention, the preferred at least one GSK3 β inhibitor is LiCl and the preferred protein kinase CK2 (CK2) inhibitor is Apigenin. The compounds may be administered in a pharmaceutically acceptable carrier medium. Wherein said combination enhances the induction of cancer cell death otherwise any of these agents separately are demonstrated not to be toxic.

In accordance with another embodiment of the invention, a method is provided for treating cancer in a patient in need comprising administering, concurrently or sequentially, a therapeutically effective amount of a combination of a selective Puc19 DNA transfection or at least one of any of the valid substitutes of puc19 transfection as listed above in combination with at least one of a selected approved chemotherapeutic agents. Wherein said Puc19 DNA transfection or administering at least one of the valid substitutes of puc19 transfection being capable of substantially enhancing anti-neoplastic effects of said proved chemotherapeutic agents, substantially reducing toxic side effects of said chemotherapeutic agents, or a combination thereof, wherein said Puc19 DNA transfection or at least one of the valid substitutes has a substantial effect on activity of said chemotherapeutic agents.

In yet another embodiment of the present invention, a method is provided for synergistically inhibiting NF- κ B-NF-KAPPAB activity in cancer cells and in a patient in need thereof by administering to the cells or patient, concurrently or sequentially, a therapeutically effective amount of at least one Dominant negative kinase dead IKK1 DNA vector (IKK1-KA) and at least one Dominant negative kinase dead IKK2 DNA vector (IKK2-KA). The at least one Dominant negative kinase dead IKK1-KA or IKK2-KA may be substituted by IKK inhibitors selected from the group consisting of (IKK inhibitor list). The compounds may be administered in a pharmaceutically acceptable carrier medium. This combinational inhibition effect may be further enhanced by adding a third agent, WST-1r or the valid substitutes of WST-1r, for further induction of cancer cell death.

Another aspect of this present invention provides a method of inducing cancer cell death, and treating a patient comprising the use of a combination therapy. The combination therapy of the present invention comprises administering an effective dose of at least a compound that inhibits NF- κ B activity and at least one compound that inhibits STAT3 in a preferred embodiment, the compound that inhibits NF- κ B activity is apigenin or an IKK inhibitor or a CK2 inhibitor and the compound that inhibits STAT is stattic. The compounds may be administered in a pharmaceutically acceptable carrier.

The present invention contemplates the use of the combination therapy to treat cancers comprising administering IKK inhibitor or apigenin and a STAT3 inhibitor, stattic. In one preferred embodiment, the cancers are selected from the group consisting of a subtype of head and neck squamous carcinoma.

BRIEF DESCRIPTION OF THE FIGURES

Fig 1. Endogenous NF-kappaB down stream gene expression levels. UM-SCC-6 cells were transfected with the pCDNA control vector, IKK1-KA, IKK β -KA and IKK1-KA and IKK β -KA vectors as indicated. mRNA levels of IkB α , p100 (A) and CK2 β (B) are shown as a ratio to

18sRNA. Data indicated that inhibiting single IKK has little or no effect on NF-KAPPAB activity. Inhibiting both IKKs can synergistically inhibit NF-KAPPAB activity.

Fig2. Cell survival from IKK-KA transfection in combination with or without WST-1 treatment. Cells were treated as described in Fig 1 followed with WST-1r treatments for 4 hours. Cell viability was measured 24 hours after WST-1r treatment with CCK8 kit. A: Quantitative cell survival by CCK8, B: Cell image showing cell death in IKK1-KA+IKK2-KA cotransfected cells and WST-1 treated cells.

Fig3. Cell Survival from combinational treatment of IKK inhibitor, IKK1-KA transfection and WST-1r. A: Inhibitor III, B: Inhibitor II, C: SC-514 from the first experiment; D: cell image and E: Cell survival by CCK8 kit for IKK inhibitor III and F: Cell survival by CCK8 kit for IKK inhibitor VII from the second experiment.

Fig 4 HT1080 cells were transfected with pUC19, pCDNA3, IKK1-KA, IKK1-KA+pUC19, or pCDNA3+pUC19 DNA vectors as indicated followed by 3, 10, or 30 μ M of IKK inhibitor III treatment and then WST-1r treatment. Cell viabilities were measured 24, 48 and 96 hours after WST-1 treatment. Top panels show the linear scale of IKK inhibitor III concentration and bottom panels show the log scale of IKK inhibitor III concentration. P3: pCDNA3, p9: pUC19, IKK1-KA: IKK1-K44A kinase dead IKK1 in pCDNA3 vector, GS: WST-1r, I-3: IKK Inhibitor III. Cells treated with 30 μ M IKK inhibitor III and transfected with any of the DNA vectors and also treated with WST-1 were permanently died at 96 hours after the treatment with WST-1r while untransfected cells fully recovered and the transfected cells without WST-1r treatment partially recovered.

Fig 5 WST-1r induces Reactive Oxygen Species generation. HT1080 cells were treated as indicated. A: Cells were labeled with CM-H2-DCFDA and then treated with WST-1r for 30 minutes. B: Cells were treated with WST-1r for 4 hours before labeled with CM-H2-DCFDA. All WST-1r treated cells in A and B showed strong labeling of the fluorescence dye indicating that WST-1 induces ROS production. Surprisingly, transfection and IKK inhibitor also induces ROS. IKK inhibitor III alone showed dose dependent increase of ROS production in A, but not in B suggesting that each of these agents may have different time course of ROS production. DNA transfection potentiates IKK inhibitor III induction of ROS (A). Only the combination of the three factors together facilitates cell death. Each agents and the different combination may be involved in different regulatory mechanisms, but only the combination of the three lead to cell death. (I-3 = IKK inhibitor III, P9 = pUC19 DNA transfection, GS = WST-1r)

Fig 6 UM-SCC-6 cells were treated with variable combination of LiCl and Apigenin as indicated followed with (Left panel) or without (Right panel) WST-1r treatment. Data suggest that some synergistic effect by combination of LiCl with Apigenin.

Fig 7 DNA transfection enhance chemotherapy drug efficacy effects. UM-SCC-6 cells were transfected with pUC19, pCDNA3, pCDNA3+pUC19, pIKK1-KA+pUC19, pIKK2-KA+pUC19, pIKK1-KA+pIKK2-KA+pUC19, pIKK1-KA, pIKK2-KA, pIKK1-KA+pIKK2-KA, DNA vectors along with no transfection as negative control and DMSO as vehicle control. Cell survival were measured by CCK8 and presented as absorption at 450nm corrected background at 690nm. A: UM-SCC-6 cells treated with 5-FU, B: UM-SCC cells treated with Cis-Platinum.

DNA transfection synergizes chemotherapy drug efficacy effect and promotes cancer cell death. HT1080 Cells were transfected with pUC19, pCDNA3, pCDNA3+pUC19, pIKK1-KA+pUC19, pIKK2-KA+pUC19, pIKK1-KA+pIKK2-KA+pUC19, pIKK1-KA+pCDNA3, pIKK2-KA+pCDNA3, pIKK1-KA+pIKK2-KA+pCDNA3, DNA vectors as indicated before the treatment of chemotherapy drugs in variable doses as indicated. A: Cis-Platinum for 72hours and 24 hours after removing the drug (96 hour), B: paclitaxel for 72hours and 24 hours after removing the drug (96 hour) C: 5-FU after 96 hours treatment, and D: Doxorubicin after 72 hours of treatment. Data showed that transfection synergize chemotherapy drug cell growth inhibition effect. Untransfected cells recovered after removing the chemotherapy drug treatment while DNA transfected cells permanent died (A and B) suggesting that DNA transfection promote irreversible cell death. pUC19 showed the strongest effect on cell growth inhibition and promoting cell death comparing to the other IKK-KA dominant negative vectors.

Fig 8 HT1080 cells transfected with IKK1-KA, IKK2-KA and IKK1-KA+IKK2-KA in combination either with pUC19 or pCDNA3. pUC19 and pCDNA3 alone as control. IkB α and IL-8 mRNA levels were measured as indicators of NF-KAPPAB transcription activity. Data were normalized by corresponding 18sRNA levels.

Fig 9 F1A Chart showing that Combination treatment with apigenin and WST-1r synergizes induced cancer cell death. F1B Test 33 Effect of Apigenin and WST-1r treatment order on cell survival

Fig 10 Chart showing Effect of Apigenin and WST-1r treatment order on cell survival

Fig 11 Chart of Time course and Dose Response of WST-1r and Dose-Response of apigenin involved in the combination treatment of WST-1r with apigenin.

Fig 12 Chart showing Effect of Combination treatment with IKK Inhibitor and WST-1r on melanoma cell lines.

Fig 13 Effects of treatment order of WST-1r and IKK inhibitor III on induced cell death

Fig 14 Teat39 Chart showing WST-1r and Apigenin combination treatment induced JNK Phosphrylation

Fig 15 Time course of ROS generation after combination treatment of WST-1r, CCK8 and apigenin and IKK inhibitor III

Fig 16 CCK8-XTT-GS Comparison cell death inducing capability of CCK8 and XTT to WST-1r in combination with apigenin treatment

Fig 17 Comparison cell capability of inducing cell death by other tetrazolium salts to that by WST-1

Fig 18 HT1080: mPMS Dose-Response on cell death

Fig 19 Differential cellular responses to mPMS treatment

Fig 20 Effect of substitution of WST-1r with WST-3 for combination treatment with apigenin on induction of cell death and Effect of substitution of WST-1r with WST-3+mPMS for combination treatment with apigenin on induction of cell death

Fig 21 Enhancement of Taxel Efficacy Effects by Combination of Puc19 DNA sequence derived siRNA with Taxel

Fig 22 Substitution of Puc19 with siRNA against TMEM182 and MAGI3 for Puc19-IKK Inhibitor-WST-1r triple combination treatment.

Fig 23 Dose Response of ROS generation after combination treatment of WST-1r, CCK8 with apigenin and IKK inhibitor III

Fig 24 As we have shown in Fig 6 that DNA transfection showed efficacy effect and it is know that IFN effect are usually involved in transfection caused effects, this experiment is to examine whether these effect can be substituted by INF. HT1080 Cells were treated as described in Fig 6 above except that instead of DNA transfection these cells were treated with Interferon (IFN) at variable doses. None treated negative control and pUC19DNA transfected positive controls were plotted in parallel by IKK inhibitor III concentration in both linear and log format as well as by concentration of IFNs in log format at 24 and 48 hours after WST-1 treatment. Total of 14 INF members, IFN α A, IFN α B, IFN α C, IFN α D, IFN α F, IFN α G, IFN α H, IFN α I, IFN α J, IFN α K, IFN α 4b, IFN α WA, IFN β , IFN γ , and IL-6 were examined. All the INF α molecules examined showed partial enhancing effects (50-80%) as compared with pUC19 transfected positive control (fig 24).

DETAILED DESCRIPTION OF THE INVENTION

1. General Description

The present invention is based on two findings: (1) The Cell Proliferation Reagent WST-1 (WST-1r), when combined with Puc19 DNA transfection and IKK inhibitor induces cell death in a synergetic manner in cancer cells and, (2) The effect of puc19 in the induction of cell death is reside in is DNA sequence.

The first discovery led to the identification of classes of chemicals and corresponding pharmaceutical compositions of using these chemical compounds as a drug for the combination treatment for cancer. The second finding further led to the discovery of several genes as potential targets for cancer treatment. These are rarely studied genes and some of them are still in hypothetical gene status. Together, these findings led to establishing several combinational treatment methods for cancer therapy.

In one embodiment of this invention, the pharmaceutical composition of WST-1r was described for the use as a drug for combinational treatment of cancer.

Yet in another embodiment of this invention, the classes of chemical compounds and the combination of these compounds that can form the formula of WST-1r and the valid substitutes of WST-1r are described for the use for the combination treatment for cancer.

In one of the embodiments, Puc19 DNA vector was found to have biological effect on mammalian and human cancer cells and was used as a drug for combination treatment with IKK inhibitor and WST-1r reagent.

In another embodiment of this invention, Puc19 DNA vector was used as a drug in combination with chemotherapeutic drug for enhancing the therapeutic effect of these chemotherapeutic drugs for the treatment of cancer.

According to the embodiments of this invention, small interfering RNAs (siRNA#1, siRNA#2, and siRNA#3), the sequence of which were derived from the nucleotide sequence of Puc19 DNA vector, were described for the use of combination treatment for cancer.

Yet also according to the embodiments of this invention, human genes (TRPC6 (SEQ ID NO: 2), MAGI-3 (SEQ ID NO: 4), TMEM182 (SEQ ID NO: 5), SH3PXD2B (SEQ ID NO: 3), or c6orf108 (SEQ ID NO: 14), and the polypeptide sequences of TRPC6 (SEQ ID NO: 6), MAGI-3 (SEQ ID NO: 8), TMEM182 (SEQ ID NO: 9), SH3PXD2B (SEQ ID NO: 7), or c6orf108 (SEQ ID NO: 15)) that were selected based on Puc19 DNA sequence analysis and the biological function of the corresponding siRNAs to be used as potential target for drug development for the treatment of cancer are described and examined with the corresponding siRNAs against these genes.

Yet another embodiment of this invention, wherein said the valid substitutes of Puc19 DNA that were selected from biological and non-biological compounds and their effects in

combination with IKK inhibitor and WST-1r or the valid substitutes of WST-1r is described. Wherein said the biological compound for the valid substitutes of Puc19 DNA include different members of Interferon and all the siRNAs mentioned above.

Yet another embodiment of this invention, wherein said the valid substitutes of Puc19 DNA that were selected from biological and non-biological compounds and their effects in combination with chemotherapeutic drugs is described. Wherein said the biological compound for the valid substitutes of Puc19 DNA include different members of Interferon and all the siRNAs mentioned above.

In another embodiment of this invention, a method of combination treatment for cancer comprising apigenin or at least one IKK inhibitor and WST-1r is described.

Yet in another embodiment of this invention, a method of combination treatment for cancer comprising at least one Protein kinase II (CK2) inhibitor, apigenin, at least one GSK3 β inhibitor, Lithium chloride, and WST-1r for enhancing treatment effect is described.

Yet in another embodiment of this invention, a method of synergetic inhibition of NF- κ B activity by cotransfeting cells with dominant negative IKK1 and IKK2 DNA into caner cells is described.

Yet in another embodiment of this invention, combination treatment comprising at least one IKK inhibitor or sat least one CK2 inhibitor and STAT3 inhibitor, Stattic, for the treatment of cancer is described.

I. Definitions

The term "pUC19 DNA" is a DNA cloning vector (SEQ ID #1) that amplifies in prokaryotic cells. DNA sequence of this vector was originally submitted to NCBI gene bank by J. Messing, Waksman Institute, NJ on 3-MAR-1986 and revised by F. Pfeiffer on 16-DEC-1986. In the present invention, pUC19 has been transfected into human cancer cells by chemical or liposome based DNA transfection reagents.

My data suggests that the DNA sequence that composes this DNA vector has biological effects in cultured human cancer cells that lead to synergistic cell death when combined with other treatments to these cells as described in this invention. Blast analysis of the DNA sequence of pUC19 against human genome and transcripts showed multiple short sequences aligned to varies locations of human genome and transcripts (Blast result is attached to this application). In the present invention, pUC19 represents the short DNA sequences, usually 15-100 bases that mapped to human transcripts and/or human genome DNA sequences, and their corresponding gene products that include but not limited to siRNA, miRNA, shRNA, peptide that are directly derived from the DNA sequence of this vector and small molecules that can

interact and/or inhibit the function and activity of these corresponding molecules as direct gene products of their gene sequences, the DNA sequences of their corresponding gene contain these short matched DNA sequences from the DNA sequence of pUC19. The matched DNA sequences don't have to be exact matches. The matched DNA sequences can vary slightly, 10%, 20%, and even up to 30-40%.

The term "pcDNA3m DNA" is a mammalian expression vector version 3.1 with modifications (SEQ ID #13). DNA sequence of this vector was originally obtained from Invitrogen, which has discontinued the production and selling of this vector. The modification includes destroy the BglII site at nucleotide 13 and Sp6 site at nucleotide 999-1016, insertion of HA tag between HindII and EcoRI. pcDNA3 has been transfected into human cancer cells by chemical or liposome based DNA transfection reagents. Our data suggests that the DNA sequence that composes this DNA vector may have biological effects in cultured human cancer cells that lead to synergistic cell death when combined with other treatments to these cells as described in this invention. Blast analysis of the DNA sequence of pcDNA3 against human genome and transcripts showed multiple short sequences aligned to various locations of human genome and transcripts (Blast result is attached to this application), each of which will subject to examination. In the present invention, pcDNA3 represents the short DNA sequences, usually 15-100 bases that mapped to human transcripts and/or human genome DNA sequences, and their corresponding gene products that include but not limited to siRNA, miRNA, shRNA, peptide that are directly derived from the DNA sequence of this vector and small molecules that can interact and/or inhibit the function and activity of these corresponding molecules as direct gene products of their gene sequences, the DNA sequences of their corresponding gene contain these short matched DNA sequences from the DNA sequence of pcDNA3. The matched DNA sequences don't have to be exact matches. The matched DNA sequences can vary up to 30-40% changes.

The term "siRNA1" is a siRNA designed based on and derived from the DNA sequence of pUC19 [SEQ ID #10]. This siRNA sequence matches to the human transcript of Homo sapiens transient receptor potential cation channel, subfamily C, member 6 (TRPC6, GeneID: 7225), mRNA (gil19923256|NM_004621.3) synonyms: TRP6, FSGS2, FLJ11098. In the present invention, siRNA1 was used for targeting TRPC6 for the treatment of cancer. TRPC6 can be targeted by any siRNA sequences that can be mapped to the transcript of TRPC6 sequence and has inhibitory effect on the expression level and function of the TRPC6. As in general the siRNA sequence can vary slightly 10%, 20% and even 30-40% of the exact sequence of the transcript. TRPC6 can also be targeted by peptide, anti-sense RNA, anti-sense DNA

oligo, dominant negative DNA vectors, antibodies and small molecule inhibitors. The siRNA sequence described above within this paragraph is the preferred sequence, but this does not limit other siRNA and other means as described immediate above within this paragraph for targeting TRPC6 from this invention. The function of TRPC6 has been previous reported as a potential target for cancer treatment, but no report regarding the use of TRPC6 as a target for a combinational cancer treatment with IKK inhibitors or chemotherapy drugs to reach the synergistic inhibition of cancer cell growth to promote cancer cell death.

The term "siRNA3" is a siRNA designed based on and derived from the DNA sequence of pUC19 [SEQ ID #12]. This siRNA sequece matches to the human transcript of Homo sapiens membrane associated guanylate kinase, WW and PDZ domain containing 3 (MAGI3, GeneID: 260425), transcript variant 2, mRNA (gil23097339|NM_152900.1) synonyms: MAGI-3, MGC163281 and the Homo sapiens transmembrane protein 182 (TMEM182, GeneID: 130827), mRNA (gil40255064|NM_144632.2). In the present invention, siRNA3 was used for targeting MAGI3 and/or TMEM182 for the treatment of cancer. MAGI3 and/or TMEM182 can be targeted by any siRNA sequences that can be mapped to each of the transcripts of MAGI3 and/or TMEM182 sequence and has inhibitory effect on the expression level and function of the MAGIKK and/or TMEM182. As in general the siRNA sequence can vary slightly, 10%, 20% and even 30-40% of the exact sequence of the transcript. MAGI3 and/or TMEM182 can also be targeted by hsRNA, peptide, anti-sense RNA, anti-sense DNA oligo, dominant negative DNA vectors, antibodies and small molecule inhibitors. The siRNA sequence described above within this paragraph is the preferred sequence, but this does not limit other siRNA and other means as described immediate above within this paragraph for targeting MAGIKK and TMEM182 from this invention. The function of MAGI3 has been previous linked to cancer, but no report regarding the use of MAGI3 as a target for a combinational cancer treatment with IKK inhibitors or chemotherapy drugs to reach the synergistic inhibition of cancer cell growth and to promote cancer cell death. TMEM182 has not been previously studied and not been linked to cancer.

The term "siRNA2" is a siRNA designed based on and derived from the DNA sequence of pUC19 [SEQ ID#2]. The 2/3 of this siRNA sequece matches to the human transcript of Homo sapiens SH3 and PX domains 2B (SH3PXD2B), mRNA. (SH3PXD2B, GeneID: 285590), mRNA (NM_001017995) synonyms: HOFI; FLJ20831; KIAA1295. In addition, this sequence also mapped to more than 45 sites within human genome. In the present invention, siRNA2 was used for targeting SH3PXD2B and all the other potential DNA sequences in the human genome for the treatment of cancer. SH3PXD2B can be targeted by any siRNA sequences that can be mapped to the transcript of SH3PXD2B sequence and has inhibitory effect

on the expression level and function of the SH3PXD2B and other gene expression. As in general the siRNA sequence can vary 30-40% of the exact sequence of the transcript. SH3PXD2B can also be targeted by shRNA, peptide, anti-sense RNA, anti-sense DNA oligo, dominant negative DNA vectors, antibodies and small molecule inhibitors. The siRNA sequence described above within this paragraph is the preferred sequence, but this does not limit other siRNA and other means as described immediate above within this paragraph for targeting SH3PXD2B and expression and function of other genes that can be altered by this siRNA sequence from this invention. SH3PXD2B has not been previously studied and has not been linked to cancer in any way.

The term “TRPC6” represents human transcript of Homo sapiens transient receptor potential cation channel, subfamily C, member 6 (TRPC6, GeneID: 7225), mRNA (gil19923256|NM_004621.3) synonyms: TRPC6, FSGS2, FLJ11098. In the present invention, TRPC6 is a potential target for the treatment of cancer. TRPC6 can be targeted by siRNA, shRNA, peptide, anti-sense RNA, anti-sense DNA oligo and small molecule inhibitors. TRPC6 can be targeted by any siRNA sequences that can be mapped to the transcript of TRPC6 sequence and has inhibitory effect on the expression level and function of the TRPC6. As in general the siRNA sequence can vary slightly, 10%, 20% and even 30-40% of the exact sequence of the transcript. TRPC6 can also be targeted by peptide, anti-sense RNA, anti-sense DNA oligo, dominant negative DNA vectors, antibodies and small molecule inhibitors. The siRNA1 sequence described above is the preferred sequence, but this does not limit other siRNA and/or other means as described immediate above within this paragraph for targeting TRPC6 from this invention. The TRPC6 has been previous reported as a potential target for cancer treatment, but no report regarding the use of TRPC6 as a target for a combinational cancer treatment with IKK inhibitors or chemotherapy drugs to reach the synergistic inhibition of cancer cell growth to promote cancer cell death.

The term “MAGI3” represents human transcript of Homo sapiens membrane associated guanylate kinase, WW and PDZ domain containing 3 (MAGIKK, GeneID: 260425), transcript variant 2, mRNA (gil23097339|NM_152900.1). Synonyms: MAGI-3, MGC163281. MAGI-3 is localized with ZO-1 and cingulin at tight junctions in epithelial cells, whereas MAGI-3 was found in E-cadherin-based cell-cell contacts and in focal adhesion sites in primary cultured astrocytes (Adamsky K, Arnold K, Sabanay H, Peles E., Junctional protein MAGIKK interacts with receptor tyrosine phosphatase beta (RPTP beta) and tyrosine-phosphorylated proteins. J Cell Sci. 2003 Apr 1;116(Pt 7):1279-89. PMID: 12615970). MAGI-3 interacts directly with LPA(2) and regulates the ability of LPA(2) to activate Erk and RhoA MAGIKK regulates LPA-

induced activation of Erk and RhoA (Zhang H, Wang D, Sun H, Hall RA, Yun CC, Cell Signal. 2007 Feb;19(2):261-8. Epub 2006 Aug 9. PMID: 16904289). The function of MAGI3 has been previously linked to cancer, but no report regarding the use of MAGI3 as a target for a combinational cancer treatment with IKK inhibitors or chemotherapy drugs to reach the synergistic inhibition of cancer cell growth and to promote cancer cell death. In the present invention, MAGIKK is a potential target for a combinational treatment of cancer. MAGIKK can be targeted by siRNA, shRNA, peptide, anti-sense RNA, anti-sense DNA oligo, dominant negative DNA vectors, antibodies and small molecule inhibitors. The siRNA3 sequence described above is the preferred sequence, but this does not limit from other siRNA sequences and other means as described in this paragraph. As in general the siRNA sequence can vary slightly, 10%, 20% and even 30-40% from the exact sequence of the transcript.

The term "TMEM182" represents Homo sapiens trans-membrane protein 182 (TMEM182, GeneID: 130827), mRNA (gil40255064|NM_144632.2). In the present invention, SH3PXD2B is a potential target for the treatment of cancer. TMEM182 can be targeted by siRNA, shRNA, peptide, anti-sense RNA, anti-sense DNA oligo, dominant negative DNA vectors, antibodies and small molecule inhibitors. The siRNA3 sequence described above is the preferred sequence, but this does not limit from other siRNA sequences and other means as described in this paragraph. As in general the siRNA sequence can vary 10%, 20% and even 30-40% from the exact sequence of the transcript. TMEM182 has not been previously studied and not been linked to cancer.

The term "SH3PXD2B" represents SH3 and PX domains 2B adaptor protein HOFI (GeneID: 285590) it contains SH3 and PX domains. SH3; Src homology 3 domains; SH3 domains bind to prolinerich ligands with moderate affinity and selectivity, preferentially to PxxP motifs; they play a role in the regulation of enzymes by intramolecular interactions, changing the subcellular localization of PX; PhoX homologous domain, present in p47phox and p40phox. Eukaryotic domain of unknown function presents in phox proteins, PLD isoforms, and a PI3K isoform. SHPXD2B has not been previously studied and not been linked to cancer. SH3PXD2B has not been previously studied and not been linked to cancer. In the present invention, SH3PXD2B is a potential target for the treatment of cancer. SH3PXD2B can be targeted by siRNA, shRNA, peptide, anti-sense RNA, anti-sense DNA oligo and small molecule inhibitors. The siRNA2 sequence described above is the preferred sequence, but this does not limit from other siRNA sequences and other means as described in this paragraph. As in general the siRNA sequence can vary 10%, 20% and even 30-40% from the exact sequence of the transcript.

The term “C6orf108” represents human C6orf108 chromosome 6 open reading frame 108 [Homo sapiens] GeneID: 10591. Official Symbol C6orf108. This gene was identified on the basis of its stimulation by c-Myc protein. The exact function of this gene is not known but studies in rat suggest a role in cellular proliferation and c-Myc-mediated transformation.

The term “Interferon” (IFN) is a group of cytokines produced by leucocytes and fibroblasts. The IFN that are described in this invention includes all type I and type II IFNs and all the subtypes of IFN including, but not limited to IFN α A, IFN α B, IFN α C, IFN α D, IFN α F, IFN α G, IFN α H, IFN α I, IFN α J, IFN α K, IFN α 4b, IFN α WA, IFN β , IFN γ and IL-6.

The term “WST-1c” representing a tetrazolium salt WST-1 {4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzenedisulfonate} was first described by Ishiyama et al in 1996 (Ishiyama M, et al Biol Pharm Bull 1996, 19:1515-20).

The term “WST-1r” represents a reagent mixture comprising WST-1c and mPMS at optimized concentration for the combination treatment.

The term “IEA” is Intermediate Electron Acceptor.

The term “mPMS” (1-methoxy-5-methyl-phenazinium methyl sulfate) is a chemical compound acts as an “electron coupling agent/intermediate electron acceptor” when combined with tetrazolium salts.

The term “Q1” is a chemical compound of IEA.

The term “valid substitutes of WST-1r” represents any compounds that can substitute the WST-1c, or the electron coupling reagent or any of the remaining components either act alone or in any type of combination among these substitutes or any type of combination with any of the component of the tetrazolium salt and IEA that comprising WST-1r to function as the WST-1r as described in this invention to reproduce the synergistic induction of cancer cell death. The term “valid substitutes of WST-1r” includes, but not limited to all the up to date available tetrazolium salt based WSTs that include, but not limited to, WST-1, WST-3, WST-4, WST-5, WST-9, WST-10 AND WST-11, MTS and XTT, an IEA, including mPMS and Q1 and the combination of these tetrazolium salts with IEA comprising WST-1+mPMS, WST-3+mPMS, WST-4+mPMS, WST-5+mPMS, WST-9+mPMS, WST-10+mPMS, WST-11+mPMS, XTT+mPMS, MTS+mPMS,, WST-3+Q1, WST-4+Q1, WST-5+ Q1, WST-9+ Q1, WST-10+ Q1, WST-11+ Q1, XTT+ Q1 MTS+ Q1.

The term “IKK inhibitors” represent all the Inhibitor kappaB Kinase (IKK) inhibitors. In the present invention the preferred IKK inhibitors are, but not limited to IKK inhibitor II, IKK inhibitor III, IKK inhibitor VI, IKK inhibitor VII and sc-514. A larger list include, but not limited to SPC839 (Signal Pharmaceutical Inc.), Anilino-Pyrimidine Derivative(Signal

Pharmaceutical Inc.), PS1145(Millennium Pharmaceutical Inc.), BMS-345541*(Bristol-Myers Squibb Pharmaceutical Research Institute, IKK inhibitor III), SC-514*(Smithkline Beecham Corp.), Amino-imidazolecarboxamide derivative(Smithkline Beecham Corp.), Ureido-thiophenecarboxamide derivatives(AstraZeneca), Diarylpyridine derivative(Bayer), Pyridooxazinone derivative(Bayer), Indolecarboxamide derivative(Aventis Pharma), Benzoimidazole carboxamide derivative(Aventis Pharma), Pyrazolo[4,3-c]quinoline derivative(Pharmacia Corporation), Imidazolylquinoline-carboxaldehyde semicarbazide derivative(Tulark Inc.), Pyridyl Cyanoguanidine derivative(Leo Pharma), I κ B Kinase Inhibitor Peptide(CalBiochem), IKK-2 Inhibitor IV [5-(p-Fluorophenyl)-2-ureido]thiophene-3-carboxamide(CalBiochem), IKK Inhibitor II, Wedelolactone(CalBiochem), IKK Inhibitor VII (CalBiochem), IKK-2 Inhibitor V N-(3,5-Bis-trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide IMD-0354(CalBiochem), IKK-2 Inhibitor VI (5-Phenyl-2-ureido)thiophene-3-carboxamide(CalBiochem), IKK-2 Inhibitor VIII ACHP 2-Amino-6-(2-(cyclopropylmethoxy)-6-hydroxyphenyl)-4-(4-piperidinyl)-3-pyridinecarbonitrile(CalBiochem).

The term “CK2 inhibitor” represents all protein kinase casein kinase2 inhibitors in the present invention. In the present invention the preferred IKK inhibitors is, but not limited to Apigenin.

The term “Apigenin” is a flavonoid present in wide range of fruits and vegetables, such as apple and celery. CAS Registry Number: 520-36-5, Chemical Abstracts Service Name: 4H-1-benzopyran-4-one,5,7-dihydroxy-2-(4-hydroxy-phenyl)- (9CI). Apigenin is described as a nonmutagenic bioflavonoid which is presented in leafy plants and vegetables (e.g., parsley, artichoke, basil, celery) and has significant chemopreventive activity against UV-radiation. Current research trials indicate that it may reduce DNA oxidative damage; inhibit the growth of human leukemia cells and induced these cells to differentiate; inhibit cancer cell signal transduction and induce apoptosis; act as an anti-inflammatory; and as an anti-spasmodic or spasmolytic. Apigenin inhibits activity of CK2, MPK, HIF, VEGF and some other molecules and regulatory pathways such as cell cycle and angiogenesis, induce p53 activity, etc. Apigenin is known to have the effects of anti-UV radiation caused oxidation, and chemoprevention for cancer.

The term “LiCl” is an inorganic salt, lithium Chloride, and is used as an inhibitor of GSK3 β . In the present invention; LiCl represents the class of inhibitors that inhibit GSK3 β .

The term “IKK” represents Inhibitory kappaB Kinase, which phosphorylate I κ B that leads to NF-KAPPAB activation. Two IKK isoforms have been identified. They are IKK1 (IKK α) and IKK2 (IKK β). Two IKK molecules, either by two of the same IKK isoforms or by

IKK1 and IKK2, form a complex with NEMO (IKK γ) to form the IKK complex. IKK is one of the important NF-kappaB upstream regulators. The term "IKK inhibitor" refers to an agent capable of inhibiting the activity of Inhiitor kappaB kinase (IKK) and thereby inhibiting the kinase activity of IKK and its function of activating nF-kB. An IKK inhibitor may be a competitive, noncompetitive, or irreversible IKK inhibitor. "A competitive IKK inhibitor" is a compound or a peptide that reversibly inhibits IKK enzyme activity at the catalytic site (for example, without limitation, need to find example); "a noncompetitive IKK Inhibitor" is a compound that reversibly inhibits IKK enzyme activity at a non-catalytic site (for example, without limitation, need to find example); and "an irreversible IKK inhibitor" is a compound that irreversibly destroys IKK enzyme activity by forming a covalent bond with the enzyme (for example, without limitation, need to find example)). The term "IKK inhibitors" of the instant invention may include, without limitation, i) compounds previously established to exhibit IKK inhibitory properties including, but not limited to: SPC839 (Signal Pharmaceutical Inc.), Anilino-Pyrimidine Derivative(Signal Pharmaceutical Inc.), PS1145(Millennium Pharmaceutical Inc.), BMS-345541*(Bristol-Myers Squibb Pharmaceutical Research Institute, IKK inhibitor III), SC-514*(Smithkilne Beecham Corp.), Amino-imidazolecarboxamide derivative(Smithkilne Beecham Corp.), Ureudo-thiophenecarboxamide derivatives(AstraZeneca), Diarylpyridine derivative(Bayer), Pyridooxazinone derivative(Bayer), Indolecarboxamide derivative(Aventis Pharma), Benzoimidazole carboxamide derivative(Aventis Pharma), Pyrazolo[4,3-c]quinoline derivative(Pharmacia Corporation), Imidazolylquinoline-carbxaldehyde semicarbazide derivative(Tulark Inc.), Pyridyl Cyanoguanidine derivate(Leo Pharma), IkB Kinase Inhibitor Peptide(CalBiochem), IKK-2 Inhibitor IV [5-(p-Fluorophenyl)-2-ureido]thiophene-3-carboxamide(CalBiochem), IKK Inhibitor II, Wedelolactone(CalBiochem), IKK Inhibitor VII (CalBiochem), IKK-2 Inhibitor V N-(3,5-Bis-trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide IMD-0354(CalBiochem), IKK-2 Inhibitor VI (5-Phenyl-2-ureido)thiophene-3-carboxamide(CalBiochem), IKK-2 Inhibitor VIII ACHP 2-Amino-6-(2-(cyclopropylmethoxy)-6-hydroxyphenyl)-4-(4-piperidiny)-3-pyridinecarbonitrile(CalBiochem). ii) In a certain embodiment, the group of IKK inhibitors may additionally include compounds discovered to have IKK inhibitory activity, in accordance with the present invention, and previously identified to have anti-tumor activity, including, but not limited to PS1145(Millennium Pharmaceutical Inc.), BMS-345541*(Bristol-Myers Squibb Pharmaceutical Research Institute).

The term "NF-kappaB" Nuclear factor kappaB is a family of rel proteins that act as transcription factors regulating gene expression. Normally NF-KAPPAB proteins forms a dimmer which also complex with an inhibitory kappa B (IkB) molecule stay in inactive form in

the cytoplasm. Upon signal activation, the I κ B is phosphorylated by IKK and dissociate from the NF-kappaB dimer, which release the NF-KAPPAB to entering the nuclear for activating transcription of a special set of genes that are regulated by NF-KAPPAB. The dissociated I κ B will be degraded by proteasomes. Activation of NF-kappaB favors cell proliferation and survival. NF-kappaB activity has been found to associate with and contribute to carcinogenesis process, tumor progression and resistance of cancer cells to chemo and radiation therapies.

The term "C-Jun N-terminal kinases" (JNKs), originally identified as kinases that bind and phosphophorylate c-Jun on Ser63 and Ser73 within its transcriptional activation domain, are mitogen-activated protein kinases which are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in T cell differentiation and apoptosis.

The term "Reactive Oxygen Species" (ROS) includes oxygen ions, free radicals and peroxides both inorganic and organic. They are generally very small molecules and are highly reactive due to the presence of unpaired valence shell electrons. ROSs form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. The effects of ROS on cell metabolism have been well documented in a variety of species. These include not only roles in programmed cell death and apoptosis, but also positive effects such as the induction of host defence genes and mobilisation of ion transport systems. This is implicating them more frequently with roles in redox signaling or oxidative signaling.

The term "Cancer Cells" represents the cells in culture that were derived from human cancer or tumors, which have malignant features, such as lost of contact inhibition.

The term "Cancer" describes a diseased state in which a carcinogenic agent or agents causes the transformation of a normal cell into an abnormal cell, the invasion of adjacent tissues by these abnormal cells, and lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant sites, i.e., metastasis.

The term "Effective dose" As used herein, the term "effective dose" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a cell, tissue, system, animal or human that is being sought, for instance, by a researcher or clinician. Furthermore, the term "therapeutically effective amount" means any amount which, as compared to a corresponding subject who has not received such amount, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function.

The term "Treatment of cancer" describes the drug or reagents administered to the cells or to a mammal, the duration of the treatment, the method used to administer these drugs, or reagents and the order and intervals of between these treatments.

The term "Synergistic effect/Synergize" refers to a combination of two or more treatments, which is more effective to produce advantageous results than the additive effects of these agents.

The term "Chemotherapy Drugs (Agent)" refers to any drugs that have cytotoxic effect on cancer cells and are currently used as a drug for treating cancer. The drugs that were tested in this invention are listed as the following. Chemotherapy Drugs that we are mentioned in this invention were not limit to this list.

The term "5-fluorouracil", 5-fluoro-2,4-(1H,3H) pyrimidinedione(5-FU), is commercially available as fluorouracil.

The term "Cis-Platinum" cis-diamminedichloroplatinum, is commercially available as PLATINOL.RTM. as an injectable solution.

The term "Paclitaxel" is a Potent anti-neoplastic drug; binds to the N-terminal region of β -tubulin and promotes the formation of highly stable microtubules that resist depolymerization, thus preventing normal cell division and arresting the cell cycle at the G₂/M phase.

The term "Doxorubicin", (8S,10S)-10-[(3-amino-2,3,6-trideoxy-.alpha.-L-lyxo-hexopyranosyl)oxy]-8-- glycoloyl, 7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as an injectable form as RUBEX.RTM. or ADRIAMYCIN RDF.RTM..

The term a "therapeutically effective amount" of a compound or a pharmaceutical composition refers to an amount sufficient to modulate cancer cell proliferation in culture, tumor growth or metastasis in an animal, especially a human, including without limitation decreasing tumor growth or size or preventing formation of tumor growth in an animal. This term may also mean the effective amount(s) needed to cause cancer cell death or selective cancer cell death while not causing side effects in normal cells.

The term "Pharmaceutically acceptable" indicates approval by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopoeia for use in animals, and more particularly in humans.

The term a "carrier" refers to, for example, a diluent, adjuvant, excipient, auxilliary agent or vehicle with which an active agent of the present invention is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil,

sesame oil and the like. Water or aqueous saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. It also include the transfection reagents as used for deliver of DNA and/or RNA into cells either in vitro or in vivo.

The term "Concurrently" means (1) simultaneously in time, or (2) at different times during the course of a common treatment schedule.

The term "Sequentially" refers to the administration of one active agent used in the method followed by administration of another active agent. After administration of one active agent, the next active agent can be administered substantially immediately after the first, or the next active agent can be administered after an effective time period after the first active agent; the effective time period is the amount of time given for realization of maximum benefit from the administration of the first active agent.

II Targets and targeting the therapeutic targets for the treatment of cancer

The present invention provides nucleotide sequences for genes that implicate and/or can be utilized as therapeutic targets for the treatment of cancer, and polypeptides encoded by such sequences and antibodies and compounds reactive with such polypeptides in methods of treating a cancer, and for agents effective in reducing the activity of cancer-linked genes and thereby treating a cancerous condition which were not previously established for anti-tumor effect(s).

The disclosed nucleotide sequences are related to and derived from a DNA cloning vector, pUC19 (SEQ ID #1), which was discovered to synergize IKK inhibition, inhibit cancer cell growth proliferation and promote cancer cell death when transfection of this vector to cancer cells was combined with IKK inhibitor treatment and followed by WST-1r or any of it valid substitutes treatment or in combination with chemotherapeutic drugs. This function of pUC19 has not been previously reported. Other potential DNA sequence may also include a modified pcDNA3 version 3.1, (SEQ ID 13) and the attached blast result entitled: "NCBI Blast_pcDNA3 Nucleotide sequence (5448 letters)".

Accordingly, the present invention discovered that this anti-cancer effect of pUC19 vector (SEQ ID #1) was primarily resides in its DNA sequences that are mapped to transcripts and/or short sequences (from 15bp up to 100 bp) that flanking the genes in human genome. The human transcripts that pUC19 DNA sequences mapped to are, but not limited to, (1) Homo sapiens transient receptor potential cation channel, subfamily C, member 6 (TRPC6, GeneID: 7225, mRNA: NM_004621.3, SEQ ID #2, #6), (2) Homo sapiens SH3 and PX domains 2B (SH3PXD2B, GeneID: 285590, mRNA: NM_001017995, SeQ ID #3, #7), (3) Homo sapiens

membrane associated guanylate kinase, WW and PDZ domain containing 3 (MAGIKK, GeneID: 260425, transcript variant 2, mRNA:NM_152900, SeQ ID #4, #8), (4) the Homo sapiens trans-membrane protein 182 (TMEM182, GeneID: 130827, mRNA: NM_144632, SeQ ID #5, #9) and (5) Homo sapiens chromosome 6 open reading frame 108 C6orf108, GeneID: 10591 SeQID #14, #15). The human genome sequences that pUC19 DNA sequences mapped to are listed in the attached file "NCBI Blast-pUC19-Human-Transcripts and genome(2686 letters)", "NCBI Blast_siRNA2 Nucleotide sequence (24 letters)" and "NCBI Blast_pcDNA3 Nucleotide sequence (5448 letters)".

The polypeptides disclosed herein incorporate various polynucleotide transcripts (SEQ ID NO: 2, 3, 4, 5 and 14) and, thus, derived amino acid sequence (SEQ ID NO: 6, 7, 8, 9 and 15) from said transcripts are available as targets for treatment of cancer, especially anti-cancer agents, including, with no limitation, antibodies specific against said polypeptides, peptide inhibitors, small molecule inhibitor, double strand siRNAs, shRNA, anti-sense RNA, anti-sense oligo and dominant negative DNA vectors. In a particular embodiment the wherein said double strand siRNAs are, but not limited to, siRNA1 (SEQ ID #10), siRNA2 (SEQ ID #11), siRNA3 (SEQ ID #12).

The nucleotides and polypeptides, as gene products, used in the processes of the present invention may comprise a recombinant polynucleotide or polypeptide, a natural polynucleotide or polypeptide, or a synthetic polynucleotide or polypeptide, or a chemically modified polynucleotide or polypeptide.

The nucleotides and polypeptides of the pUC19 vector, that are mapped to the human genome, flanking genes in the human genome used in the processes of the present invention may comprise a recombinant polynucleotide or polypeptide, a natural polynucleotide or polypeptide, or a synthetic polynucleotide or polypeptide.

Fragments of such polynucleotide and polypeptides as are disclosed herein may also be useful in practicing the processes of the present invention. For example, a fragment, derivative or analog of the polynucleotide (SEQ ID# 2, 3, 4, 5 and 14) may be substituted by (i) any part of these sequences and/or with mismatches for up to 40% of the total sequences been used for, (ii) fused into a DNA vector or any type of carriers, (iii) nucleotide sequences with modified nucleotides.

Fragments of such polynucleotides and polypeptides as are disclosed herein may also be useful in practicing the processes of the present invention. For example, a fragment, derivative or analog of the polypeptide (SEQ ID NO: 6, 7, 8, 9 and 15) may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue

(more preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substitute group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretor sequence or a sequence which is employed for purification of the mature polypeptide (such as a histidine hexapeptide) or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Substituting these siRNAs (SEQ ID 10, 11, 12) as are disclosed herein above may also be useful in practicing the processes of the present invention. Examples may include, but not limited to, (i) a siRNA that mapped to another part of the sequence of the coding sequence of the gene, (ii) variations of the siRNA sequences that still capable to target the same gene and reduce its expression level, (iii) any type of modifications of the siRNA either at the nucleotides or the whole siRNA, (iv) put the siRNA sequence into any type of carriers, such as a vector or a chemical for the delivery of the sequence.

The nucleotide sequence of the complete mRNA and open reading frame of the transcripts and amino acid sequences, as discussed above, can be found in the NCBI GenBank database with the Gene ID or accession numbers listed above.

The present invention is based, at least in part, on the discovery of inhibitory effect of pUC19 vector in cancer cell growth and proliferation and inducing cancer cell death when combined with IKK inhibitor WST-1r treatment as well as in combination with chemotherapeutic drugs to treat cancer cells. This inhibitory effect of pUC19 DNA transfection may be substituted by siRNA, compounds or small molecule inhibitor, peptide inhibitor, antibody, shRNA, anti-sense RNA, anti-sense oligo, and antibody and dominant negative DNA vectors targeting the gene to alter its expression level, the corresponding transcripts and/or protein as described above in this section and at least in partial by IFN.

Cancers that may be treated using the present discovery include, but are not limited to: cancers of the prostate, colorectum, pancreas, cervix, stomach, endometrium, brain, liver, bladder, ovary, testis, head, neck, skin (including melanoma and basal carcinoma), mesothelial lining, esophagus, breast, muscle, connective tissue, lung (including small-cell lung carcinoma and non-small-cell carcinoma), adrenal gland, thyroid, kidney, or bone; glioblastoma, mesothelioma, renal cell carcinoma, gastric carcinoma, sarcoma, choriocarcinoma, cutaneous

basocellular carcinoma, and testicular seminoma.

III. Therapy for the Treatment of Cancer

The present invention provides pharmaceutical compositions comprising WST-1r and its valid substitutes which have not previously been established as having an anti-tumor effect. The anti-cancer effect of WST-1r was initially found when it was used as a cell proliferation detection reagent, Cell proliferation-WST-1. When combined with (1) apigenin, or its valid substitutes, (2) An INN inhibitor, or (3) transfection of Puc19 or its valid substitutes, plus at least one of the IKK inhibitors, WST-1r synergize the induction of cancer cell death. Such a pharmaceutical composition may be administered, in a therapeutically effective amount, in optimized concentration in phosphate buffered saline or any of the valid pharmaceutical acceptable medium, to a patient in need for the treatment of cancer.

The Cell Proliferation WST-1 is composed of a tetrazolium salt, WST-1 {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzenedisulfonate} (WST-1, Ishiyama M, et al Biol Pharm Bull 1996, 19:1515-20; Berridge MV, et al Biotechnology Annual Review, Vol. II:127-152, 2005), and an intermediate electron acceptor (IEA), 1-methoxy-5-methylphenazinium methyl sulfate (mPMS, Berridge MV, et al Biotechnology Annual Review, Vol. II:127-152, 2005) diluted in phosphor buffered saline.

In accordance with this invention, the active gradient of WST-1r for the treatment of cancer in this invention can be either the WST-1 or the mPMS or the combination of the two components in optimized concentration or optimized ratio. The WST-1r that is described in this invention represents a group of chemical compound or mixes of combinations of a tetrazolium salts and an IEA that have similar activity and function, but not limited to, trans-plasma membranes electron transferring, capable to induce generation of reactive oxygen species (ROS).

The valid substitutes of WST-1 include, but not limited to other tetrazolium salts, examples as WST-3, WST-4, WST-5, XTT at optimized concentration in a pharmaceutical acceptable medium.

The valid substitutes of mPMS include, other IEAs, examples may be as, but not limited to Q1 (Berridge MV, et al Biotechnology Annual Review, Vol. II:127-152, 2005) at optimized concentration in a pharmaceutical acceptable medium.

The WST-1r includes compositions of at least one tetrazolium salt, WST-1, and at least one IEA, mPMS at optimized concentration in a pharmaceutical acceptable medium.

The valid substitute of WST-1r includes, but not limited to (1) the combination of at least one tetrazolium salt with at least one IEA. Examples as, but not limited to: WST-1+mPMS,

WST-3+mPMS, WST-4+mPMS, XTT+mPMS; (2) at least one of the tetrazolium salt, such as, with no limitation, WST-3; (3) at least one IEA, such as, with no limitation, mPMS at optimized concentration in a pharmaceutically acceptable medium.

It is yet another object of this invention to treat cancer cells with WST-1r and apigenin or all other valid substitutes simultaneously and sequentially for each of the above and following embodiments, forming a more preferred embodiment.

It is yet another object of this invention to treat cancer cells with WST-1r with at least one IKK inhibitor simultaneously and sequentially for each of the above and following embodiments, forming a more preferred embodiment.

It is yet another object of this invention to treat cancer cells with (1) the DNA transfection, or IFN, or siRNA transfection or all other valid substitutes and, then, (2) one of the IKK, or CK2 or GSK3 β inhibitors and, then, sequentially treat with WST-1r for each of the above and following embodiments, forming a more preferred embodiment

It is yet another object of this invention to treat with (1) the DNA transfection, or IFN, or siRNA transfection or all other valid substitutes and, then, (2) one of the IKK, or CK2 or GSK3 β inhibitors and, then, sequentially treat with electron coupling reagent of the WST-1r for each of the above and following embodiments, forming a more preferred embodiment

It is yet another object of this invention to treat with (1) the DNA transfection, or IFN, or siRNA transfection or all other valid substitutes and, then, (2) one of the IKK, or CK2 or GSK3 β inhibitors and, then, sequentially treat with all the remaining subcomponent of the WST-1r for each of the above and following embodiments, forming a more preferred embodiment

It is yet another object of this invention to treat with (1) the DNA transfection, or IFN, or siRNA transfection or all other valid substitutes and, then, (2) one of the IKK, or CK2 or GSK3 β inhibitors and, then, sequentially treat with any valid substitution for WST-1r for each of the above and following embodiments, forming a more preferred embodiment

It is yet another object of this invention to treat with (1) the DNA transfection, or IFN, or siRNA transfection or all other valid substitutes and, then, (2) one of the IKK, or CK2 or GSK3 β inhibitors and, then, sequentially treat with any valid substitution for WST-1c for each of the above and following embodiments, forming a more preferred embodiment

It is yet another object of this invention to treat with (1) the DNA transfection, or IFN, or siRNA transfection or all other valid substitutes and, then, (2) one of the IKK, or CK2 or GSK3 β inhibitors and, then, sequentially treat with any valid substitution for electron coupling reagent of the WST-1r for each of the above and following embodiments, forming a more preferred embodiment.

It is yet another object of this invention to treat with (1) the DNA transfection, or IFN, or siRNA transfection or all other valid substitutes and, then, (2) one of the IKK, or CK2 or GSK3 β inhibitors and, then, sequentially treat with any valid substitution for the remaining subcomponent of the WST-1r for each of the above and following embodiments, forming a more preferred embodiment.

It is yet another object of this invention to treat with (1) the DNA transfection, or IFN, or siRNA transfection or all other valid substitutes and, then, (2) one of the IKK, or CK2 or GSK3 β inhibitors and, then, sequentially treat with any valid substitution as any type of combination of the valid substitutes and the subcomponent of the WST-1r for each of the above and following embodiments, forming a more preferred embodiment

Moreover, the present invention provides a method for the treatment of cancer by administering to a patient, in need thereof, a therapeutically effective amount of at least one of the WST-1r component or its valid substitutes mentioned immediately above.

The optimized concentration may or may not be the same concentration as that of the Cell Proliferation WST-1 reagent and may vary from each of the compositions and their valid substitutes and between in vitro and in vivo usage. The preferred optimized in vitro concentration range for mPMS is between 20-60 μ M and the preferred optimized in vitro concentration range for WST-1 is between 0.1-1mM, and the preferred optimized in vitro concentration range for WST-3 is between 0.1-1mM in a pharmaceutically acceptable medium.

WST-1r, WST-3+mPMS and WST-3 are the most preferred embodiment because it is the component for which we have the most valid data.

Moreover, the present invention provides a method for the treatment of cancer by administering to a patient, in need thereof, a therapeutically effective amount of at least one of the WST-1r component or its valid substitutes mentioned immediately above.

In a particular embodiment, the preferred treatment of WST-1r is in contact with cells for 15 minutes to 8 hours. The more preferred treatment time for WST-1r is between 30 min to 4 hours. The even more preferred treatment time for WST-1r is between 2-4 hours.

Cancers that may be treated using the present protocol include, but are not limited to: carcinoma such as the prostate, colorectum, pancreas, cervix, stomach, endometrium, brain, liver, bladder, ovary, testis, head, neck, skin (including melanoma and basal carcinoma), mesothelial lining, white esophagus, breast, muscle, connective tissue, lung (including small-cell lung carcinoma and non-small-cell carcinoma), adrenal gland, thyroid, kidney, or bone; glioblastoma, mesothelioma, renal cell carcinoma, gastric carcinoma, sarcoma, choriocarcinoma, cutaneous basocellular carcinoma, and testicular seminoma, and sarcomas.

IV. Combinatorial Therapies with inhibitor and WST-1r for the Treatment of Cancer

The present invention provides additional methods for inducing cancer cell death and tumor suppression. In accordance with the present invention, it has been discovered that the combination of pUC19 DNA transfection and/or its valid substitutes with an IKK inhibitor for synergistic suppression of cancer cell growth and tumor growth and promote cancer cell death. Accordingly, the present invention provides a pharmaceutical composition and protocol for the treatment of cancer in a patient comprising at least pUC19 DNA transfection or its valid substitutes in combination with at least one IKK inhibitor and WST-1r or at least one of the valid substitutes of the WST-1r. Also provided is a method for treating cancer in a patient by IFN in combination with administering an effective amount of at least one IKK inhibitor and WST-1r or at least one of the valid substitutes of the WST-1r. Also provided is a method for treating cancer in a patient by transfection of the cells with siRNA in combination with administering an effective amount of at least one IKK inhibitor and WST-1r or at least one of the valid substitutes of the WST-1r.

The DNA transfection may be substituted by (i) administering a suitable dose of at least one IFN, or (ii) transfection of at least one specific siRNA targeting at least one of the target transcripts as described previously in this invention, or (iii) chemical compounds or small molecule inhibitors that targets at least one of the target genes and/or its gene products as described previously in this invention, or (iv) antibody targeting at least one of the target genes products as described previously in this invention, (v) anti-sense RNA targeting at least one of the target transcripts as described previously in this invention, (vi) shRNA targeting at least one of the target transcripts as described previously in this invention, (vii) anti-sense oligo targeting at least one of the target transcripts as described previously in this invention, (viii) A dominant negative DNA vector targeting at least one of the target genes as described previously in this invention, (ix) peptides targeting at least one of the target genes products as described previously in this invention.

The target genes are, but not limited to, (1) Homo sapiens transient receptor potential cation channel, subfamily C, member 6 (TRPC6, GeneID: 7225), mRNA (gil19923256|NM_004621.3) synonyms: TRP6, FSGS2, FLJ11098 (SEQ ID #2, #6), (2) Homo sapiens SH3 and PX domains 2B (SH3PXD2B), mRNA (. (SH3PXD2B, GeneID: 285590), mRNA (NM_001017995) synonyms: HOFI; FLJ20831; KIAA1295 (SEQ ID #3, #7), (3) Homo sapiens membrane associated guanylate kinase, WW and PDZ domain containing 3 (MAGIKK, GeneID: 260425), transcript variant 2, mRNA (gil23097339|NM_152900.1) synonyms: MAGI-

3, MGC163281 (SEQ ID #4, #8), and (4) the Homo sapiens transmembrane protein 182 (TMEM182, GeneID: 130827), mRNA (gil40255064|NM_144632.2) (SEQ ID #5, #9).

The gene products include, but not limited to, the transcripts from these genes and proteins above.

The siRNA sequences and the targets of the siRNA sequences may also include the human genomic sequences that flanking the genes as listed in the attached file entitled: "NCBI Blast-pUC19-Human-Transcripts and genome(2686 letters)", "NCBI Blast_siRNA2 Nucleotide sequence (24 letters)" and "NCBI Blast_pcDNA3 Nucleotide sequence (5448 letters)". NCBI Blast-pUC19-Human-Transcripts and genome.

The at least one IFN may be selected from the subfamily of type I IFN including, but not limited to: IFN α A, IFN α B, IFN α C, IFN α D, IFN α F, IFN α G, IFN α H, IFN α I, IFN α J, IFN α K, IFN α 4b, IFN α WA, and IFN α .

The effective concentration of IFN that were used for treating cancer cells was 10 unit/ml or lower for each IFN used.

Suitable IKK inhibitors include any compound which exhibits IKK inhibitory activity.

The at least one IKK inhibitor may be selected from compounds of the group consisting of, without limitation, i) compounds previously established to exhibit IKK inhibitory properties including, but not limited to: SPC839 (Signal Pharmaceutical Inc.), Anilino-Pyrimidine Derivative(Signal Pharmaceutical Inc.), PS1145(Millennium Pharmaceutical Inc.), BMS-345541*(IKK inhibitor III, Bristol-Myers Squibb Pharmaceutical Research Institute), SC-514*(Smithkline Beecham Corp.), Amino-imidazolecarboxamide derivative(Smithkline Beecham Corp.), Ureido-thiophenecarboxamide derivatives(AstraZeneca), Diarylpyridine derivative(Bayer), Pyridooxazinone derivative(Bayer), Indolecarboxamide derivative(Aventis Pharma), Benzoimidazole carboxamide derivative(Aventis Pharma), Pyrazolo[4,3-c]quinoline derivative(Pharmacia Corporation), Imidazolylquinoline-carboxaldehyde semicarbazide derivative(Tulark Inc.), Pyridyl Cyanoguanidine derivate(Leo Pharma), I κ B Kinase Inhibitor Peptide(CalBiochem), IKK-2 Inhibitor IV [5-(p-Fluorophenyl)-2-ureido]thiophene-3-carboxamide(CalBiochem), IKK Inhibitor II (Wedelolactone(CalBiochem), IKK Inhibitor VII (CalBiochem), IKK-2 Inhibitor V (N-(3,5-Bis-trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide IMD-0354, CalBiochem), IKK-2 Inhibitor VI (5-Phenyl-2-ureido)thiophene-3-carboxamide, CalBiochem), IKK-2 Inhibitor VIII (ACHP 2-Amino-6-(2-(cyclopropylmethoxy)-6-hydroxyphenyl)-4-(4-piperidinyl)-3-pyridinecarbonitrile, CalBiochem). ii) In a certain embodiment, the group of IKK inhibitors may additionally include compounds discovered to have IKK inhibitory activity, in accordance with the present invention, and

previously identified as anti-tumor agents, including, but not limited to PS1145(Millennium Pharmaceutical Inc.), BMS-345541*(IKK inhibitor III, Bristol-Myers Squibb Pharmaceutical Research Institute).

Suitable WST-1r and the at least one of the valid substitutes of the WST-1r, as noted herein above, include, but are not limited to WST-1r and each of the individual components that are comprises the WST-1r, the valid substitutes for tetrazolium and that for IEA of the WST-1r and all possible combination among these valid substitutes of WST-1 and mPMS or the combination of these valid substitutes and the individual component of the WST-1r, the tetrazolium salt and IEA, at optimized concentrations in pharmaceutically acceptable medium.

In a specific embodiment of the present invention, the preferred order of treatment in this invention is to administer the pUC19 DNA transfection or its valid substitutes, at least one IKK inhibitor and WST-1r or at least one of the valid substitutes of WST-1r sequentially in any type of order. However, the pUC19 DNA transfection or IFN treatment, or siRNA transfection or its other valid substitutes, at least one IKK inhibitor and the WST-1r or the at least one valid substitutes of WST-1r may be administered to the cells or patient concurrently or sequentially. In other words, the pUC19 DNA transfection may be treated first, the at least one IKK inhibitor may be administered first, the WST-1r or the at least one substitute of WST-1r may be administered first, or the pUC19 DNA transfection, the at least one IKK inhibitor and the at least one substitute of WST-1r may be administered at the same time. Additionally, when the pUC19 DNA transfection is replaced by siRNA transfection, IFN administration, or small molecule targeting the target genes as described in this invention above, in combination with at least one IKK inhibitor and WST-1r or at least one valid substitute of WST-1r is used, the compounds may be administered in any order.

Cancers that may be treated using the present combinatorial protocol are carcinomas and sarcomas include, but are not limited to those cancers described herein above. However, the suitable cancer cells and tumors that may be more susceptible to this treatment are those with aberrant NF- κ B/NF-KAPPAB activities.

The present invention also provides additional methods for inducing cancer cell death and suppressing tumor in cancer patients. In accordance with the present invention, it has been discovered that the combination of a flavonoid, apigenin, or its valid substitutes, or an IKK inhibitor at effective concentration with the WST-1r or the valid substitutes at effective concentration for synergistic induction of cancer cell death. Accordingly, the present invention provides a pharmaceutical composition and protocol for the treatment of cancer in a patient in need with effective dose comprising of at least one flavonoid, specifically, apigenin, or its valid

substitutes, or an IKK inhibitor with WST-1r or at least one of the valid substitutes of the WST-1r in a pharmaceutical acceptable medium.

A removal of the treatment is required for all the above and following embodiments to induce programmed cell death of the treated cells by this method.

Suitable flavonoids include, but not limited to, apigenin and valid substitutes of apigenin in pharmaceutically acceptable medium.

The valid substitutes of apigenin include the compounds that exhibit inhibitory activity as apigenin does in pharmaceutically acceptable medium.

Suitable IKK inhibitors are as listed above and following embodiments include any compound which exhibits IKK inhibitory activity in pharmaceutically acceptable medium. The at least one IKK inhibitor may be selected from compounds of the group consisting of, without limitation, i) compounds previously established to exhibit IKK inhibitory properties including, but not limited to: SPC839 (Signal Pharmaceutical Inc.), Anilino-Pyrimidine Derivative(Signal Pharmaceutical Inc.), PS1145(Millennium Pharmaceutical Inc.), BMS-345541*(IKK inhibitor III, Bristol-Myers Squibb Pharmaceutical Research Institute), SC-514*(Smithkline Beecham Corp.), Amino-imidazolecarboxamide derivative(Smithkline Beecham Corp.), Ureido-thiophenecarboxamide derivatives(AstraZeneca), Diarylpyridine derivative(Bayer), Pyridooxazinone derivative(Bayer), Indolecarboxamide derivative(Aventis Pharma), Benzoimidazole carboxamide derivative(Aventis Pharma), Pyrazolo[4,3-c]quinoline derivative(Pharmacia Corporation), Imidazolylquinoline-carboxaldehyde semicarbazide derivative(Tulark Inc.), Pyridyl Cyanoguanidine derivate(Leo Pharma), IkB Kinase Inhibitor Peptide(CalBiochem), IKK-2 Inhibitor IV [5-(p-Fluorophenyl)-2-ureido]thiophene-3-carboxamide(CalBiochem), IKK Inhibitor II (Wedelolactone(CalBiochem), IKK Inhibitor VII (CalBiochem), IKK-2 Inhibitor V (N-(3,5-Bis-trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide IMD-0354, CalBiochem), IKK-2 Inhibitor VI (5-Phenyl-2-ureido)thiophene-3-carboxamide, CalBiochem), IKK-2 Inhibitor VIII (ACHP 2-Amino-6-(2-(cyclopropylmethoxy)-6-hydroxyphenyl)-4-(4-piperidinyl)-3-pyridinecarbonitrile, CalBiochem). ii) In a certain embodiment, the group of IKK inhibitors may additionally include compounds discovered to have IKK inhibitory activity, in accordance with the present invention, and previously identified as anti-tumor agents, including, but not limited to PS1145(Millennium Pharmaceutical Inc.), BMS-345541*(IKK inhibitor III, Bristol-Myers Squibb Pharmaceutical Research Institute).

Suitable WST-1r and the at least one of the valid substitutes of the WST-1r, as noted herein above, include, but are not limited to WST-1r and each of the individual components that

are comprises the WST-1r, the valid substitutes for tetrazolium and that for IEA of the WST-1r and all possible combination among these valid substitutes of WST-1 and mPMS or the combination of these valid substitutes and the individual component of the WST-1r, the tetrazolium salt and IEA, at optimized concentrations in pharmaceutically acceptable medium.

The effective concentration of apigenin that were used may vary depending on cell type. The preferred dose is at the range of 10-100 μ M in vitro.

For all the above and following embodiments, the effective concentration of WST-1r and the valid substitutes may vary depending on the individual composition and the effective concentration of each of the composition may or may not be the same concentration as that in the Cell Proliferation WST-1 reagent and may vary from each of the compositions and their valid substitutes and between in vitro and in vivo usage. The preferred in vitro concentration range for Cell Proliferation WST-1 is 3-10% in a pharmaceutical acceptable medium. The preferred in vitro concentration range for mPMS is between 20-60 μ M in a pharmaceutical acceptable medium. The preferred in vitro concentration range for WST-1 is between 0.1-1mM when mixed with mPMS in a pharmaceutical acceptable medium. The preferred in vitro concentration range for WST-3 is 0.1-1mM in a pharmaceutical acceptable medium.

In a specific embodiment of the present invention, the administration of the WST-1r or at least one valid substitutes of WST-1r, the apigenin or at least one of the valid substitutes of apigenin or the at least one IKK inhibitor can be in any type of order. Specifically, the WST-1r or at least one valid substitutes of WST-1r, and the apigenin or at least one of the valid substitutes of apigenin or the at least one IKK inhibitor may be administered to the cells or patient concurrently or sequentially. In other words, the apigenin or at least one of the valid substitutes of apigenin or the at least one IKK inhibitor may be administered first, the WST-1r or the at least one substitute of WST-1r may be administered first, or the WST-1r or at least one valid substitutes of WST-1r, and the apigenin or at least one of the valid substitutes of apigenin or the at least one IKK inhibitor may be administered at the same time. The preferred order of the treatment in this invention is to administer the WST-1r or the valid substitutes of WST-1r and the apigenin or the valid substitutes of apigenin or at least one IKK inhibitor simultaneously and then, after removal of the WST-1r, add apigenin or IKK inhibitor again and keep in contact with cells for another 24 hours.

In a particular embodiment, the treatment of WST-1r is in contact with cells for 15 minutes to 8 hours. The preferred time is between 30 min to 4 hours. The more preferred time is between 2-4 hours. A removal of the WST-1r or its valid substitute's from treatment is required

for all the above and following embodiments to induce programmed cell death of the treated cells by this method thereof.

Moreover, the present invention provides a method for the treatment of cancer by administering to a patient, in need thereof, a therapeutically effective dose of at least one of the WST-1r or its valid substitutes and apigenin or at least one of its valid substitutes mentioned above in pharmaceutical acceptable medium.

Also, the present invention provides a method for the treatment of cancer by administering to a patient, in need thereof, a therapeutically effective dose of at least one of the WST-1r or its valid substitutes and at least one IKK inhibitor mentioned above in pharmaceutical acceptable medium.

Cancers that may be treated using the combinatorial protocol with WST-1r or its valid substitutes in combination with apigenin include, but are not limited to those carcinomas and sarcomas that may be treated using the present protocol include, but are not limited to: cancers of the prostate, colorectum, pancreas, cervix, stomach, endometrium, brain, liver, bladder, ovary, testis, head, neck, skin (including melanoma and basal carcinoma), mesothelial lining, esophagus, breast, muscle, connective tissue, lung (including small-cell lung carcinoma and non-small-cell carcinoma), adrenal gland, thyroid, kidney, or bone; glioblastoma, mesothelioma, renal cell carcinoma, gastric carcinoma, sarcoma, choriocarcinoma, cutaneous basocellular carcinoma, and testicular seminoma, soft tissue sarcoma.

Cancers that may be treated using the combinatorial protocol with WST-1r or its valid substitutes in combination with at least one IKK inhibitor include, but are not limited to a subset of carcinomas and sarcomas cancer cells, such as, SK-Mel-5, T294 melanoma cancer cell and cancer cell and cancers that response to this combination treatment.

The present invention provides additional methods for synergistic inhibition of NF- κ B activity in cancer cells. In accordance with the present invention, it has also been discovered that the pUC19 DNA transfection may also synergize the inhibition of NF- κ B activity in cancer cells when both IKK1-KA and IKK2-KA kinase dead dominant negative vector were used simultaneously. This inhibitory effect can be further enhanced by the combination of additional treatment of WST-1r or at least one of the valid substitutes for WST-1r.

Accordingly, pUC19 DNA transfection may be substituted by treating the cells or a mammal with (i) administering a suitable dose of at least one IFN, or (ii) transfection of at least one specific siRNA or shRNA targeting at least one of the target transcripts as described previously in this invention, or (iii) small molecule inhibitors that targets at least one of the target genes products as described previously in this invention, or (iv) antibody targeting at least one of

the target genes products as described previously in this invention, (v) anti-sense RNA targeting at least one of the target transcripts as described previously in this invention, (vi) anti-sense oligo targeting at least one of the target gene's transcripts as described previously in this invention in combination with the treatment of at least one IKK inhibitors that can inhibit both IKK1 and IKK2 kinase activities.

The at least one IFN may be selected from the subfamily of IFN including, but not limited to: IFN α A, IFN α B, IFN α C, IFN α D, IFN α F, IFN α G, IFN α H, IFN α I, IFN α J, IFN α K, IFN α 4b, IFN α WA, IFN β , IFN γ or IL-6.

The transcripts, and proteins as the targets of the siRNA, shRNA, small molecule inhibitor, peptide inhibitor, antibody, anti-sense RNA, anti-sense oligo, and antibody are, but not limited to, (1) Homo sapiens transient receptor potential cation channel, subfamily C, member 6 (TRPC6, SEQ ID 2, 6), (2) Homo sapiens SH3 and PX domains 2B (SH3PXD2B, SeQ ID #3, #7), (3) Homo sapiens membrane associated guanylate kinase, WW and PDZ domain containing 3 (MAGIKK, SeQ ID #4, #8), (4) the Homo sapiens transmembrane protein 182 (TMEM182, SeQ ID #5, #9) and (5) the C6orf108 (Seq ID #14, #15).

Suitable WST-1r and the at least one of the valid substitutes of the WST-1r, as noted herein above, include, but are not limited to WST-1r and each of the individual tetrazolium components that are comprises the WST-1r, the valid substitutes of each component of the WST-1r and any type of combination among these valid substitutes or the combination among these valid substitutes and the individual component of the WST-1 and mPMS.

The at least one IKK inhibitor may be selected from compounds of the group consisting of: i) compounds previously established to exhibit IKK inhibitory properties including, but not limited to: SPC839 (Signal Pharmaceutical Inc.), Anilino-Pyrimidine Derivative (Signal Pharmaceutical Inc.), PS1145 (Millennium Pharmaceutical Inc.), BMS-345541* (Bristol-Myers Squibb Pharmaceutical Research Institute), SC-514* (Smithkline Beecham Corp.), Amino-imidazolecarboxamide derivative (Smithkline Beecham Corp.), Ureido-thiophenecarboxamide derivatives (AstraZeneca), Diarylpyridine derivative (Bayer), Pyridoxazine derivative (Bayer), Indolecarboxamide derivative (Aventis Pharma), Benzoimidazole carboxamide derivative (Aventis Pharma), Pyrazolo[4,3-c]quinoline derivative (Pharmacia Corporation), Imidazolylquinoline-carboxaldehyde semicarbazide derivative (Tulark Inc.), Pyridyl Cyanoguanidine derivative (Leo Pharma), I κ B Kinase Inhibitor Peptide (CalBiochem), IKK-2 Inhibitor IV [5-(p-Fluorophenyl)-2-ureido]thiophene-3-carboxamide (CalBiochem), IKK Inhibitor II, Wedelolactone (CalBiochem), IKK Inhibitor VII K Inhibitor VII (CalBiochem), IKK-2 Inhibitor V N-(3,5-Bis-trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide IMD-

0354(CalBiochem), IKK-2 Inhibitor VI (5-Phenyl-2-ureido)thiophene-3-carboxamide(CalBiochem), IKK-2 Inhibitor VIII ACHP 2-Amino-6-(2-(cyclopropylmethoxy)-6-hydroxyphenyl)-4-(4-piperidinyl)-3-pyridinecarbonitrile(CalBiochem). In a certain embodiment, the group of IKK inhibitors may additionally include compounds discovered to have IKK inhibitory activity, in accordance with the present invention, and previously identified as anti-tumor agents, including, but not limited to PS1145 (Millennium Pharmaceutical Inc.), BMS-345541*(Bristol-Myers Squibb Pharmaceutical Research Institute). The preferred IKK inhibitors are the IKK inhibitors that can inhibit both IKK1 and IKK2 kinase activities.

The present invention provides additional methods for inducing cancer cell death and tumor suppression. In accordance with the present invention, it has been discovered that the combination of a GSK3 β inhibitor with a CK2 inhibitor in combination with WST-1r or at least one of the valid substitutes for WST-1r act synergistically to suppress tumor growth. Accordingly, the present invention provides a pharmaceutical composition for the treatment of cancer in a subset of cancer cells and/or in a patient comprising at least one GSK3 β inhibitor, at least one CK2 inhibitor and WST-1r or the at least one of the valid substitutes for WST-1 in a pharmaceutically acceptable carrier. Also provided is a method for treating cancer in a patient by administering an effective amount of at least one GSK3 β inhibitor in combination with at least one CK2 inhibitor. Suitable GSK3 β inhibitors include any compound which exhibits GSK3 β inhibitory activity, for example, LiCl. Suitable CK2 inhibitors, include, but are not limited to: Apigenin.

The at least one CK2 inhibitor may be selected from compounds of the group consisting of, but not limited to: TBB, TBBz, emodin, CK2 inhibitor III (sigma).

Suitable WST-1r and the at least one of the valid substitutes of the WST-1r, as noted herein above, include, but are not limited to WST-1r and each of the individual components that comprises the WST-1r, the valid substitutes of each component of the WST-1r and any type of combination among these valid substitutes or the combination among these valid substitutes and the individual component of the WST-1r.

In a specific embodiment of the present invention, the at least one GSK3 β inhibitor and at least one CK2 inhibitor may be administered to the cancer cells or patient concurrently or sequentially. In other words, the at least one GSK3 β inhibitor may be administered first, the at least one CK2 inhibitor may be administered first, or the at least one GSK3 β inhibitor and the at least one CK2 inhibitor may be administered at the same time. Additionally, when more than one GSK3 β inhibitor and/or CK2 inhibitor are used, the compounds may be administered in any order.

Cancer cells that may be treated using the present combinatorial protocol include, but are not limited to UM-SCC-6 cells. Cancers that may be treated using the present combinatorial protocol include, but are not limited to, those cancers described herein.

The present invention provides additional methods for enhancing or synergizing the efficacy effects of chemotherapy drugs for the treatment of cancer. In accordance with the present invention, it has also been discovered that the Puc19 DNA transfection also synergizes suppression of tumor growth and promotes cancer cell death. Accordingly, the present invention provides a pharmaceutical composition for the treatment of cancer in a patient comprising puc19 DNA transfection or at least one of its valid substitutes and at least one chemotherapeutic agent. This induction of cancer cell death effect may be further enhanced by additional combination with WST-1r or at least one of the valid substitutes of WST-1r in a pharmaceutically acceptable carrier. Also provided is a method for treating cancer cells or cancer in a patient by administering an effective dose of at least one DNA transfection or at least one of the valid substitutes for DNA transfection in combination with at least one chemotherapeutic agent. In a preferred embodiment, the preferred DNA for transfection is pUC19 DNA cloning vector as described previous in this application (Sequence #1).

The at least one valid substitute for the pUC19 DNA transfection may include, but not limited to, (i) administering a suitable dose of at least one IFN, or (ii) transfection of at least one specific siRNA targeting at least one of the target transcripts as described previously in this invention, or (iii) at least one chemical compounds or small molecule inhibitors that targets at least one of the target genes and/or its gene products as described previously in this invention, or (iv) at lease one antibody targeting at least one of the target genes products as described previously in this invention, or (v) anti-sense RNA targeting at least one of the target transcripts as described previously in this invention, (vi) shRNA targeting at least one of the target transcripts as described previously in this invention, (vii) anti-sense oligo targeting at least one of the target transcripts as described previously in this invention, (viii) A dominant negative DNA vector targeting at least one of the target genes as described previously in this invention, (ix) peptides targeting at least one of the target genes products as described previously in this invention.

Suitable IFN may be selected from any IFN subfamily members, which include, but not limited to, IFN α A, IFN α B, IFN α C, IFN α D, IFN α F, IFN α G, IFN α H, IFN α I, IFN α J, IFN α K, IFN α 4b, WA, IFN β , IFN γ and Interlukine-6 (IL-6). In a preferred embodiment of this invention, the preferred IFN are subfamily members of IFN α , IFN β . The effective concentration of IFN is 10 unit/ml or lower for each IFN.

The target genes to be targeted by the at least one chemical compounds or small molecule inhibitors, at least one specific siRNA, shRNA, anti-sense RNA, anti-sense oligo, dominant negative DNA vector, at least one peptide, at least one antibody, at least one inhibitor are, but not limited to, (1) TRPC6, (SEQ ID #2, #6), (2) SH3PXD2B, (SEQ ID #3, #7), (3) MAGIKK, (SEQ ID #4, #8), (4) TMEM182, , (SEQ ID #5, #9), and (5) C6orf108 (Seq ID #14, #15).

The gene products include, but not limited to, the nucleotide sequence of the transcripts from the gene and amino acid sequence of the protein that derived from these genes.

The siRNA and or shRNA sequences and the targets of the siRNA sequences may also include the nucleotide sequence that mapped to the human genomic sequences that flanking the genes as listed in the attached file "NCBI Blast-pUC19-Human-Transcripts and genome(2686 letters)" and "NCBI Blast_siRNA2 Nucleotide sequence (24 letters)".

Accordingly, Suitable siRNAs include siRNA1 (SEQ ID #10), siRNA 2(SEQ ID #11), and siRNA 3(SEQ ID #12) as described previous in this application and all the potential siRNAs that may be derived from pUC19 DNA sequence that mapped to human genome and/or transcripts in short pieces (10-100 bp and more). These nucleotide sequences and their corresponding genes are listed in the attached file "NCBI Blast-pUC19-Human-Transcripts and genome(2686 letters)" and "NCBI Blast_siRNA2 Nucleotide sequence (24 letters)". As in general, these siRNA sequences can be vary up to 40% from the exact sequences of the gene. Additionally, the function of these siRNAs can be substituted by any of the siRNA and/or shRNA that mapped to other part sequences of the corresponding target gene, small molecule inhibitors, peptide inhibitors, antibodies, anti-sense RNAs, anti-sense oligos and dominant negative DNA vectors that can effectively target the gene products as targets, which are the target of the siRNAs as described above in this paragraph and are include, but not limited to, (1) TRPC6, (SEQ ID #2, #6), (2) SH3PXD2B, (SEQ ID #3, #7), (3) MAGIKK, (SEQ ID #4, #8), (4) TMEM182, , (SEQ ID #5, #9), and (5) C6orf108 (Seq ID #14, #15).

The WST-1r or at least one of the valid substitutes of WST-1r, as noted herein above, include, but are not limited to WST-1r and each of the individual components that are comprises the WST-1r, the valid substitutes of each component of the WST-1r and any type of combination among these valid substitutes or the combination among these valid substitutes and the individual component of the WST-1r.

Suitable chemotherapeutic agents include, but are not limited to: paclitaxel (Taxol.RTM.), cisplatin, docetaxol, carboplatin, vincristine, vinblastine, methotrexate, cyclophosphamide, CPT-11, 5-fluorouracil (5-FU), gemcitabine, estramustine, carmustine, adriamycin (doxorubicin), etoposide, arsenic trioxide, irinotecan, and epothilone derivatives.

The preferred chemotherapeutic agents are paclitaxel (Taxol.RTM.), cisplatin, , 5-fluorouracil (5-FU), and

In a specific embodiment of the present invention, the preferred order is to transfect the pUC19 DNA or at least one of its valid substitutes first and, then, administering the chemotherapy drugs after the transfection of pUC19DNA. However, the pUC19 DNA transfection or at least one of its valid substitutes and administering the chemotherapy drugs may be administered to the cancer cells or patient concurrently or sequentially. In other words, the pUC19 DNA transfection may be administered first; the chemotherapy drugs may be administered first.

Cancers that may be treated using the present combinatorial protocol include, but are not limited to those carcinomas and sarcomas set forth herein above.

In addition to all the above, the present invention provides additional methods for inhibiting NF- κ B activity and tumor suppression. In accordance with the present invention, it has been discovered that the simultaneous transfection of kinase dead IKK1 and IKK2 (KKI-KA and IKK2-KA) that knockout both IKK1 and IKK2 kinase activities act synergistically to suppress NF-KAPPAB activity and tumor growth. The cancer cell growth was further suppressed when this double knockout was combined with the treatment of WST-1r and the at least one of the valid substitutes of the WST-1r. Accordingly, the present invention provides a pharmaceutical composition for inhibition of NF-KAPPAB activity and/or the treatment of cancer in a patient comprising at least one IKK1-KA and at least one IKK2-KA in combination with WST-1r and the at least one of the valid substitutes of the WST-1r in a pharmaceutically acceptable carrier. Also provided is a method for inhibiting of NF-KAPPAB activity and/or the treatment of cancer in a patient by effectively inhibiting IKK1 and IKK2 kinase activity. The effect of knockout IKK1 and IKK2 may be substituted by (1) siRNAs (2) anti-sense RNA, (3) anti-sense oligo, (4) small molecule inhibitors, (5) peptide inhibitors, (6) antibodies, that target IKK1 and/or IKK2 individually or as a whole or in any type of combination. In a particular embodiment, the WST-1r and the at least one of the valid substitutes of the WST-1r is WST-1r.

In a certain embodiment, the group of IKK inhibitors in accordance with the present invention, including, but not limited to SPC839 (Signal Pharmaceutical Inc.), Anilino-Pyrimidine Derivative(Signal Pharmaceutical Inc.), PS1145(Millennium Pharmaceutical Inc.), BMS-345541*(Bristol-Myers Squibb Pharmaceutical Research Institute), SC-514*(Smithkline Beecham Corp.), Amino-imidazolecarboxamide derivative(Smithkline Beecham Corp.), Ureido-thiophenecarboxamide derivatives(AstraZeneca), Diarylpyridine derivative(Bayer), Pyridooxazinone derivative(Bayer), Indolecarboxamide derivative(Aventis Pharma),

Benzoimidazole carboxamide derivative(Aventis Pharma), Pyrazolo[4,3-c]quinoline derivative(Pharmacia Corporation), Imidazolylquinoline-carboxaldehyde semicarbazide derivative(Tulark Inc.), Pyridyl Cyanoguanidine derivative(Leo Pharma), IκB Kinase Inhibitor Peptide(CalBiochem), IKK-2 Inhibitor IV [5-(p-Fluorophenyl)-2-ureido]thiophene-3-carboxamide(CalBiochem), IKK Inhibitor II, Wedelolactone(CalBiochem), IKK Inhibitor VII K Inhibitor VII(CalBiochem), IKK-2 Inhibitor V N-(3,5-Bis-trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide IMD-0354(CalBiochem), IKK-2 Inhibitor VI (5-Phenyl-2-ureido)thiophene-3-carboxamide(CalBiochem), IKK-2 Inhibitor VIII ACHP 2-Amino-6-(2-(cyclopropylmethoxy)-6-hydroxyphenyl)-4-(4-piperidiny)-3-pyridinecarbonitrile(CalBiochem)

In a specific embodiment of the present invention, the IKK inhibition by IKK1-KA and IKK2-KA knockout or any other means as described above and the WST-1r and the at least one of the valid substitutes of the WST-1r may be administered to the cells or patients concurrently or sequentially. In other words, the IKK-KA transfection or other means of IKK inhibition may be administered first, the NOX substrate/activator may be administered first, or the both of them may be administered at the same time. Additionally, when more than one means of IKK inhibition are used, the treatment may be administered in any order.

Cancers that may be treated using the present combinatorial protocol include, but are not limited to those cancers described hereinabove.

The present invention provides additional methods for inducing cancer cell death and tumor suppression. In accordance with the present invention, it has been discovered that the combination of a IKK inhibitor or a CK2 inhibitor in combination with Stat inhibitor, static, or at least one of the valid substitutes for static act synergistically to induce cancer cell death and to suppress tumor growth. Accordingly, the present invention provides a pharmaceutical composition for the treatment of cancer in a subset of cancer cells and/or in a patient comprising at least one IKK inhibitor or at least one CK2 inhibitor and static or the at least one of the valid substitutes for static in a pharmaceutically acceptable carrier. Also provided is a method for treating cancer in a patient by administering an effective amount of at least one IKK inhibitor or at least one CK2 inhibitor in combination with static or valid substitutes. Suitable IKK inhibitors are as listed above. Suitable CK2 inhibitors, include, but are not limited to: Apigenin. Suitable Stat inhibitors are the inhibitors that inhibit stat phosphorylation, activation and nuclear translocation, include, but not limited to static. The administration of the IKK inhibitors or the CK2 inhibitors and the static may be administered in any order. The preferred order is to administrate the inhibitors concurrently.

V. Administration of Pharmaceutical Compositions and Compounds

The pharmaceutical compositions of the present invention can be administered by any suitable route, for example, by injection, by oral, pulmonary, nasal or other methods of administration. In general, pharmaceutical compositions of the present invention comprise, among other things, pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions can include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; and additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol). The compositions can be incorporated into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc., or into liposomes. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of components of a pharmaceutical composition of the present invention. See, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, Pa. 18042) pages 1435-1712 which are herein incorporated by reference. The pharmaceutical composition of the present invention can be prepared, for example, in liquid form, or can be in dried powder form (e.g., lyophilized). Particular methods of administering pharmaceutical compositions are described hereinabove.

In yet another embodiment, the pharmaceutical compositions of the present invention can be delivered in a controlled release system, such as using an intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In a particular embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Rev. Biomed. Eng.* (1987) 14:201; Buchwald et al., *Surgery* (1980) 88:507; Saudek et al., *N. Engl. J. Med.* (1989) 321:574). In another embodiment, polymeric materials may be employed (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Press: Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley: New York (1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* (1983) 23:61; see also Levy et al., *Science* (1985) 228:190; During et al., *Ann. Neurol.* (1989) 25:351; Howard et al., *J. Neurosurg.* (1989) 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the target tissues of the animal, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, (1984) vol. 2, pp. 115-138). In particular, a controlled release device can be introduced into an animal in proximity of the site of inappropriate immune activation or a

tumor. Other controlled release systems are discussed in the review by Langer (Science (1990) 249:1527-1533).

The conclusion that this is programmed cell death is formed by the observation that normal cells had no cytotoxic reaction and further, that a 100% kill rate is more than substantial evidence of a significant find. Therein, because this invention touches a programmed cancer cell death pathway that was prior untouched, or in the alternative, that this invention activates a known pathway in a manner not able to be duplicated by other inventions, the very sequence of events defined in this invention activates programmed cell death in the cancer cells and as such, presents a valid model for further study. In other words, through processes known to those of skill, the very core molecular event leading to the 100% kill rate, can be explored because we have the working model to induce such events. Therein, the invention is also claimed as an important model for further research, study and pathway illumination/elucidation.

Although above and below I have shown specific experimentation and data, One of skill in the art of cancer preclinical and clinical protocol structure, execution and analysis will recognize upon reading this document, through variation of the dosages of the named components, the order in which they are applied and the time frames between applications, valid substitutions of the named components there are a myriad of variable applications which may result in the same or similar outcome. To the extent that these variables can be applied to any cancer in any mammal, the inventor notes than nothing contained within this document or any subsequent documentation provided by the inventor is intended to be limiting. The inventor also notes that this invention is intended to work alone, and reduce cytotoxic effects of traditional cancer therapy, such as chemotherapy and radiation, however, nothing herein is intended to limit the use of this invention to the extent that chemotherapeutic and radiation combination therapies can be utilized in combination with this invention. Further, that the use of chemotherapy and radiation therapy combinations, in conjunction with this invention, may reduce the cytotoxicity of the chemotherapy or radiation therapy because the dosages of the chemotherapy and radiation therapy can be reduced when used in combination with this invention. And finally, that the named invention may further sensitize cancer cells selectively over normal cells such that subsequent application of chemotherapy and radiation, as well as combination chemo/radiation therapies, will work more efficiently again, allowing for the reduction of chemotherapy and radiation and combination chemo/radiation dosages.

The foregoing description of the present invention provides illustration and description, but is not intended to be exhaustive or to limit the invention to the precise one disclosed. Modifications and variations consistent with the above teachings may be acquired from practice

of the invention. Thus, it is noted that the scope of the invention is defined by the claims and their equivalents.

The present invention will now be illustrated in more detail in the following examples. It is to be understood that these examples serve only to describe the specific embodiments of the present invention, but do not in any way intend to limit the scope of the claims. It is of further note to one of skill that unique sequence data has been provided in this application. To the extent that each of these new sequence data represent novel targets for the development of cancer therapeutics, nothing contained herein is intended to be limiting. Said targets are noted as potential targets for further development under this application using the above methods and other methods known to those of skill. Although not mentioned in this specification elsewhere, use of radiation as a distinct step, or other small molecule drugs, DNA, RNA, siRNA and all other methods for cancer therapy known to those of skill are noted as possible adjuvant to these protocols.

EXAMPLES

Example 1

Synergistic inhibition of NF-KAPPAB activity

Overview: Normally, NF-kappaB activity is measured by reporter assay, electronic gel mobility shift assay and more recently, DNA binding ELISA. However, all of these methods employ exogenous DNA oligo or constructs carrying consensus NF-kappaB response element sequences for measuring specific NF-KAPPAB DNA binding and transcriptional activity. Additionally, the NF-KAPPAB consensus response element is different from the real promoter sequences that also need complex interaction with multiple molecules and may introduce artificial effects.

Method: UM-SCC-6 cells were transfected with effectene (Qiagen) i) 20% dominant negative IKK1-KA (K44A) and 80% pUC19, ii) 20% dominant negative IKK2-KA (K44A) and 80% pUC19, iii) 20% dominant negative IKK1-KA (K44A), 20% dominant negative IKK2-KA (K44A) and 60% pUC19, iv) 20% pCDNA3 and 80% pUC19 as negative control, for 72 hours. At the end of transfection, cells were lysed with lysis solution from GeneSpectra kit (Panomics). The $\text{I}\kappa\text{B}\alpha$, p100, CSNK2B mRNA levels were measured with the GeneSpectra kit. The expression levels of each transcript from different transfections were normalized by their 18sRNA level as measured at the same time.

Previously, we observed partial inhibition of NF-KAPPAB reporter activity by ~50% caused by cotransfection of kinase dead K44A-IKK1 or K44A-IKK2 into UM-SCC-6 cells and

other head and neck squamous carcinoma cells. By measuring expression levels of endogenous NF-KAPPAB downstream gene, $I\kappa B\alpha$, p100 and CK2 β , as an indicator of NF-KAPPAB activity, we observed little inhibitory effect on NF-KAPPAB activity from K44A-IKK1 transfected cells (~20%) and no inhibitory effect from K44A-IKK2 transfected cells. In contrast, when inhibiting both IKK1 and IKK2 molecules by cotransfecting dominant negative K44A-IKK1 and K44A-IKK2 simultaneously, we observed ~90% inhibition at all three target gene expression levels that we measured (Fig 1 A and B). This result is different from our data obtained from NF-KAPPAB reporter assay, which showed partial inhibition of NF-KAPPAB activity by inhibiting each of the IKK molecule and this difference may be caused by the artificial effect from the reporter assay. Additionally, our data contradict to current concept that IKK1 and IKK2 activate NF-KAPPAB independently through different pathways. Instead, our data showed synergistic inhibitory effect of combination of K44A-IKK1 and K44A-IKK2 on constitutive NF-KAPPAB activity in these cancer cells, suggesting potential interchangeable function between these two IKKs.

Example 2

Simultaneous Inhibition of IKK1 and IKK2 also lead to cancer cell death

In addition to the inhibition of NF-KAPPAB activity, we also observed cell death associated with cotransfection of K44A-IKK1 and K44A-IKK β from UM-SCC-6 cells (Fig 2). 48 hours after transfection, K44A-IKK1 and K44A-IKK2 co-transfected cells showed 85% reduction in cell number (Fig 2 WST-1 no no) and dramatic cell death (Fig 2B). The data represents the average of 7 sets of duplicates. This result indicates that inhibition of NF-KAPPAB activity does lead to cancer cell death and that this can be reached only by inhibiting both IKK1 and IKK2 simultaneously. Additionally, we also observed that cotransfection of K44A-IKK1 and K44A-IKK2 sensitize UM-SCC-6 cells to Cis-Platinum and 5-FU by 10 to 100 folds (data not shown).

Example 2

Tetrazolium dye WST-1 enhances cancer cell killing caused by double inhibition of IKKs

During this study, we experienced difficulties in measuring cell survival and proliferation by regular WST-1 assay, which measures mitochondria potential. It turned out that the inhibition of IKK kinase activity and NF-KAPPAB activity interfere this measurement. Furthermore, we have found that treating cells with following inhibition of both IKK1 and IKK2 synergized cell death (Fig 2). In Fig 2A we show ~80% reduction of cell number in K44A-IKK1 and K44A-IKK2 cotransfected cells and over 95% reduction when these cells were treated with WST-1 in addition to cotransfection of K44A-IKK1 and K44A-IKK β . Data represents an

average of 7 sets of duplicates. Fig 4B shows cell death from double transfected cells and partial cell death from K44A-IKK1 or K44A-IKK2 single transfected cells.

Example 3

Tetrazolium dye WST-1 facilitate synergistic cancer cell killing caused by DNA transfection in combination with IKK inhibitors in UM-SCC-6 cells

Overview: We further examined whether IKK inhibitors can have similar effect as that by cotransfection of K44A-IKK1 and K44A-IKK2 on cancer cell death. We combined transfection of K44A-IKK1 with IKK2 inhibitors for inhibiting NF-KAPPAB activity. We detected significant cell death at 10 μ M for Inhibitor III, 20 μ M for Inhibitor IV, VI and VII, 30 μ M for Inhibitor II and sc-514 and 60 μ M for MLN120b. There are over all lower cell number in cells treated with combination of K44A-IKK1 and IKK2 inhibitor comparing to IKK inhibitor only, but the differences were limited. However, when cells were treated with the WST-1 following the K44A-IKK1 transfection and IKK inhibitor, a synergistic cell death was observed. WST-1 sensitizes these cancer cells to combined inhibition of IKKs for more than a thousand fold less than that of IKK2 inhibitor concentration used. This synergistic effect applied to 6 of the 7 IKK2 inhibitors that have been tested. Fig 3 shows this synergistic cancer cell death caused by combination of K44A-IKK1 transfection with Inhibitor III and VII. Similar effects have been observed in Inhibitor II, VI, sc-514 and MLN120b treated cells. These data suggest that IKK inhibitors may only make the cancer cells vulnerable and a third hit with WST-1 is required to trigger cancer cell death. Thus our data showed that triple combination of inhibition of IKK1, IKK2 and the following treatment with WST-1 can synergistically facilitate cancer cell death and will be implicated in cancer treatment.

On the other hand, the data also showed that the differences between the control vector (p3+pUC19) and the IKK1-KA vector (IKK1-KA + pUC19) were very limited. More detailed analysis found that this combination effect is because of pUC19 DNA transfection in combination with IKK inhibitors and WST-1.

Example 4

WST-1 promote HT1080 human sarcoma cell death in triple combination treatment

Methods: HT1080 cells were cultured in 96 well plates and treated with DNA transfection with one of the pUC19, pCDNA3, IKK1-KA, IKK1-KA+pUC19, and pCDNA3+pUC19 DNA vectors, IKK Inhibitor III and WST-1 sequentially. Each set of the cells were transfected with one of the DNA vectors as described immediate above in this paragraph except for untransfected control for 24 hours followed by IKK Inhibitor III treatment at concentration of 3-30 μ M for another 24hours and, then, with WST-1 for 4 hours and cultured

overnight before detection. The same treatments of cells were measured at 24, 48 and 96 hours after WST-1 treatment. Cell viability was measured by Cell Count Kit 8 (CCK8) kit.

Data showed (1) significant IKK inhibitor III dose dependent inhibition of cell growth and decreased cell survival from the cells that were transfected with any of the DNA vectors at 24, 48 and 96 hour after WST-1 treatment comparing to non transfected control cells, but no significant difference between pUC19 vector only form IKK1-KA vector (Fig 4); (2) further induced cell death and decreased cell survival detected from the cells that were treated with WST-1 at 24, 48 and 96 hours after WST-1 treatment comparing to the no WST-1 treated cells; (3) at 96 hours after the treatment of WST-1 all the non transfected cells grow back to the same amount as the untrated control, while partial recovery of the cells from no WST-1 treated, but trasnfected cells; and (4) at 96 hours after all the treatment, only the transfected cells and also treated with WST-1 remained died with no recovery, which means that the treatment by either IKK inhibitor and/or DNA transfection make these cells sick and the WST-1 in the triple combination further promote these sick cells to 100% death. The difference in the absorption at 24 hour after WST-1 treatment were caused, in partial, by decreased response from the WST-1 treated cells to the CCK8 detection. This effect reduced in 48 hours after the removal of WST-1 treatment and diminished at 96 hours after the removal of WST-1 treatment. Morphology examination of the cells found that at 24 hours after the WST-1 treatment majority of the 30 μ M IKK inhibitor III treated cells died after the treatment. However, the survival cells that were not treated with WST-1 grow back up again. Conversely, the deaths of all the cells that were transfected with DNA vectors and with the same IKK inhibitor III treatment and treated with WST-1 were 100%. These data demonstrate the effect of WST-1 enhances the IKK inhibitor III induce cancer cell death effect and promote cell death of these cells and that pUC19 vector may also contribute to the combined effect of inhibiting cell growth.

Example 7

IFN substitute pUC19 transfection to enhance IKK inhibitor III and WST-1 effect

Overview: Our previous data suggest that DNA transfection plays a role in the triple combination treatment for synergistic cancer cell death. Moreover, Interferon (IFN) responses have been reported to be involved in transfection effects. We examined whether IFN can be a substitute for the DNA transfection effect for in vivo treatment.

Methods: HT1080 cells were cultured in 96 well plates and treated with IFN, IKK inhibitor III and WST-1 sequentially. Each set of the cells were treated with one of the IFN members at the concentration ranging from 2-1000units/ml for 24 hours followed by IKK inhibitor III treatment at concentration of 3-30 μ M for another 24 hours and, then, with WST-1

for 4 hours and cultured overnight before detection. Cell viability was measured by CCK8 kit at 24 and 48 hours after WST-1 treatment. Total of 15 IFNs were tested. They are IFN α A, IFN α B, IFN α C, IFN α D, IFN α F, IFN α G, IFN α H, IFN α I, IFN α J, IFN α K, IFN α 4b, and IFN α WA, IFN β , IFN γ and IL-6.

Data (Fig 4) showed IFN dose dependent and IKK Inhibitor III dependent decrease of cell growth and enhancement of cell death comparing to that without IFN treatment. Comparing to pUC19 DNA transfection, which synergized the inhibition of cell growth and promotes cell death, IFN reached 80-90% inhibitory effects caused by pUC19 DNA transfection when combined with 30 μ M I-3 and WST-1 at 48 hours after WST-1 treatment.

Example 5

WST-1 induces ROS generation

Overview: WST-1 was first described by Ishiyama et al in 1996 (Ishiyama M, et al Biol Pharm Bull 1996, 19:1515-20). It is a cell proliferation detection reagent manufactured by Roche. WST-1 is composed of tetrazolium salt WST-1 {4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolol]-1, 3-benzenedisulfonate} and an electron coupling reagent diluted in phosphate buffered saline. WST-1 can be cleaved by mitochondrial succinate-tetrazolium-reductase system. This cleavage has been used as the basis of the measurement of live cell. However, WST-1 has been found impermeable to cell membrane and their reduction occurs at the cell surface or at the level of the plasma membrane via trans-plasma membrane electron transport (Berridge, MV et al, Biotechnol Annu Rev, 2005; 11:127052). Alternatively, WST-1 can be reduced by cell surface NAD(P)H-Oxidase (Berridge, MV, et al, Antioxid Redox Signal, 2000, 2:231-42, Scalett, DJ, et al, Biofactors, 2004, 20:199-206). In the present invention WST-1 has been found to synergize the inhibitory effect on cell growth and promote cancer cell death when it is used in combination with at least one of the DNA transfection, or IFN, or siRNA transfection and one of the IKK, or combination of CK2 and GSK3 β inhibitors. Theoretically, it has been proposed that the balance between JNK activation and NF-KAPPAB activity determines cell fate to death or a live. Prolonged JNK activation induces programmed cell death. Generation of ROS induces JNK activation while NF-KAPPAB activity leads to suppress ROS level (Luo, JL, et al, J. Clin. Invest, (2005) 115:2625-32, Shen, HM, et al, Free Radical Biology & Medicine 2006, 40:928-939). The present invention has found that WST-1 induces ROS production in these cancer cells and promotes cell death.

Methods: HT1080 cells were cultured in cover slices and transfected with pUC19 and treated with IKK inhibitor III in sequential. Following these treatment, the cells, thus, treated, were either labeled with CM-H2-DCFDA, a fluorescence dye that can label ROS in cells,

and, then, treated with WST-1 for 30 minutes (Fig 10-A) or treated with WST-1 for 2 hours and then labeled with CM-H2-DCFDA (Fig 5-B). The results were recorded by a digital camera with Spotlight software. Manual exposure levels were used to maintain the same exposure level for comparison.

In both experiments, significant WST-1 induced ROS generation has been documented (Fig 5 A and B). In Fig 8-A, we also observed IKK inhibitor III dose dependent labeled ROS from the cells that were transfected with pUC19, and treated with IKK, but with no exposure to WST-1. This may suggest that IKK Inhibitor III may also induce ROS generation.

Example 6

LiCl + Apigenin synergized SCC-6, WST-1 enhance further

Overview: LiCl is known to inhibit GSK3 β activity and Apigenin is a CK2 inhibitor. The activity of both GSK3 β and CK2 are known to influence NF-KAPPAB activity. I have examined whether combination of LiCl and Apigenin can substitute DNA transfection for the synergistic inhibitory effect.

Methods: UM-SCC-6 cells were cultured in 96 well plates and treated with LiCl (1, 3, 10, 30, 100mM) and Apigenin (1, 3, 10, 30, and 100 μ M) in different combination of their doses for 24 hours followed by WST-1 treatment. Cell viability was measured with CCK8 kit.

Data showed that combination of LiCl and Apigenin dose dependent decrease of cell growth and enhancement of cell death comparing to untreated control cells. 10 μ M AP and 30mM LiCl showed synergistic increase of cell death (Fig 6A). The subsequent treatment of WST-1 further enhanced this inhibitory effect (Fig 6B).

Example 7

pUC19 DNA transfection synergize chemotherapeutic drug effect in UM-SCC-6 cells

UM-SCC-6 cells were transfected with pUC19 DNA, pCDNA3, pUC19+pCDNA3, IKK1-KA+pUC19, IKK2-KA+pUC19, and IKK1-KA+ IKK2-KA+pUC19, IKK1-KA+pCDNA3, IKK2-KA+pCDNA3, or IKK1-KA+ IKK2-KA+pCDNA3, for 48 hours and, treated with variable doses of 5-FU (Fig 7A) or Cis-Platinum (Fig 7B) for 96 or 72 hours respectively. Cell viability was measured in 96 and 72 hours respectively after drug treatment. Data showed that pUC19 transfected cells showed the strongest inhibitory effects on cell growth.

Example 8

pUC19 DNA transfection synergize chemotherapeutic drug effect in HT1080 cells

HT1080 Cells were transfected with pUC19 DNA, pCDNA3, pUC19+pCDNA3, IKK1-KA+pUC19, IKK2-KA+pUC19, and IKK1-KA+ IKK2-KA+pUC19, IKK1-KA+pCDNA3, IKK2-KA+pCDNA3, or IKK1-KA+ IKK2-KA+pCDNA3, for 48 hours before the treatment of

chemotherapy drugs at various doses. Cell viability was measured in 72 and 96 hours after drug treatment.

Drug treatment: Cis-Platinum 30ng/ml – 3 μ g/ml (Fig 7D), Paclitaxel 1nM – 10 μ M (Fig7C), 5-FU 50nM- 500 μ M (data not shown), Doxorubicin 30nM – 3.3 μ M (data not shown).

Variable enhancement and synergistic effects were shown by the transfection of these DNA vectors. pUC19 DNA alone transfection showed the strongest synergistic efficacy effect to these chemotherapy drugs comparing to other DNA vectors tested. IC₅₀ of the drugs were lowered approximately 10 fold when combined with pUC19 DNA transfection comparing to untransfected cells of drug treatment. Furthermore, at 96 hours after of the cis-Platinum or paclitaxel treatment untransfected cells recovered and grown up while the transfected cells, especially pUC19 vector alone transfected cells were irreversible, meaning they were 100% died. These data suggest that these chemotherapy drugs inhibit cancer cell growth, but may not kill these cells. The combination of transfection of pUC19 DNA promote cell to death.

Example 9

pUC 19 transfection synergize Dominant negative IKK-KA inhibition of NF-KAPPAB activity

Fig 1 shows the synergistic inhibition of NF-KAPPAB activity by inhibiting both IKK1 and IKK2 kinase activities. pUC19 was used as a carrier DNA for cotransfection. To examine whether pUC19 played a role in the synergistic inhibition of NF-KAPPAB activity, pCDNA3 was used in parallel with pUC19 for cotransfecting HT1080 cells 20% dominant negative IKK1-KA (K44A) and 80% pUC19, ii) 20% dominant negative IKK2-KA (K44A) and 80% pUC19, iii) 20% dominant negative IKK1-KA (K44A), 20% dominant negative IKK2-KA (K44A) and 60% pUC19, iv) 20% pCDNA3 and 80% pUC19 as negative control, v) 100% pUC19 as another negative control, and in another set of transfection: vi) 20% dominant negative IKK1-KA (K44A) and 80% pCDNA3, vii) 20% dominant negative IKK2-KA (K44A) and 80% pCDNA3, viii) 20% dominant negative IKK1-KA (K44A), 20% dominant negative IKK2-KA (K44A) and 60% pCDNA3 for 72 hours. At the end of transfection, cells were lysed with lysis solution from GeneSpectra kit (Panomics). pUC19 and pCDNA3 alone as negative control. The I κ B α (Fig 17A), CSNK2B (Fig 8B) and p100 (Fig 8C)mRNA levels were measured as indicators of NF-KAPPAB transcription activity with the GeneSpectra kit. The expression levels of each transcript from different transfections were normalized by their 18sRNA level as measured at the same time. Data showed that cotransfection of pUC19 has stronger inhibitory effects on the expression levels of the I κ B α (Fig 8A), CSNK2B (Fig 8B) and p100 (Fig 8C)mRNA comparing to those cotransfected with pCDNA3 (Fig 8).

Example 10

Combination treatment of apigenin with WST-1r synergizes induced cancer cell death

Method: UM-SCC6, MDA-MB-231, Cal27, HT1080, B6-5 and A431 cells were treated with 3% of WST-1r or 100μM apigenin or combination of 3% WST-1r with 100μM apigenin in parallel with untreated control cells and DMSO control for 4 hours, then, the treatments were removed and the cells, thus, treated were changed to normal growth medium and maintained in culture for another 24 hours. DMSO was used as vehicle control. Cell viabilities were measured by CCK8 Kit and normalized to % of untreated control calls.

Result: Data showed that the combination of WST-1r and apigenin induced 75% to 95% cell death of all six tested cancer cell lines comparing to untreated controls (Fig 9).

Example 11

Effect of Apigenin and WST-1r treatment order on induced cell death

Method: UM-SCC6 (Fig 10 A) and HT1080 (Fig 10 B) cells were treated with 10% of WST-1r and 100μM apigenin in combination in different order as indicated. Cell viabilities were measured by CCK8 Kit and normalized as % of that of untreated control calls. Control: Cells treated with apigenin only at the indicated doses for 24 hours and, then, changed to normal growth medium for another 24 hours before measuring cell viability. Ap→GS: Cells treated with apigenin only at the indicated doses for 24 hours and, then, added WST-1r to 10% final concentration for 4 hours, then removing the treatment and changed to normal growth medium for another 24 hours before measuring cell viability. GS+Ap: Cells treated with 10% WST-1r and apigenin at the indicated doses for 4 hours and, then, removing the treatment and changed to normal growth medium for another 24 hours before measuring cell viability. GS+Ap→Ap: Cells treated with 10% WST-1r and apigenin at the indicated doses for 4 hours and, then, removing the treatment and added apigenin at the indicated doses in normal growth medium for another 24 hours before measuring cell viability. GS→Ap: Cells treated with 10% WST-1r for 4 hours and, then, removing the treatment and added apigenin at the indicated doses in normal growth medium for another 24 hours before measuring cell viability. Data showed that induction of cell death occurred in all four combination orders (Fig 10 A, B), but the most effective order of treatment may vary depending on specific cell lines. The GS+Ap→Ap showed the best effects from both of the two cell lines.

Example 12

Time course and Dose Response of WST-1r and Dose-Response of apigenin involved in the combination treatment of WST-1r with apigenin

Methods: Cal27 (C1), HT1080 (C2) and UM-SCC6 (C3) cells were treated with variable concentration (1%, 3% and 10%) of WST-1r as indicated for 0.5, 1, 2, and 4 hours in

combination with different doses of apigenin (3, 10, 30 and 100 μ M) as indicated and, then, treated with the same concentration of apigenin for another 24 hours. Cell viabilities were measured by CCK8 Kit and normalized as % of that of untreated control calls.

Results: Data showed WST-1r time and dose dependent and apigenin dose dependent cell death from all three tested cell lines (Fig 11-C1-3). Synergetic induction of cell death (over 80%) occurred at 10% WST-1r treatment for 0.5 hour in combination with 100 μ M apigenin treated Cal27 and UM-SCC6 cells (Fig 11-C1, 3) and at 3% WST-1r treatment for 1 hour in combination with 100 μ M apigenin treated HT1080 cells (Fig 11-C2). By increasing WST-1r treatment time, 80-90% cell death can be reached at 30 μ M apigenin in combination with 4 hour 10% WST-1r treatment from HT1080 and UM-SCC6 cells (Fig11-C2-3) and at 30 μ M apigenin in combination with 1 hour 10% WST-1r treatment from Cal 27 cells (Fig11-C1).

Example 13

Effect of Combination treatment with IKK Inhibitor and WST-1r on melanoma cell lines

Method: SK-Mel-5 and T294 human melanoma cells were treated with WST-1r at 1% and 3% final concentration respectively as indicated for 4 hours, then, removed WST-1r by changing to normal growth medium and added IKK inhibitor III for another 24 hours. After 24 hours treatment with 3 μ M and 10 μ M IKK inhibitor III respectively as indicated, cells were changed to grow in normal growth medium for 48 hours before measuring cell viability by CCK8 Kit.

Result: Data showed that synergetic induction of cell death occurred at the treatment with 3% WST-1r in combination with 10 μ M IKK inhibitor III (Fig12-A1-2).

Example 14

Effects of treatment order of WST-1r and IKK inhibitor III on induced cell death

Method: T294 cells were treated with 3% of WST-1r in combination with 3 or 10 μ M IKK Inhibitor III respectively in different order as indicated. Cell viabilities were measured by CCK8 Kit and normalized as % of that of untreated control calls. **Control:** Cells were either untreated or treated with IKK Inhibitor III only at the indicated doses for 24 hours and, then, changed to normal growth medium for another 24 hours before measuring cell viability. **Ap→GS:** Cells treated with IKK Inhibitor III only at the indicated doses for 24 hours and, then, added WST-1r at 3% final concentration for 4 hours, then removing the treatment and changed to normal growth medium for another 24 hours before measuring cell viability. **GS+Ap:** Cells treated with 3% WST-1r and IKK Inhibitor III at the indicated doses for 4 hours and, then, removing the treatment and changed to normal growth medium for another 24 hours before measuring cell viability. **GS+Ap→Ap:** Cells treated with 3% WST-1r and IKK Inhibitor III at the indicated doses for 4 hours and, then, removing the treatment and added IKK Inhibitor III at the indicated

doses in normal growth medium for another 24 hours before measuring cell viability. GS→Ap: Cells treated with 3% WST-1r for 4 hours and, then, removing the treatment and added IKK Inhibitor III at the indicated doses in normal growth medium for another 24 hours before measuring cell viability.

Result: Data showed that GS+Ap and GS+Ap→Ap treatment orders synergized induction of cell death (Fig13 F2-B).

Example 15

WST-1r and Apigenin combination treatment induced JNK Phosphorylation

Method: UM-SCC6 cells were treated with WST-1r and apigenin at the indicated doses for 4 hours, and then phosphorylated JNK and total JNK were measured in parallel with FACE Kit (Qiogen). The resulting data were normalized to total cell number measured by crystal violet staining. The phosphorylated JNK from each measurement were normalized to the ratio of phosphorylated JNK over total JNK values.

Result: Data showed WST-1r dose dependent and apigenin dose dependent induction of phosphorylation of JNK in UM-SCC6 cells (Fig 14). Combination of WST-1r and apigenin further increased JNK phosphorylation. The most significant increase of JNK phosphorylation from UM-SCC6 cells occurred at the treatment of 100μM apigenin in combination with 3% and 10% WST-1r. One observed exception was 10μM apigenin showed inhibitory effect on JNK phosphorylation. This result supports the hypotheses that the combination of WST-1r with apigenin treatment induced JNK activation.

Example 16

Dose Response of ROS generation after combination treatment of WST-1r and apigenin and IKK inhibitor III

Method: UM-SCC6 cells were labeled with 10μM CM-H2-DCFDA for 15 minutes and then treated with WST-1r or CCK8 at the indicated amounts in combination with variable doses of apigenin (A) or IKK Inhibitor III (B) for 4 hours. Fluorescence at Ex485/Em535 were measured for detecting ROS generation that labeled by the CM-H2-DCFDA.

Result: Data showed WST-1r dose dependent induction of ROS generation (Fig23A and B). On the other hand, CCK8 induced low and very limited level of ROS generation with no relation to the CCK8 treatment dose at 4 hours after the treatment. Apigenin alone showed no effect on ROS generation. However, combination of apigenin with 1% and 3% WST-1r did show apigenin dose dependent, limited, but, steady increase on ROS generation from thus treated cells when comparing to that of the corresponding doses of WST-1r only treated cells. (Fig23-A) Conversely, combination of 10% of WST-1r with apigenin resulted in decrease of ROS

levels(Fig23-B). In addition, when combined with CCK8, apigenin also increase the ROS generation(Fig23-A). This effect is apigenin dose dependent.

Similarly, IKK inhibitor III alone and combination of WST-1r with IKK inhibitor III (Fig23-B) showed similar effect as apigenin did, where 5 μ M IKK Inhibitor increased ROS levels while 10 μ M IKK Inhibitor III decreased it (Fig23-B). However, IKK Inhibitor III had no combined effect with CCK8 on ROS levels.

Example 17

Time course of ROS generation after combination treatment of WST-1r and apigenin and IKK inhibitor III

Method: UM-SCC6 cells were labeled with 10 μ M CM-H2-DCFDA for 15 minutes and then treated with WST-1r or CCK8 at the indicated amounts in combination with variable doses of apigenin or IKK inhibitor III for the time period from 15 minute up to 4 hours. At each time points as indicated, fluorescence at Ex485/Em535 were measured for detecting ROS generation that labeled by the CM-H2-DCFDA.

Result: Data showed that WST-1r induced ROS generation continued increase and lasted at least for more than 4 hours (Fig15-B and D), whereas, CCK8 only induced low level and transience increase of ROS(Fig15-A and C).

Example 18

CCK8-XTT-WST-1 Comparison

Comparison cell death inducing capability of CCK8 and XTT to WST-1r in combination with apigenin treatment

Method: HT1080 and UM-SCC6 cells were treated with 10% of WST-1r, CCK8 or XTT in combination with variable doses of apigenin for 4 hours and, then changed to normal growth medium for another 24 hours. Cell viability was measured with CCK8 kit.

Result: Data showed that CCK8 had no effect on cell death while XTT showed intermediate induction of cell death effect comparing to WST-1r on both UM-SCC6 and HT1080 cells (Fig16).

Example 19

Comparison cell capability of inducing cell death by other tetrazolium salts to that by WST-1

Method: HT1080 and UM-SCC6 cells were treated with 1mM WST-1, 0.4mM WST-3, 0.5mM WST-4, 0.5mM WST-5 or 0.12mM mPMS alone or each of the WST-3, WST-4, and WST-5 at the same concentration in combination with 0.12mM mPMS (0.4mM WST-3+0.12mM mPMS, 0.5mM WST-4+0.12mM mPMS, 0.5mM WST-5+0.12mM mPMS) plus

apigenin for 4 hours and, then changed to normal growth medium for another 24 hours. Cell viability was measured with CCK8 Kit.

Result: Data showed that WST-3 alone, WST-3+mPMS and WST-4+mPMS in combination with apigenin showed similar effect on inducing cell death that equivalent to that WST-1r does (Fig17 A-B). WST-1, WST-4, and WST-5 alone showed no such effect (Fig17 A,B). WST-3+mPMS are more potent than WST-1r on cell death induction.

Example 20 HT1080: mPMS Dose-Response on cell death

Method: HT1080 cells were treated with variable concentrations of mPMS as indicated in combination with 1mM WST-1 and 10, 30 or 100 μ M apigenin for 4 hours and, then, changed to normal growth medium for another 24 hours. Cell viabilities were measured by CCK8 Kit. 1 mM WST-1 only, 0.12mM mPMS only and 10% WST-1r were used as parallel control.

Result: Data showed mPMS and apigenin dose dependent cell death (Fig 18).

Fig 19 plotted the same cell survival data as response to apigenin doses along with all the controls. Data label: Ctrl: untreated control, GS: WST-1r, mPMS: 1.2mM mPMS alone, WST-1: WST-1 alone, 1: 1mM WST-1r plus 0.12 mM mPMS, 2: 1mM WST-1r plus 0.1 mM mPMS, 3: 1mM WST-1r plus 0.08 mM mPMS, 4: 1mM WST-1r plus 0.06 mM mPMS, 5: 1mM WST-1r plus 0.04 mM mPMS, 6: 1mM WST-1r plus 0.02 mM mPMS.

Data showed mPMS dose dependent cell death. mPMS 0.12mM is very toxic to cells Combination with WST-1 attenuated this toxicity. When mPMS was combined with WST-1, data showed mPMS and apigenin dose dependent cell death. 0.08mM WST-1 plus 0.12mM mPMS showed similar cell death effect as WST-1r did. WST-1 1mM and mPMS 0.06mM Ap100 caused 100% cell death.

Example 21

Effect of substitution of WST-1r with WST-3 for combination treatment with apigenin on induction of cell death

Method: UM-SCC6, HT1080, Cal27, SK-Mel-5, and HEKa cells were treated with WST-3 alone, in combination with 10 or 30 μ M apigenin for 4 hours and, then, changed to normal growth medium and remained culture in this medium for another 24 hours. After the 24 hours culture, cell viabilities were measured with CCK8 Kit. Data were normalized to % of untreated control cells.

Result: Data showed that over 80% cell death observed from combination of 30 μ M apigenin with 50 μ M WST-3 treated Cal27, UM-SCC6, HT1080 and SK-Mel-5 cells (Fig 20). On the other hand, under this treatment condition, HEKa, primary cultured human keratinocytes,

were resistant to this treatment. This difference in sensitivity to this combination treatment may provide a window for differential targeting cancer cells and to control toxicity to normal cells.

Example 22

Effect of substitution of WST-1r with WST-3+mPMS for combination treatment with apigenin on induction of cell death

Method: UM-SCC6, HT1080, Cal27, SK-Mel-5, and HEKa cells were treated with 0.1mM WST-3 plus 30 μ M mPMS, or WST-3 only, or untreated control in combination with 10 or 30 μ M apigenin for 4 hours and, then, changed to normal growth medium and remained culture in this medium for another 24 hours. After the 24 hours culture, cell viabilities were measured with CCK8 Kit. Data were normalized to % of untreated control cells.

Result: Data showed that over 90% induced cell death observed from thus treated Cal27, UM-SCC6, and SK-Mel-5 cells that were treated in combination of 0.1mM WST-3, 30 μ M mPMS and 30 μ M apigenin (F3-F2). On the other hand, under this treatment condition, HEKa, primary cultured human keratinocytes, were relative resistant to this treatment. This difference in sensitivity to this combination treatment may provide a window for differentiating targeting cancer cells and to control toxicity to normal cells.

Example 23

Enhancement of Taxel Efficacy Effects by Combination of Puc19 DNA sequence derived siRNA with Taxel

Method: HT1080 cells transfected with siRNAs that were derived from Puc19 DNA sequence and the siRNAs that targeting the corresponding genes that are the targets of the Puc19 derived siRNAs for 24 hours, then, treated with Taxel at 3, 10, 30, and 100nM for 48hours. After the 48 hours of Taxel treatment, cells in culture were changed to normal growth medium for 24 to 72 hours. Cell viability was monitored by CCK8 Kit every 24hours. Data are normalized to % of untreated control cells. The siRNAs that used for this study includes siRNA#1, siRNA#2, siRNA#3, siRNA targeting TRPC6, SH3PXD2B, C6orf108, TTBK1, MAGI3, and TMEM182 as well as combination of siRNA32+#3, and siRNA#1+#2+#3+#4+#5 (siRNA Σ 1-5).

Result: Data showed represent the measurements of 72 hours after the treatment. The cell survival data showed Taxel dose dependent cell death and enhanced cell death by Puc19 trasnfecion and majority of the siRNA trasfactions (Fig 21). The IC50 of taxel was reduced more than three fold by Puc19 DNA trasnfecion and by the trasnfeciton of siRNAs targeting TRPC6, SH3PXD2B, C6orf108, TTBK1, MAGI3, and TMEM182 as well as by the transfection of combination of siRNA#2+#3, and siRNA#1+#2+#3+#4+#5. Over 2.5 fold decrease of IC50

of taxel was observed from siRNA#2, siRNA#3, and siRNA targeting SH3PXD2B, C6orf108. These data support the hypothesis that the DAN sequences of Puc19 DNA vector may code for some short functional sequences that can target and interrupt the expression levels of the corresponding genes and the cellular functions. At least the genes (TRPC6, SH3PXD2B, C6orf108, MAGI3, and TMEM182) that have been tested can be used as a target for anti-cancer drug design for enhancing the efficacy effect of chemotherapy drugs. The siRNAs targeting these corresponding genes that were identified may also be used as a tool to reach this goal. In addition, these combined treatments induce cancer cell death rather simple inhibition of cell growth. 72 hours after treatment, the treated cells did not grow back. This feature adds to lasting effect of the treatment.

Example 24

Substitution of Puc19 with siRNA against TMEM182 and MAGI3 for Puc19-IKK Inhibitor-WST-1r triple combination treatment

Method: HT1080 cells were transfected with siRNA#3, or the siRNA targeting MAGI3, and TMEM182 that were derived from Puc19 DNA sequence and the siRNAs that targeting the corresponding genes that are the targets of the Puc19 derived siRNAs for 24 hours, then, treated with IKK inhibitor III for 24 hours followed by adding WST-1r for another 4 hour. After the 4 hours WST-1r treatment, cells in culture were changed to normal growth medium for 24. Cell viability was monitored by CCK8 Kit every 24hours. Data are normalized to % of untreated control cells. AllStar siRNA was used negative siRNA transfection control. Puc19 DNA vector transfection was used as positive control.

Result: Data showed IKK inhibitor III dose dependent cell death and that siRNA targeting MAGI3 and TMEM182 synergize the cell death (Fig 22). Again, these data showed that targeting TMEM182 and MAGI3 may enhance effect on cancer treatment.

SEQUENCE LISTING

Seq ID

#1 pUC19 nucleotide sequence

#2 TRPC6 nucleotide sequence

>gi|19923256|ref|NM_004621.3| Homo sapiens transient receptor potential cation channel, subfamily C, member 6 (TRPC6), mRNA

#3 SH3PXD2B nucleotide sequence

>gi|63055058|ref|NM_001017995.1| Homo sapiens SH3 and PX domains 2B (SH3PXD2B), mRNA

#4 MAGI3 nucleotide sequence

>gi|23097339|ref|NM_152900.1| Homo sapiens membrane associated guanylate kinase, WW and PDZ domain containing 3 (MAGIKK), transcript variant 2, mRNA

#5 TMEM182 nucleotide sequence

>gi|40255064|ref|NM_144632.2| Homo sapiens transmembrane protein 182 (TMEM182), mRNA

#6 TRPC6 peptide sequence
>gi|5730102|ref|NP_004612.2| transient receptor potential cation channel, subfamily C, member 6 [Homo sapiens]
#7 SH3PXD2B Polypeptide
>gi|63055059|ref|NP_001017995.1| SH3 and PX domains 2B [Homo sapiens]
#8 MAGI-3 membrane associated guanylate kinase, WW and PDZ domain containing 3 Polypeptide sequence
>gi|23097340|ref|NP_690864.1| membrane-associated guanylate kinase-related 3 isoform 2 [Homo sapiens]
#9 TMEM182 polypeptide sequence
>gi|40255065|ref|NP_653233.2| hypothetical protein LOC130827 [Homo sapiens]
#10 siRNA1
Sense: UGA AUU CGA GCU CGG UAC CCG GGG A
Antisense: UCC CCG GGU ACC GAG CUC GAA UUC A
#11 siRNA 2
Sense: CAG GAA AGA ACA UGU GAG CAA AAG
Antisense: CUU UUG CUC ACA UGU UCU UUC CUG
#12 siRNA3
Sense: CUU UUA AAU UAA AAA UGA AGU UUU A
Antisense: UAA AAC UUC AUU UUU AAU UUA AAA G
#13 pcDNA3m
SEQ ID NO#14 C6orf108
>gi|40354200|ref|NM_199184.1| Homo sapiens chromosome 6 open reading frame 108 (C6orf108), transcript variant 2, mRNA
SEQ ID NO#15 C6orf108
>gi|40354201|ref|NP_954653.1| putative c-Myc-responsive isoform 2 [Homo sapiens]

What is claimed

1. A method for inducing cell death in a malignant cell population and treating cancer in a mammal or a cancer patient in need comprising administering a combination of at least one compound and at least one of WST-1r and any valid substitutes of WST-1r to a host in need in pharmaceutical effective amount simultaneous, separate or sequential use in any order during a course of treatment that is therapeutically synergistic in the induction of cancer cell death for the treatment of said cancer.
2. The method of claim 1, where said at least one compound is selected from the group of flavonoids comprising apigenin and the group of IKK inhibitors.
3. The method of claim 2 wherein said the IKK inhibitors selected from the group consisting of SPC839, Anilino-Pyrimidine Derivative, PS1145, BMS-345541*, , Amino-imidazolecarboxamide derivative, Ureido-thiophenecarboxamide derivatives, Diarylpyridine derivative, Pyridooxazinone derivative, Indolecarboxamide derivative, Benzoimidazole carboxamide derivative, Pyrazolo[4,3-c]quinoline derivative, Imidazolylquinoline-carboxaldehyde semicarbazide derivative, Pyridyl Cyanoguanidine derivate, IκB Kinase Inhibitor Peptide, IKK-2 Inhibitor IV [5-(p-Fluorophenyl)-2-ureido]thiophene-3-carboxamide, IKK Inhibitor II, Wedelolactone, IKK Inhibitor VII, IKK-2 Inhibitor V N-(3,5-Bis-trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide IMD-0354, IKK-2 Inhibitor VI (5-Phenyl-2-ureido)thiophene-3-carboxamide, IKK-2 Inhibitor VIII ACHP 2-Amino-6-(2-(cyclopropylmethoxy)-6-hydroxyphenyl)-4-(4-piperidiny)-3-pyridinecarbonitrile, and SC-514*.
4. The method of claim 1, wherein said WST-1r or at least one of any valid substitutes of WST-1r is selected from (1) a combination of compounds comprising at least one tetrazolium salt and at least one intermediate electron acceptor (IEA) that is selected from a group comprising WST-1+mPMS, WST-3+mPMS, WST-4+mPMS, WST-5+mPMS, XTT+mPMS, MTS+mPMS, WST-1+Q1, WST-3+Q1, WST-4+Q1, WST-5+Q1, XTT+Q1, MTS+Q1; or (2) at least one tetrazolium salts that is selected from a group comprising WST-3, WST-10, WST-11, XTT and MTS or (3) at least one IEA that is selected from a group comprising mPMS and Q1 in pharmaceutical acceptable carrier medium.
5. The method of claim 1 wherein said administering a combination of apigenin and at least one of WST-1r and any valid substitutes of WST-1r to a host may additionally include

- the addition of a third agent, which can approve the combination treatment of apigenin and at least one of WST-1r and any valid substitutes of WST-1r.
6. The method of claim 5 wherein said the third agent is selected from a group of GSK3b inhibitor including Lithium Chloride.
 7. The method of claim 1, wherein said at least one of WST-1r and any valid substitutes of WST-1r is administered in contact with cancer cells and tumor in pulsed manner for 30 minutes to 4 hours.
 8. The method of claim 1 wherein said the cancer and the cells of malignant population are carcinomas and sarcomas.
 9. A combination composition comprising at least one compound and at least one of WST-1r and any valid substitutes of WST-1r in pharmaceutical acceptable carrier medium and formulated in pharmaceutical effective amount for simultaneous, separate or sequential administering to a cancer patient in need for synergistically inducing cancer cell.
 10. The method of claim 9, where said at least one compound is selected from the group of flavonoids consisting apigenin and the group of IKK inhibitors in pharmaceutical acceptable carrier medium.
 11. The pharmaceutical composition of claim 9, wherein said at least one WST-1r and any valid substitutes of WST-1r is selected from the group of (1) the combination of compounds comprising at least one tetrazolium salt and at least one intermediate electron acceptor (IEA) comprising WST-1+mPMS, WST-3+mPMS, WST-4+mPMS, WST-5+mPMS, XTT+mPMS, MTS+mPMS, WST-1+Q1, WST-3+Q1, WST-4+Q1, WST-5+Q1, XTT+Q1, MTS+Q1; (2) a group of tetrazolium salt comprising WST-1, WST-3, WST-4, WST-5, WST-9, WST-10, WST-11, XTT and MTS; and (3) a group of IEA comprising mPMS and Q1 in pharmaceutical acceptable carrier medium.
 12. A method for inducing cell death in a malignant cell population and treating cancer in a mammal or a cancer patient in need comprising administering a combination of at least one compound, at least one IKK inhibitors, and at least one of WST-1r and any valid substitutes of WST-1r to a host in need in pharmaceutical effective amount simultaneous, separate or sequential use in any order during a course of treatment that is therapeutically synergistic in the induction of cancer cell death for the treatment of said cancer.
 13. The method of claim 12 wherein said a compound is selected from the group of polynucleotide, synthetic polynucleotide, protein, polypeptide, peptides, antibody, antibody fragment that can (1) alter or interfere expression level, structure, function and activity of TPCR6 (SEQ ID NO:2/6), SH3PXD2B (SEQ ID NO:3/7), MAGI3 (SEQ ID

- NO:4/8), TMEM182(SEQ ID NO:5/9) and C6orf108(SEQ ID NO:14/15); (2) mimic biological function of puc19(SEQ ID NO:1), organic or inorganic inhibitors.
14. The method of claim C1A wherein said a polynucleotide is puc19 DNA vector (SEQ ID NO:1).
 15. The method of claim 13 wherein said synthetic polynucleotide is further selected from the group of siRNA comprising siRNA#1 (SEQ ID NO:10), siRNA#2 (SEQ ID NO:11), siRNA#3 (SEQ ID NO:12), the sequence of which were derived from the puc19 DNA sequence, and siRNA against human transcripts of TPCR6 (SEQ ID NO:2), SH3PXD2B(SEQ ID NO:3), MAGI3(SEQ ID NO:4), TMEM182(SEQ ID NO:5) and C6orf108(SEQ ID NO:14).
 16. The method of claim 13 wherein said a protein is selected from the group of type I interferon comprising IFN α A, IFN α B, IFN α C, IFN α D, IFN α F, IFN α G, IFN α H, IFN α I, IFN α J, IFN α K, IFN α 4b, IFN α WA, IFN β , IFN γ and IL-6.
 17. The method of claim 12 wherein said the at least one IKK inhibitor is selected from the group consisting of SPC839, Anilino-Pyrimidine Derivative, PS1145, BMS-345541*, , Amino-imidazolecarboxamide derivative, Ureido-thiophenecarboxamide derivatives, Diarylpyridine derivative, Pyridoxazine derivative, Indolecarboxamide derivative, Benzoimidazole carboxamide derivative, Pyrazolo[4,3-c]quinoline derivative, Imidazolylquinoline-carboxaldehyde semicarbazide derivative, Pyridyl Cyanoguanidine derivative, I κ B Kinase Inhibitor Peptide, IKK-2 Inhibitor IV [5-(p-Fluorophenyl)-2-ureido]thiophene-3-carboxamide, IKK Inhibitor II, Wedelolactone, IKK Inhibitor VII, IKK-2 Inhibitor V N-(3,5-Bis-trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide IMD-0354, IKK-2 Inhibitor VI (5-Phenyl-2-ureido)thiophene-3-carboxamide, IKK-2 Inhibitor VIII ACHP 2-Amino-6-(2-(cyclopropylmethoxy)-6-hydroxyphenyl)-4-(4-piperidyl)-3-pyridinecarbonitrile, and SC-514*.
 18. The method of claim 12, wherein said WST-1r or at least one of any valid substitutes of WST-1r is selected from (1) a combination of compounds comprising at least one tetrazolium salt and at least one intermediate electron acceptor (IEA) that is selected from a group comprising WST-1+mPMS, WST-3+mPMS, WST-4+mPMS, WST-5+mPMS, XTT+mPMS, MTS+mPMS, WST-1+Q1, WST-3+Q1, WST-4+Q1, WST-5+Q1, XTT+Q1, MTS+Q1; or (2) at least one tetrazolium salts that is selected from a group comprising WST-1, WST-3, WST-4, WST-5, WST-9, WST-10, WST-11, XTT and MTS or (3) at least one IEA that is selected from a group comprising mPMS and Q1 in pharmaceutical acceptable carrier medium.

19. The method of claim 12, wherein said at least one of WST-1r and any valid substitutes of WST-1r is administered in contact with cancer cells and tumor in pulsed manner for 30 minutes to 4 hours.
20. The method of claim 12 wherein said the cancer and the cells of malignant population are carcinomas and sarcomas.
21. A combination composition comprising at least one compound, at least one IKK inhibitor, and at least one of WST-1r and any valid substitutes of WST-1r in pharmaceutical acceptable carrier medium and in pharmaceutical effective amount for simultaneous, separate or sequential administering to a cancer patient in need for synergistically inducing cancer cell and enhancing antineoplastic effects.
22. The pharmaceutical composition of claim 21 wherein said the at least one compound is selected from the group of (1) polynucleotide consisting puc19 DNA vector (SEQ ID NO:1), (2) synthetic polynucleotide further selected from the group of siRNA comprising siRNA#1 (SEQ ID NO:10), siRNA#2 (SEQ ID NO:11), siRNA#3 (SEQ ID NO:12), the sequence of which were derived from the puc19 DNA sequence, and siRNA against human transcripts of TPCR6 (SEQ ID NO:2), SH3PXD2B(SEQ ID NO:3), MAGI3(SEQ ID NO:4), TMEM182(SEQ ID NO:5) and C6orf108(SEQ ID NO:14), and (3) selected from the group of type I interferon comprising IFN α A, IFN α B, IFN α C, IFN α D, IFN α F, IFN α G, IFN α H, IFN α I, IFN α J, IFN α K, IFN α 4b, IFN α WA, IFN β , IFN γ and IL-6, (4) all other biologic, non-biologic organic and inorganic that are capable of inhibiting expression level, structure, function activity of TPCR6 (SEQ ID NO:2), SH3PXD2B(SEQ ID NO:3), MAGI3(SEQ ID NO:4), TMEM182(SEQ ID NO:5) and C6orf108(SEQ ID NO:14), or mimic puc19 effect of combination treatment with IKK inhibitor and WST-1r and the valid substitutes of WST-1r in pharmaceutical acceptable medium.
23. The pharmaceutical composition of claim 21 wherein said the at least one IKK inhibitors selected from the group consisting of SPC839, Anilino-Pyrimidine Derivative, PS1145, BMS-345541*, , Amino-imidazolecarboxamide derivative, Ureido-thiophenecarboxamide derivatives, Diarylpyridine derivative, Pyridoxazine derivative, Indolecarboxamide derivative, Benzoimidazole carboxamide derivative, Pyrazolo[4,3-c]quinoline derivative, Imidazolylquinoline-carboxaldehyde semicarbazide derivative, Pyridyl Cyanoguanidine derivative, I κ B Kinase Inhibitor Peptide, IKK-2 Inhibitor IV [5-(p-Fluorophenyl)-2-ureido]thiophene-3-carboxamide, IKK Inhibitor II, Wedelolactone, IKK Inhibitor VII, IKK-2 Inhibitor V N-(3,5-Bis-

trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide IMD-0354, IKK-2 Inhibitor VI (5-Phenyl-2-ureido)thiophene-3-carboxamide, IKK-2 Inhibitor VIII ACHP 2-Amino-6-(2-(cyclopropylmethoxy)-6-hydroxyphenyl)-4-(4-piperidiny)-3-pyridinecarbonitrile, and SC-514*.

24. The pharmaceutical composition of claim 21, wherein said at least one WST-1r and any valid substitutes of WST-1r is selected from the group of the combination of compounds comprising (1) at least one tetrazolium salt and at least one intermediate electron acceptor (IEA) comprising WST-1+mPMS, WST-3+mPMS, WST-4+mPMS, WST-5+mPMS, XTT+mPMS, MTS+mPMS, WST-1+Q1, WST-3+Q1, WST-4+Q1, WST-5+Q1, XTT+Q1, MTS+Q1; (2) a group of tetrazolium salt comprising WST-1, WST-3, WST-4, WST-5, WST-9, WST-10, WST-11, XTT and MTS; and (3) a group of IEA comprising mPMS and Q1.
25. A pharmaceutical composition formulated for WST-1r and any valid substitutes of WST-1r is a reagent of combination of compounds comprising tetrazolium salt and IEA in pharmaceutical acceptable carrier medium.
26. The pharmaceutical composition according to claim 25, wherein said WST-1r is a combination of compounds comprising a tetrazolium salt, WST-1, and an IEA, mPMS, in pharmaceutical acceptable carrier medium.
27. The pharmaceutical composition according to claim 25, wherein said tetrazolium salt is selected from a group of tetrazolium salt comprising WST-3, WST-4, WST-5, WST-9, WST-10, WST-11, XTT and MTS in pharmaceutical acceptable carrier medium.
28. The pharmaceutical composition according to claim 25, wherein said IEA is selected from the group comprising mPMS and Q1 in pharmaceutical acceptable carrier medium.
29. The pharmaceutical composition according to claim 25, wherein said any valid substitutes of WST-1r is selected from the groups of (1) a combination of compounds comprising at least one tetrazolium salt and at least one IEA, or (2) at least one tetrazolium salt alone, or (3) at least one IEA alone in pharmaceutical acceptable carrier medium.
30. The pharmaceutical composition according to claim 29, wherein said the any valid substitutes of WST-1r is selected from (1) a group of combination of compounds of at least one tetrazolium salt, and at least one IEA are further selected from the group comprising WST-1+mPMS, WST-3+mPMS, WST-4+mPMS, WST-5+mPMS, XTT+mPMS, MTS+mPMS, WST-1+Q1, WST-3+Q1, WST-4+Q1, WST-5+Q1, XTT+Q1, MTS+Q1 in pharmaceutical acceptable carrier medium.

31. The pharmaceutical composition according to claim 29, wherein said any valid substitutes of WST-1r are selected from groups of tetrazolium salts alone is further selected from a group of tetrasolium salts comprising WST-1, WST-3, WST-4, WST-5, WST-9, WST-10, WST-11, XTT and MTS Q1 in pharmaceutical acceptable carrier medium.
32. The pharmaceutical composition according to claim 31, wherein said the at least one tetrozolium salt is WST-3 in pharmaceutical acceptable carrier medium.
33. The pharmaceutical composition according to claim 29, wherein said the at least one IEA is selected from a group of IEA alone are further selected from the group comprising mPMS and Q1 in pharmaceutical acceptable carrier medium.
34. A method for inducing cell death in a malignant cell population and for treating cancer in a mammal or a patient in need thereof comprising a combination of administering to said patient, a therapeutically effective amount of at least one compound and at least one cytotoxic chemotherapeutic drug concurrently or sequentially in any order, during a course of treatment. That is therapeutically synergistic in the induction of cancer cell death for the treatment of said cancer.
35. The method of claim 34 wherein said at least one compound is selected from the group consisting of biological and non-biological organic or non-organic compounds that can targeting and interfering the expression levels, molecule structure, function, and activity of the gene products of TPRC6 (SEQ ID NO:2, NO:6), SH3PXD2B (SEQ ID NO:3, NO:7), MAGI3 (SEQ ID NO:4, NO:8), TMEM182 (SEQ ID NO:5, NO:9), and C6orf108 (SEQ ID NO:14, NO:15).
36. The method according to claim 34 wherein said biological compounds are further selected from the group of polynucleotides, synthetic polynucleotides, proteins, peptides, polypeptide, antibodies, and antibody fragments.
37. The method according to claim 36 wherein said polynucleotides is purified DNA vector puc19 (SEQ ID NO: 1).
38. The method according to claim 35 wherein said synthetic polynucleotides is selected from the group of siRNA comprising siRNA#1 (SEQ ID NO:10), siRNA#2 (SEQ ID NO:11), siRNA#3 (SEQ ID NO:12), the sequence of which were derived from the puc19 DNA sequence, and siRNA against human transcripts of TPCR6 (SEQ ID NO:2), SH3PXD2B(SEQ ID NO:3), MAGI3(SEQ ID NO:4), TMEM182(SEQ ID NO:5) and C6orf108(SEQ ID NO:14).

39. The method of claim 34, wherein said at least one cytotoxic chemotherapeutic drug is selected from the group comprising of paclitaxel (Taxol.RTM.), cisplatin, docetaxol, carboplatin, vincristine, vinblastine, methotrexate, cyclophosphamide, CPT-11, 5-fluorouracil (5-FU), gemcitabine, estramustine, carmustine, adriamycin (doxorubicin), etoposide, arsenic trioxide, irinotecan, and epothilone derivatives in a pharmaceutically acceptable carrier medium.
40. The method of claim 34, wherein said cancer is selected from the group consisting of cancers of carcinomas the prostate, colorectum, pancreas, cervix, stomach, endometrium, brain, liver, bladder, ovary, testis, head, neck, skin (including melanoma and basal carcinoma), mesothelial lining, esophagus, breast, muscle, connective tissue, lung (including small-cell lung carcinoma and non-small-cell carcinoma), adrenal gland, thyroid, kidney, or bone; glioblastoma, mesothelioma, renal cell carcinoma, gastric carcinoma, sarcoma, choriocarcinoma, cutaneous basocellular carcinoma, and testicular seminoma, and sarcomas, soft tissue sarcoma.
41. A Pharmaceutical composition for the treatment of cancer comprising an effective amount of at least one compound that can that can (1) alter or interfere expression level, structure, function and activity of TPCR6 (SEQ ID NO:2/6), SH3PXD2B(SEQ ID NO:3/7), MAGI3(SEQ ID NO:4/8), TMEM182(SEQ ID NO:5/9) and C6orf108(SEQ ID NO:14/15); (2) mimic biological function of puc19(SEQ ID NO:1); and at least one chemotherapeutic agent in a pharmaceutically acceptable carrier medium.
42. The pharmaceutical composition of claim 41, wherein said at least one chemotherapeutic agent is selected from the group consisting of paclitaxel (Taxol.RTM.), cisplatin, docetaxol, carboplatin, vincristine, vinblastine, methotrexate, cyclophosphamide, CPT-11, 5-fluorouracil (5-FU), gemcitabine, estramustine, carmustine, adriamycin (doxorubicin), etoposide, arsenic trioxide, irinotecan, and epothilone derivatives in a pharmaceutically acceptable carrier medium.
- D32 The pharmaceutical composition of claim 41 wherein said the at least one biological compound is selected from the group of (1) polynucleotide consisting of puc19 DNA vector (SEQ ID NO:1), (2) synthetic polynucleotide further selected from the group of siRNA comprising siRNA#1 (SEQ ID NO:10), siRNA#2 (SEQ ID NO:11), siRNA#3 (SEQ ID NO:12), the sequence of which were derived from the puc19 DNA sequence, and siRNA against human transcripts of TPCR6 (SEQ ID NO:2), SH3PXD2B(SEQ ID NO:3), MAGI3(SEQ ID NO:4), TMEM182(SEQ ID NO:5) and C6orf108(SEQ ID NO:14) in a pharmaceutically acceptable carrier medium.

43. A method of inducing cancer cell death for the treatment of cancer in a mammal or a patient in need thereof comprising administering to said patient a combination therapy comprising a therapeutically effective amount of Stattic, in combination with a therapeutically effective amount of at least one of NF- κ B inhibitor simultaneous, separate and sequential use for the treatment of cancer substantially enhancing the induction of cancer cell death.
44. The method of claim 43, wherein said NF- κ B inhibitor is selected from groups of IKK inhibitors and CK2 inhibitors.
45. The method in claim 43, said cancer is a subset of head and neck squamous carcinoma that sensitive to the treatment.
46. Isolated polynucleotids consisting of the nucleotide sequences defined by SEQ ID: NO 2, 3, 4, 5, and 14 encoding the polypeptide consisting of the amino acid sequence of SEQ ID NO: 6, 7, 8, 9 and 15 correspondingly as drug target for the treatment of cancer. The said transcripts and their corresponding proteins as potential target for developing anti-cancer agents comprised of a) Homo sapiens transient receptor potential cation channel, subfamily C, member 6 TRPC6 mRNA (SEQ ID No. 2 and 6), b) Homo sapiens SH3 and PX domains 2B (SH3PXD2B), mRNA (SEQ ID No. 3 and 7), c) Homo sapiens membrane associated guanylate kinase, WW and PDZ domain containing 3 (MAGIKK, (SEQ ID No. 4 and 8) the Homo sapiens transmembrane protein 182 (TMEM182, SEQ ID No. 5, and 9), the homo sapiens chromosome 6 open reading frame 108 (C6orf108, SEQ ID No.: 14 and 15).
47. The valid substitutes of double strand DNA, Puc19, of claim 18 and claim 19 are synthetic single-stranded polynucleotide comprising 22 to 25 bases of the regions of the Puc19 of claim 19 defined by SEQ ID NO:1, wherein said polynucleotide is synthetic siRNA of SEQ ID NO: 10 (Puc19 5' 396-418, 22 bases), 11(Puc19 5' 797-820, 23 bases), and 12(Puc19 5' 1561-1586, 25 bases) and the siRNAs, anti-sense RNAs, anti-sense-oligos, antibodies, peptide inhibitors, small molecule inhibitors, or dominant negative DAN vectors targeting the said transcripts of the polynucleotide sequences and amino acid sequences of the genes in claim 46.

Fig 1 NF-kB Down Stream Gene Expression Levels

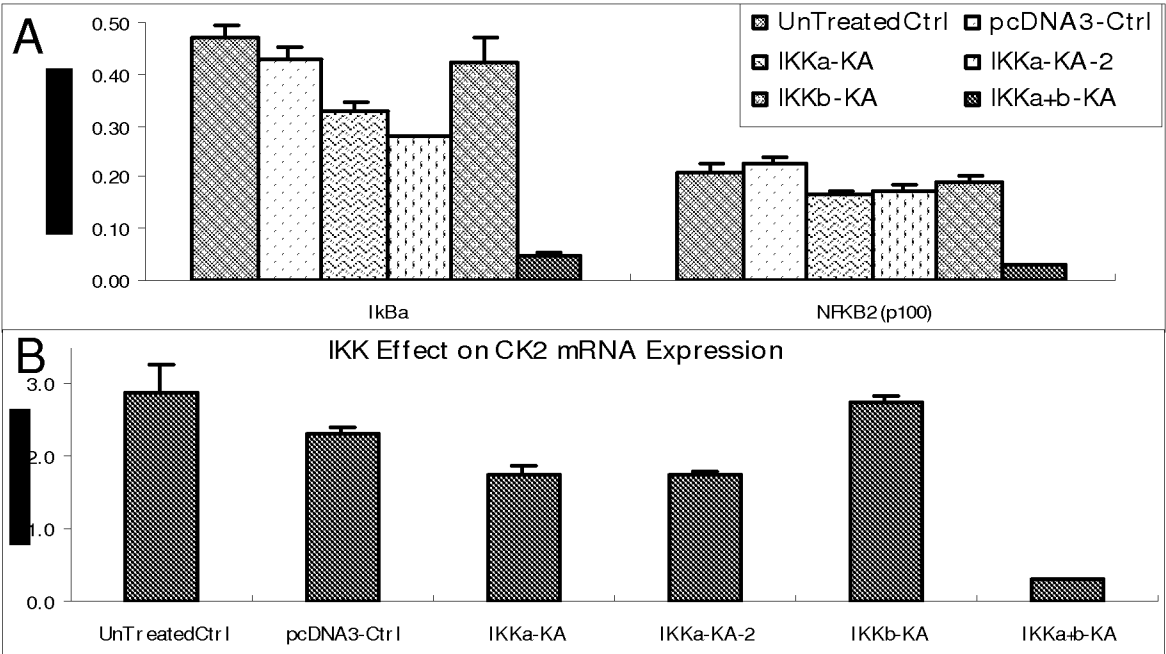


Fig 2 Combinational Effect of IKK-KA Transfection and WST-1 Treatment

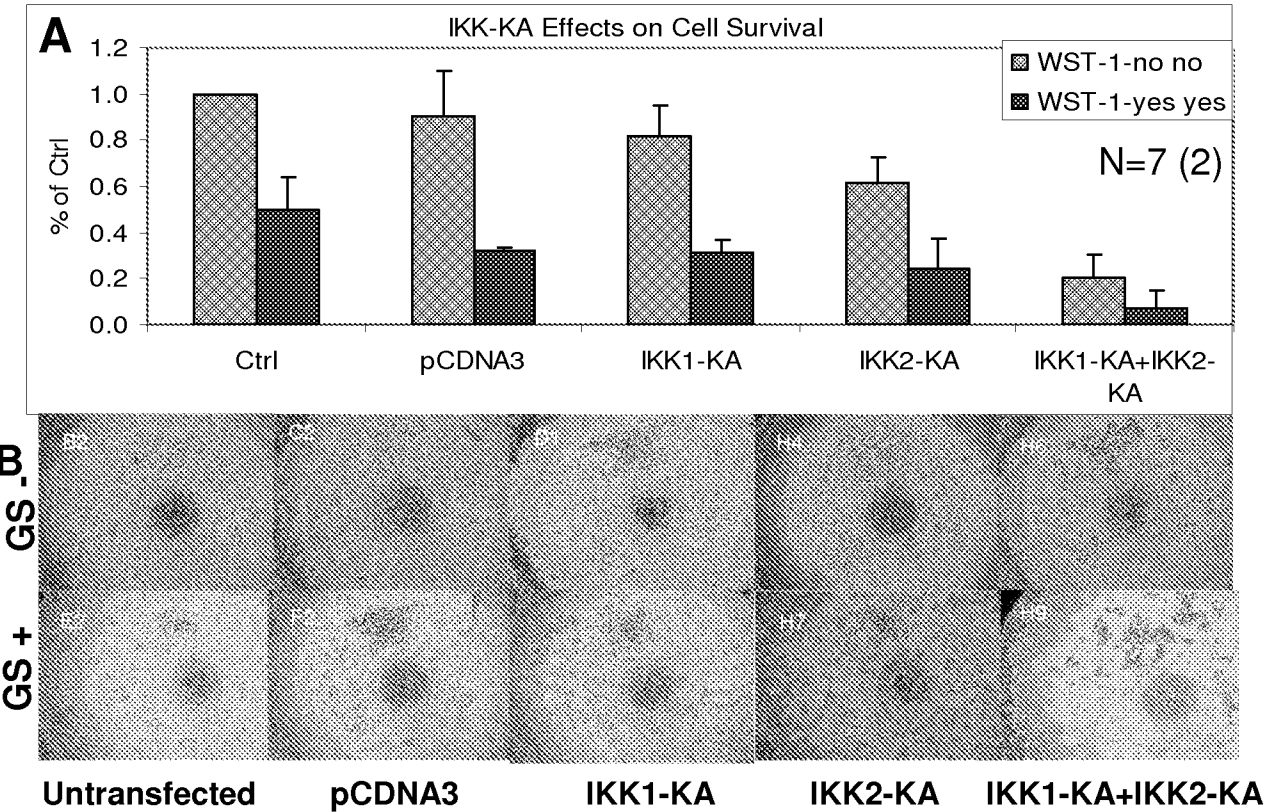
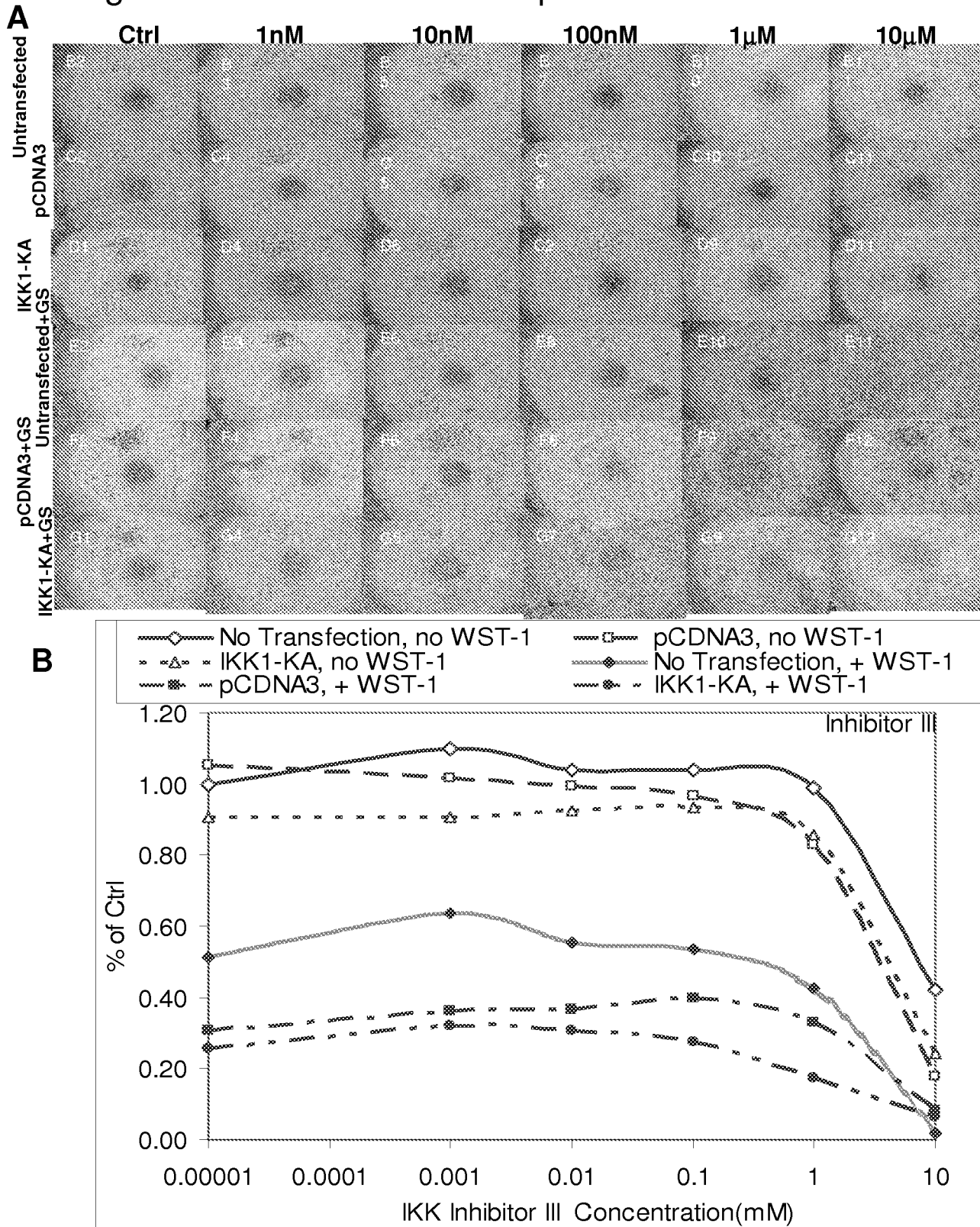


Fig 3 IKK inhibitors Dose response and WST-1 effects



Continue Fig3

IKK inhibitors Dose response and WST-1 effects

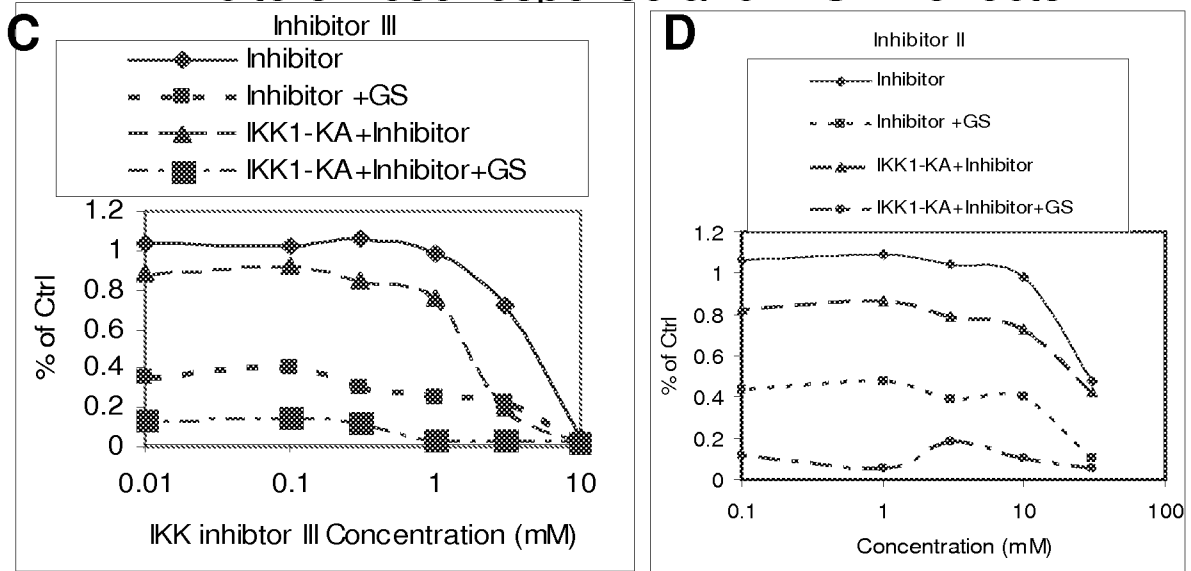


Fig 4 WST-1 promotes HT1080 Human Sarcoma cell death by triple combination Treatment

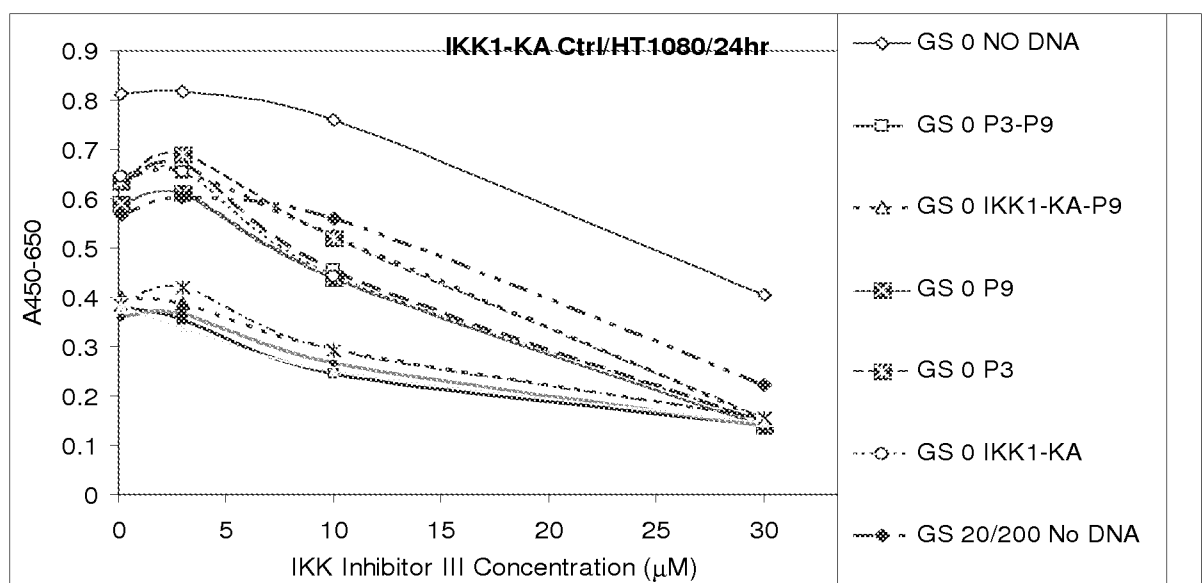


Fig 5 WST-1 induces ROS generation
B HT1080 Cell labeled with CM-H2-DCFDA , then,
Treated with WSTGT-1r for 30 min

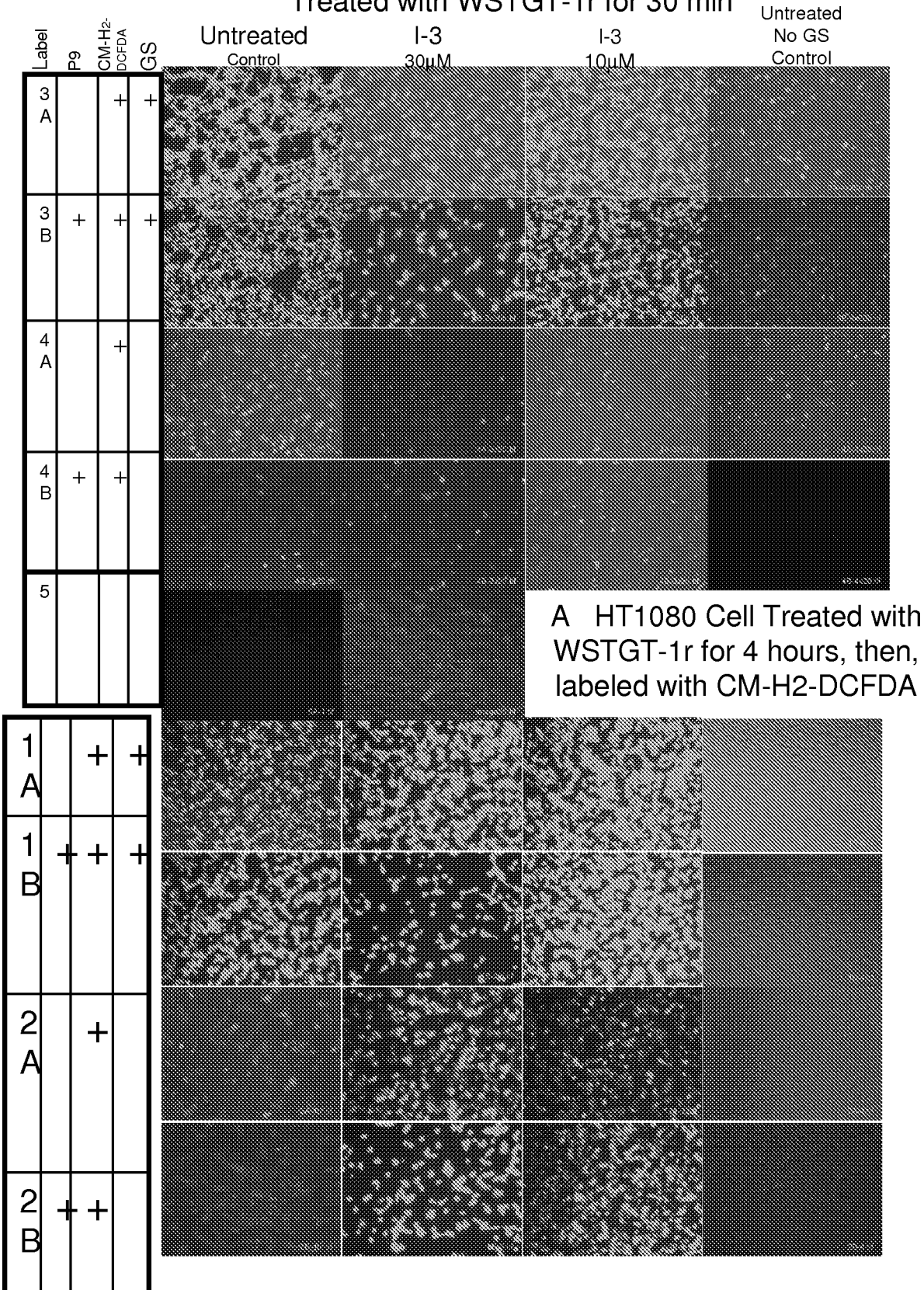


Fig 6 LiCl + Apigenin synergized
SCC-6, WST-1 enhance further

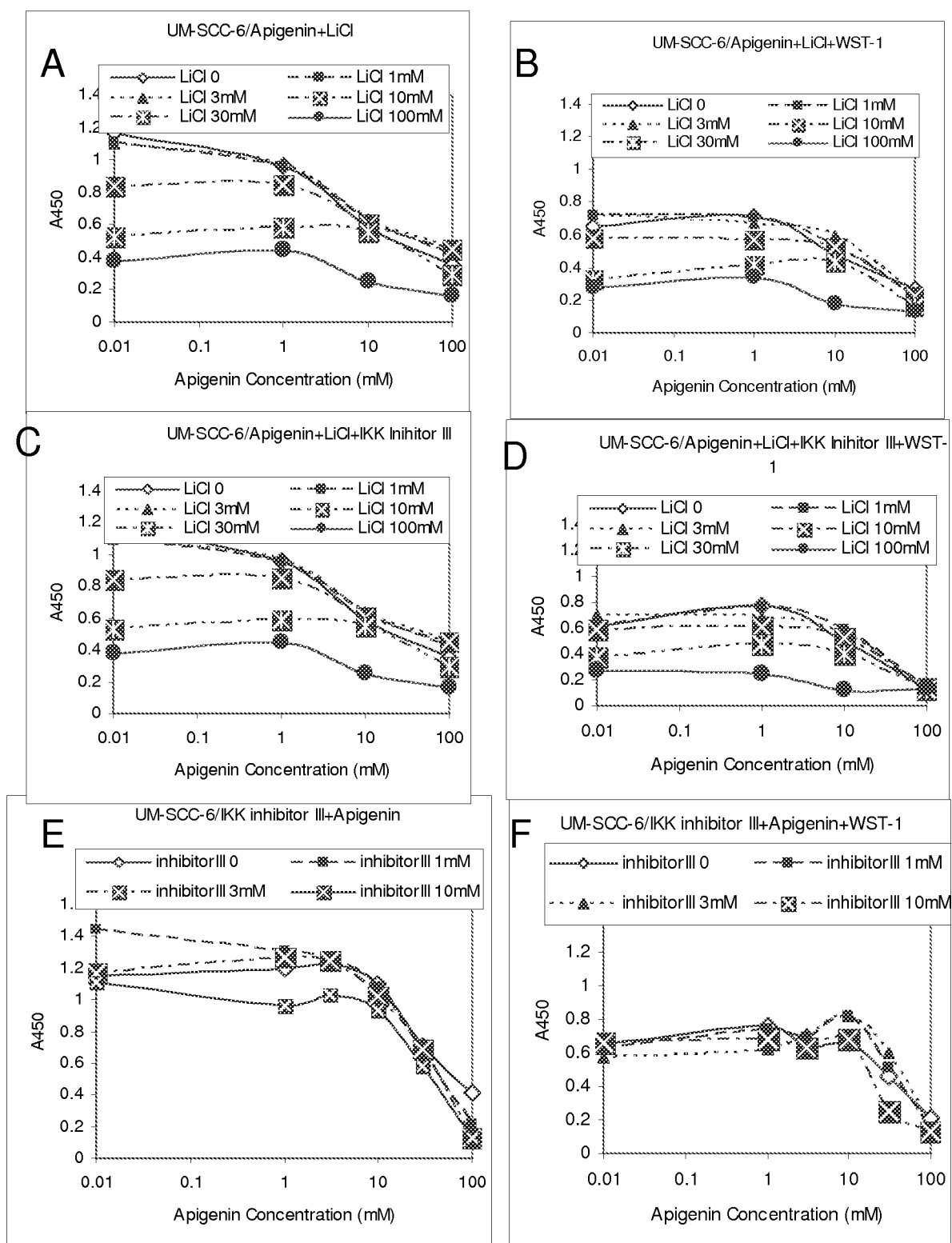


Fig 7 pUC19 DNA transfection synergize
chemotherapeutic drug effect

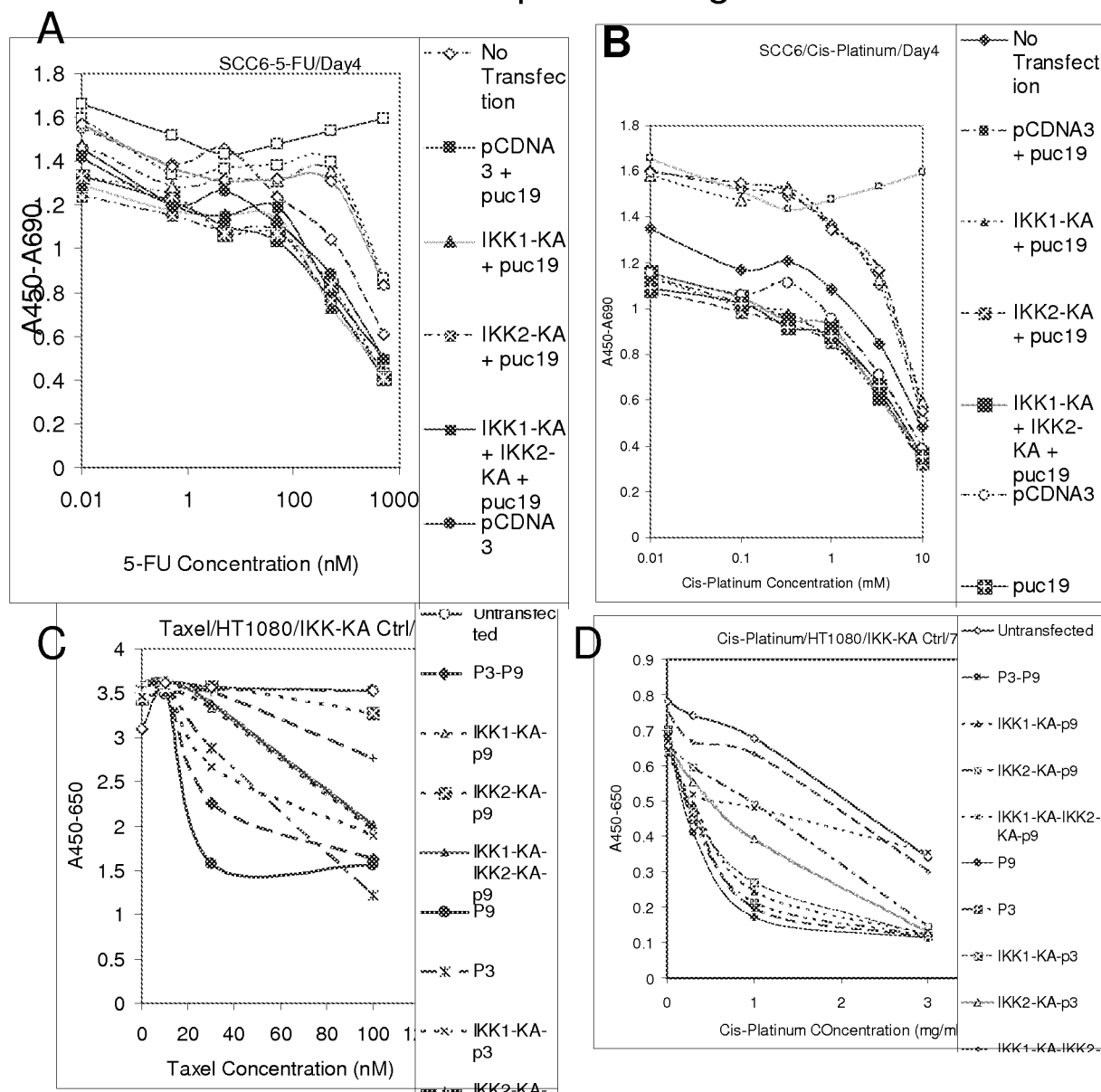
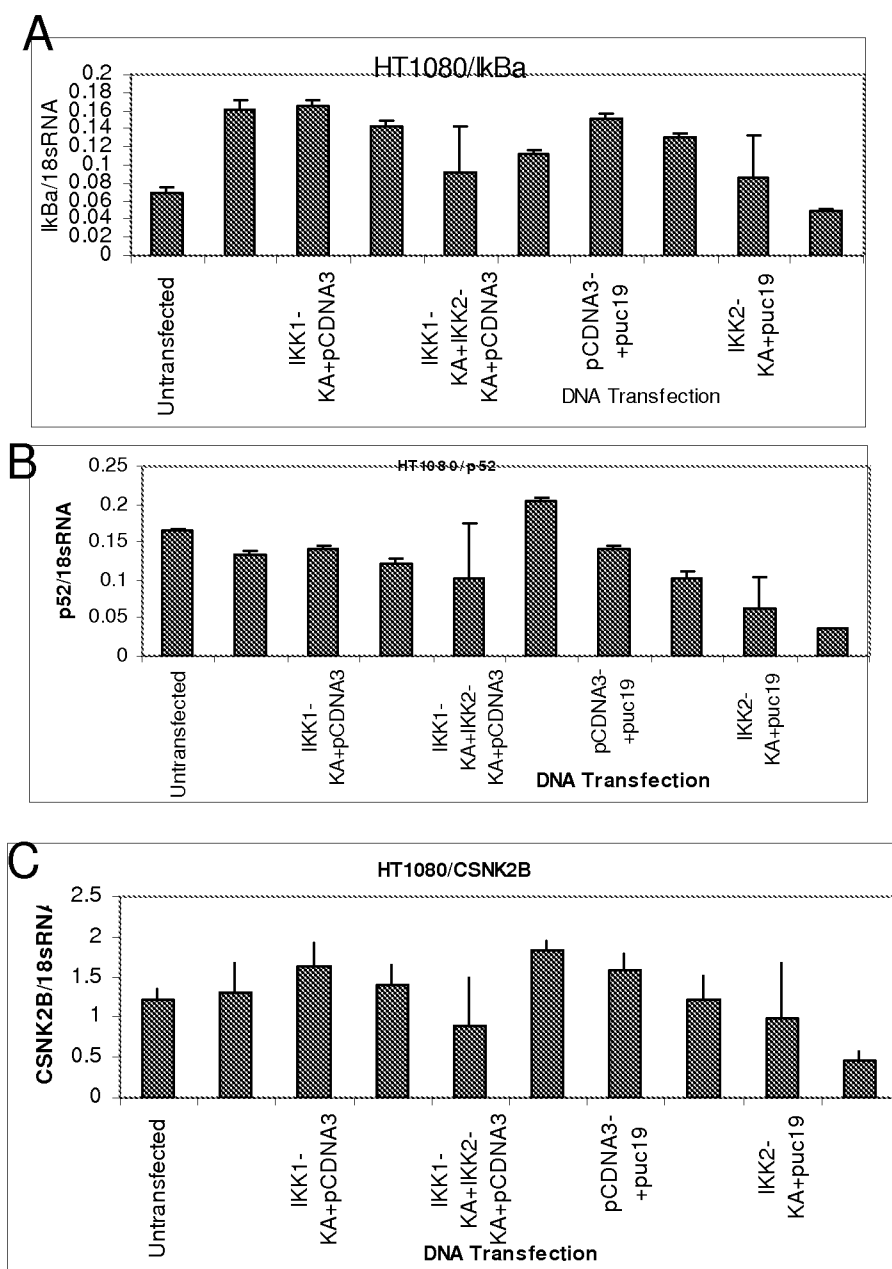


Fig8 pUC 19 transfection synergize Dominant negative IKK-KA inhibition of NF-KAPPAB activity



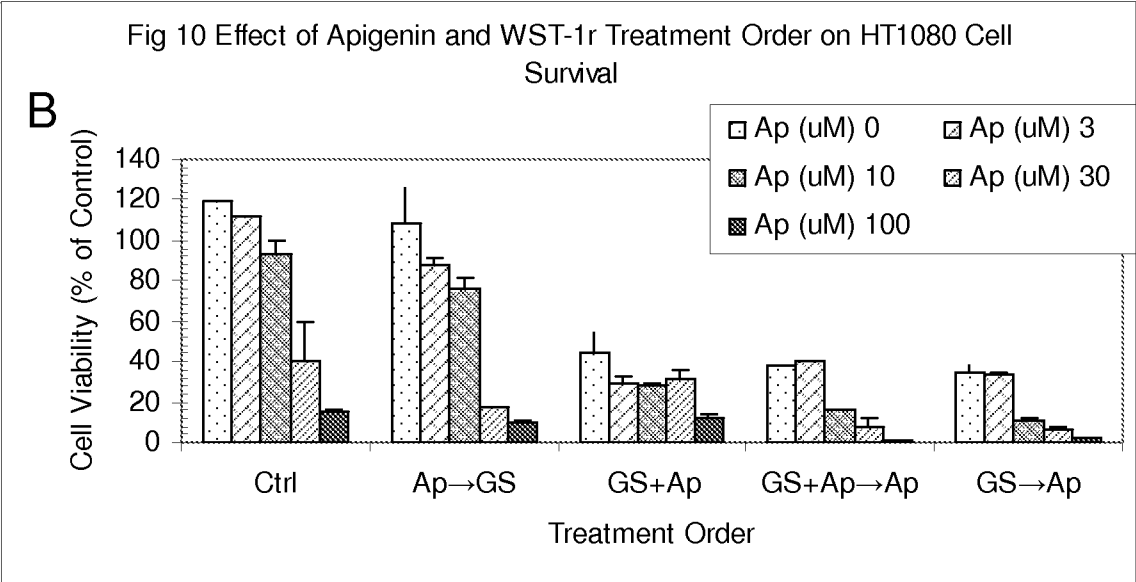
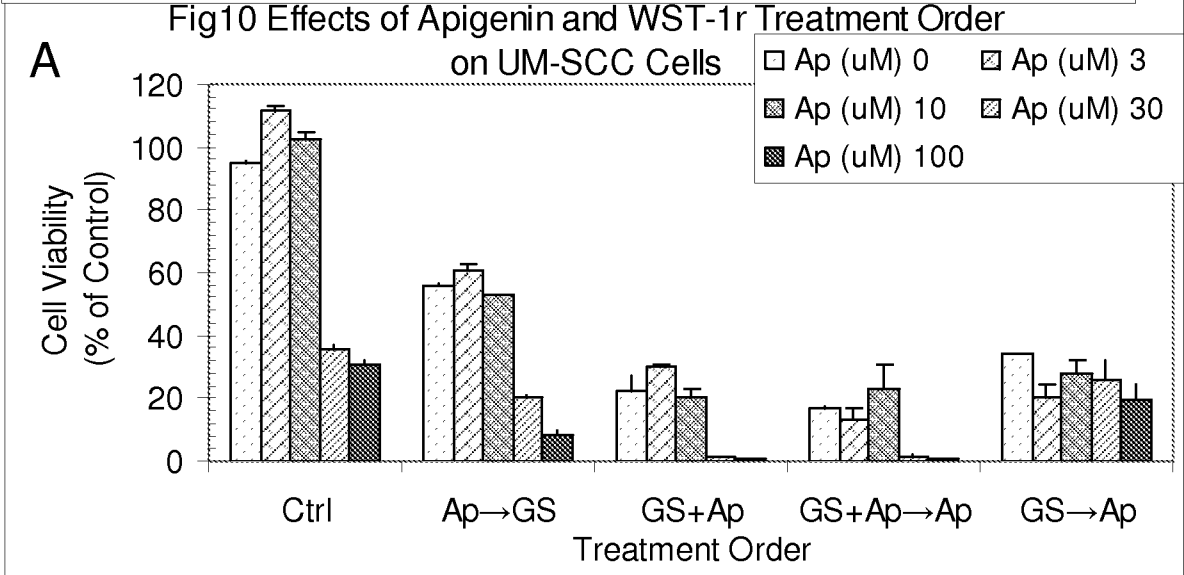
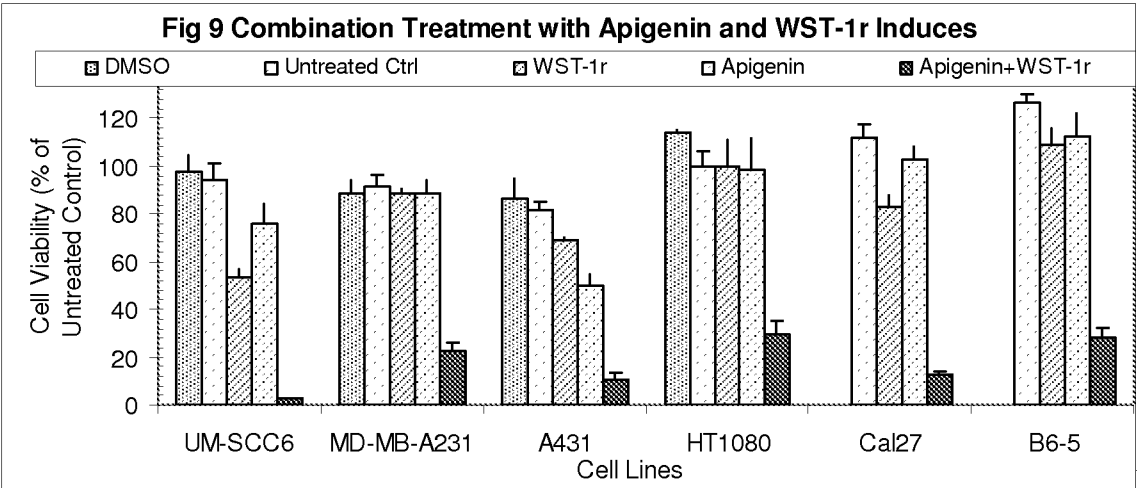


Fig 11 Time course and Dose Response of WST-1r and Dose-Response of apigenin involved in the combination treatment of WST-1r with apigenin

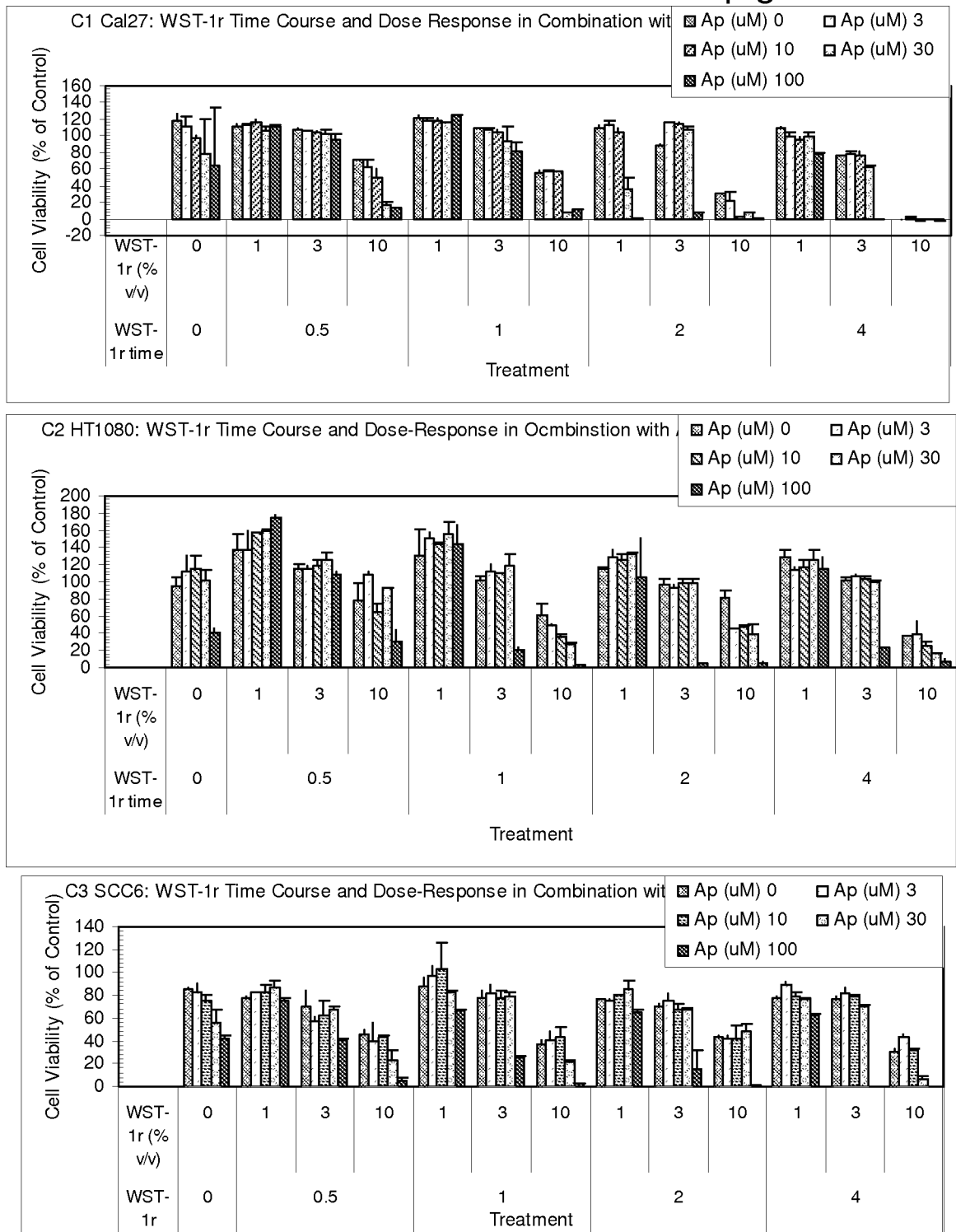


Fig 12

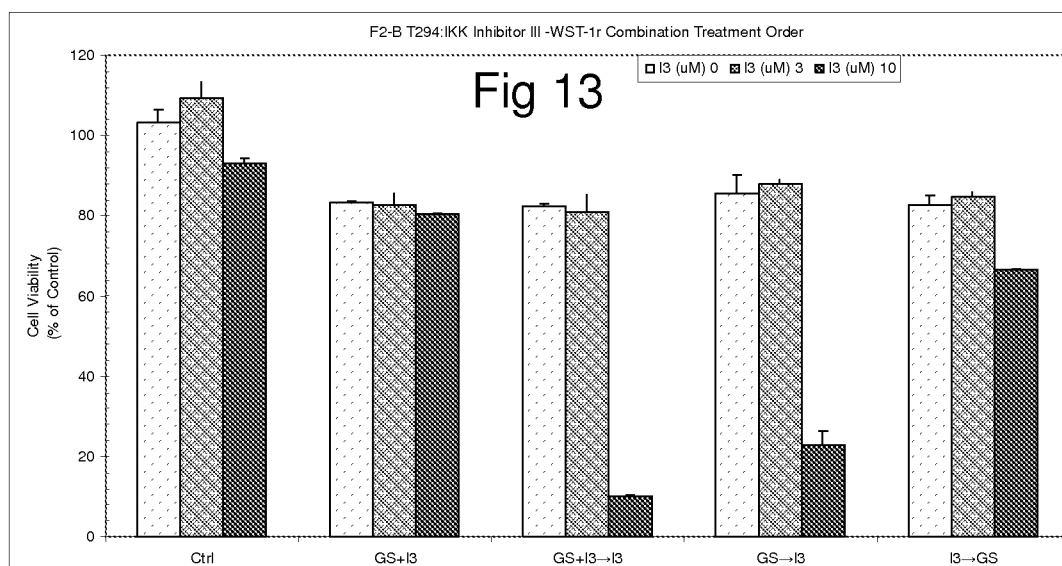
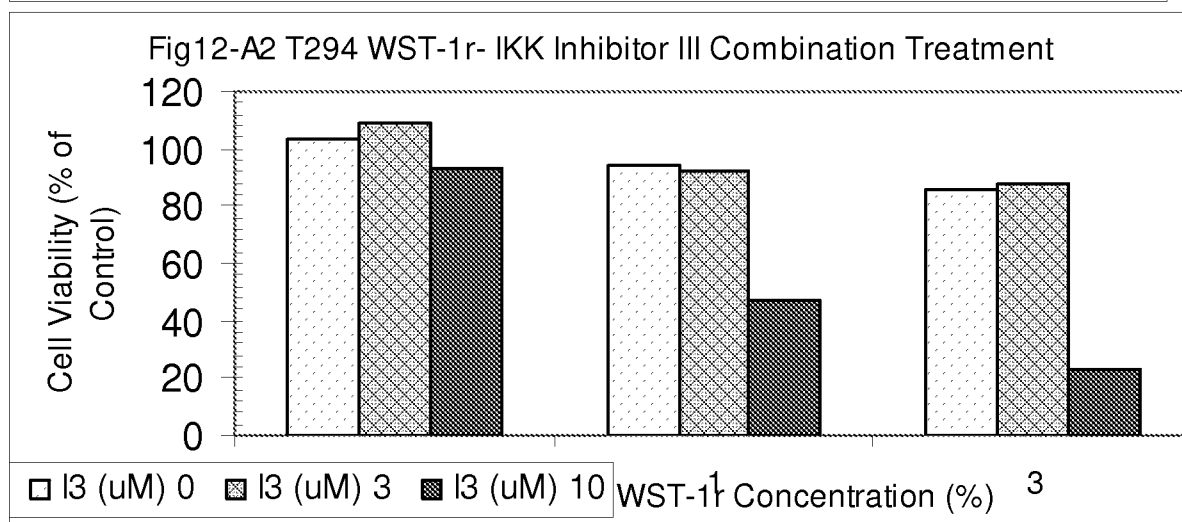
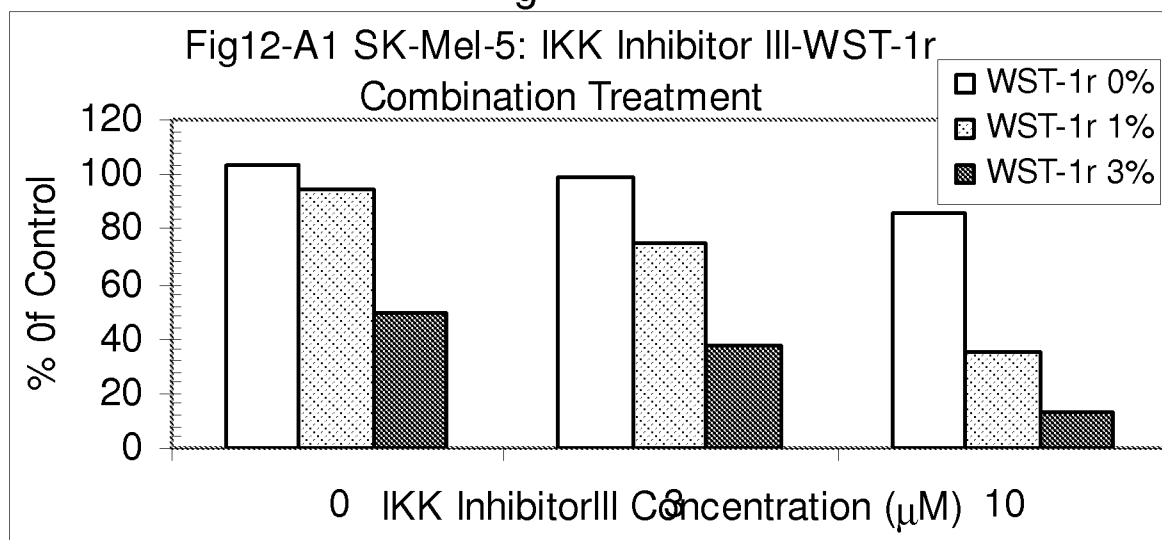


Fig14 UM-SCC6 Cell JNK Activation

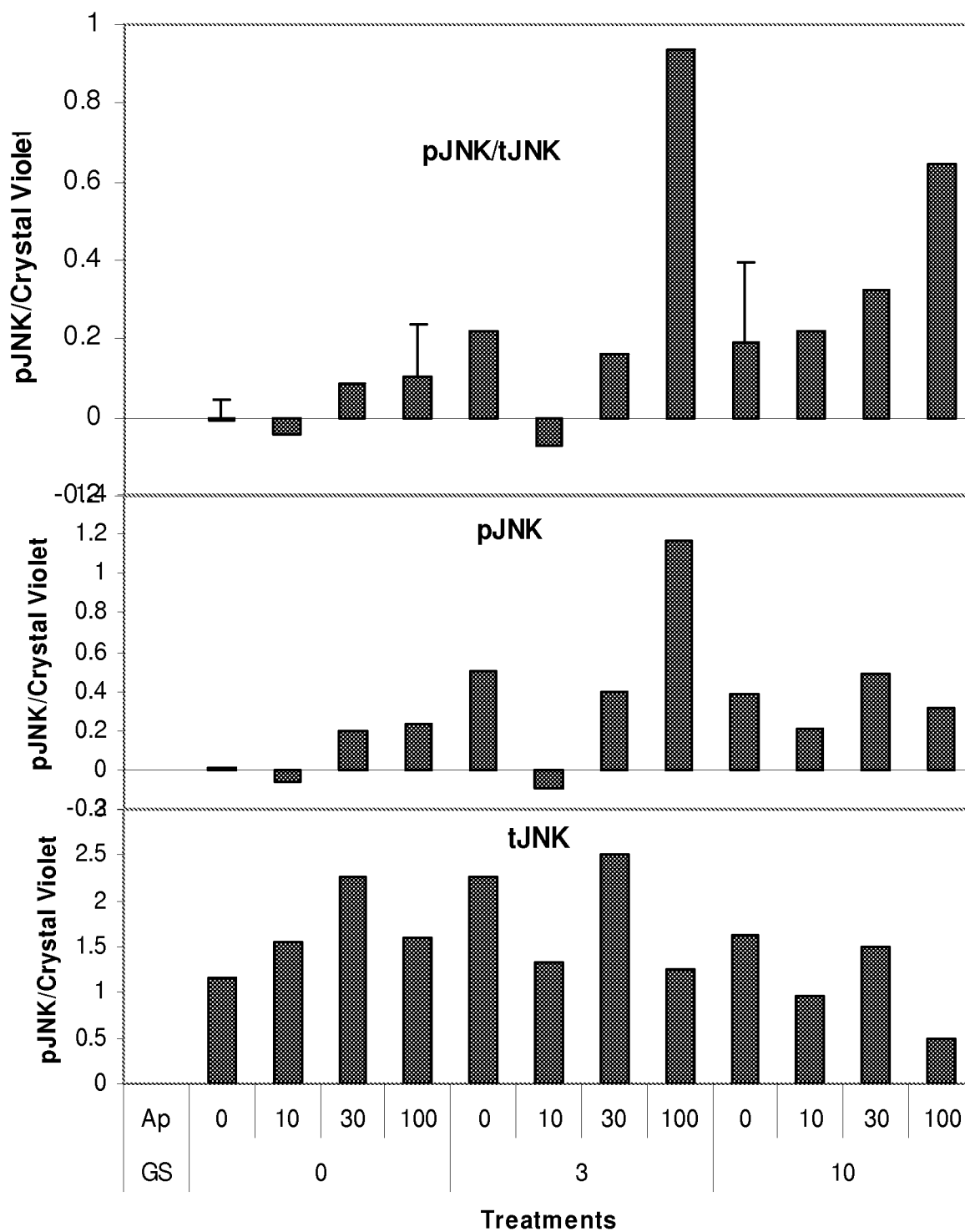


Fig15

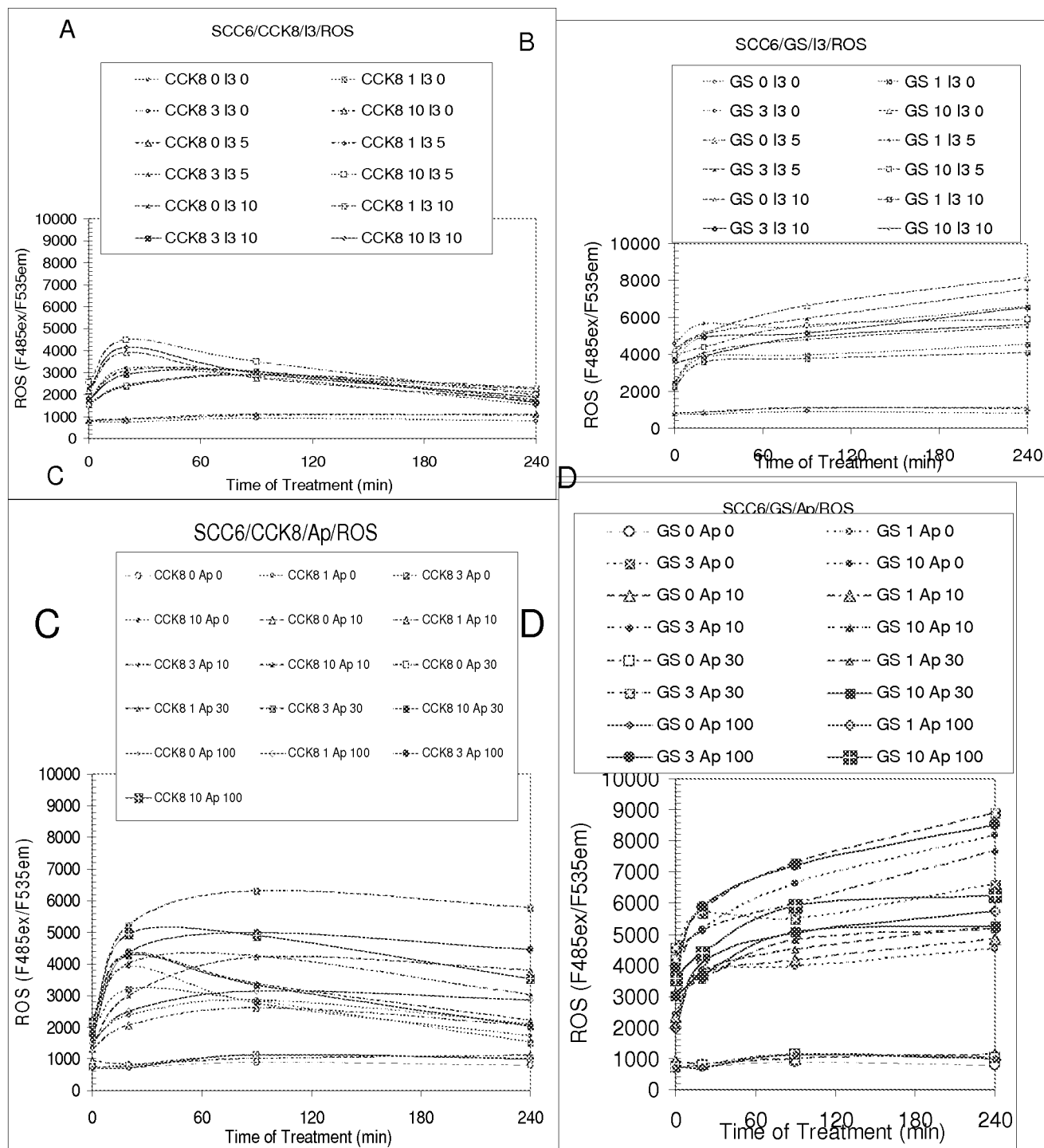


Fig 16

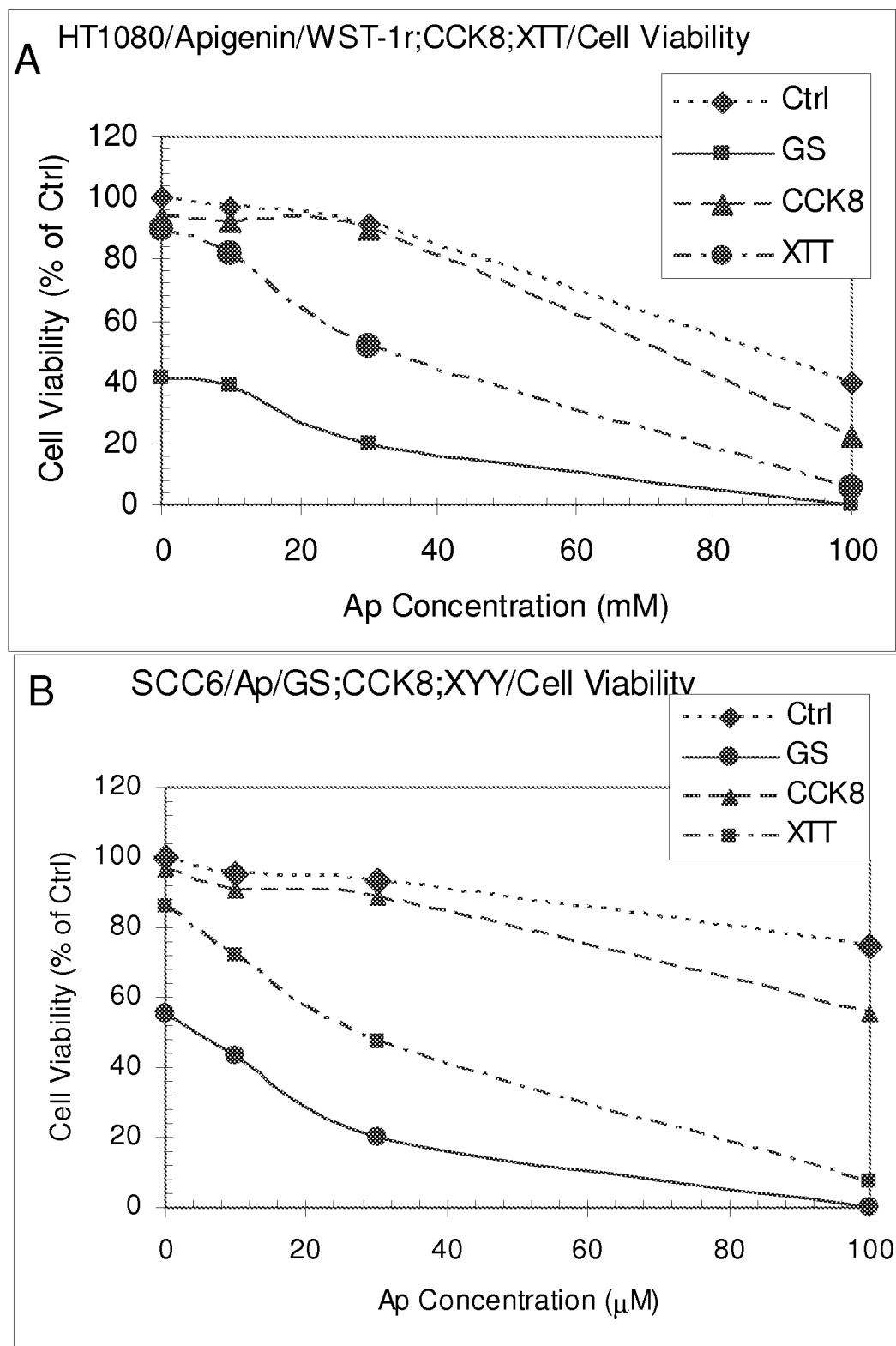


Fig 17

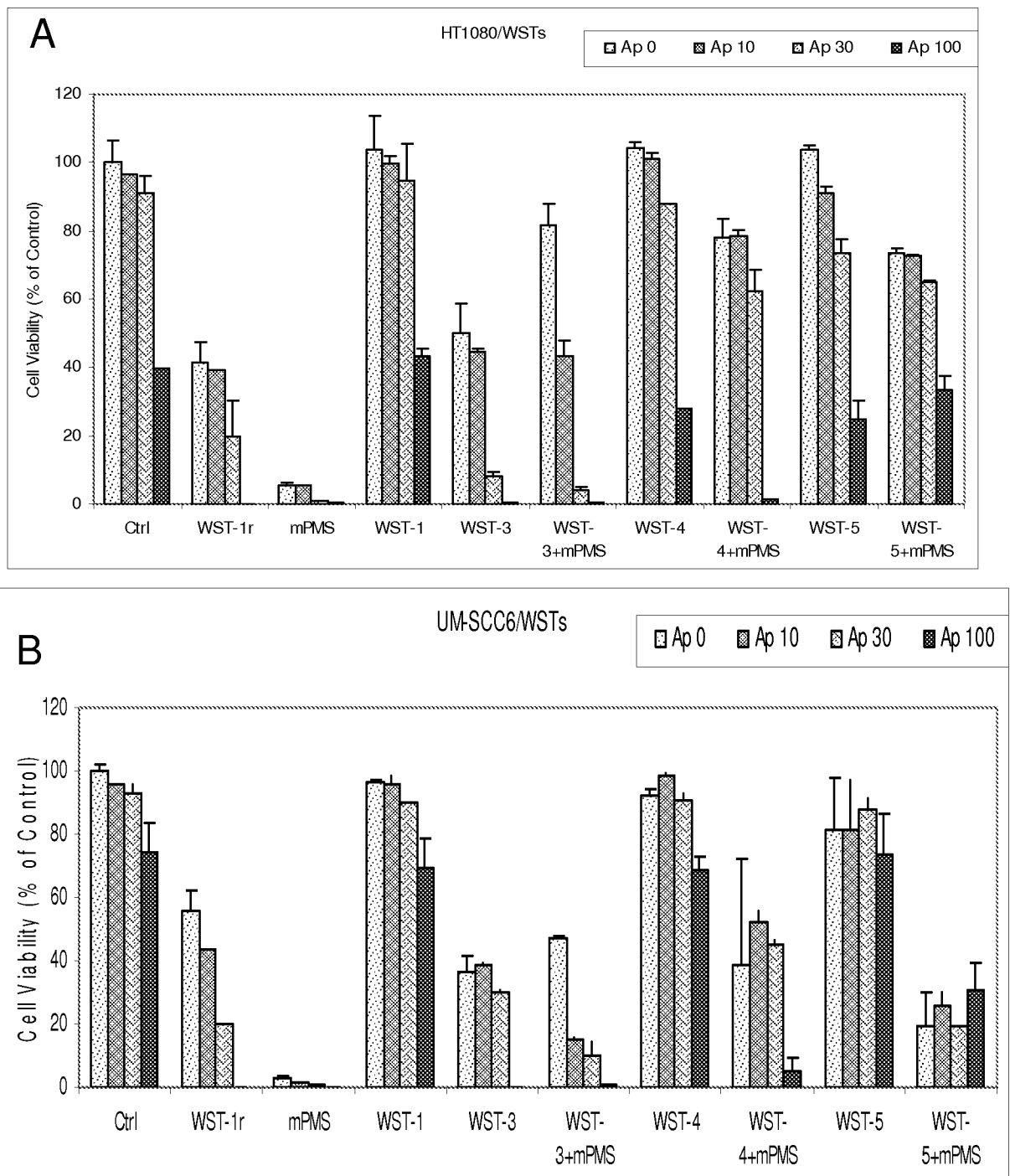


Fig 18

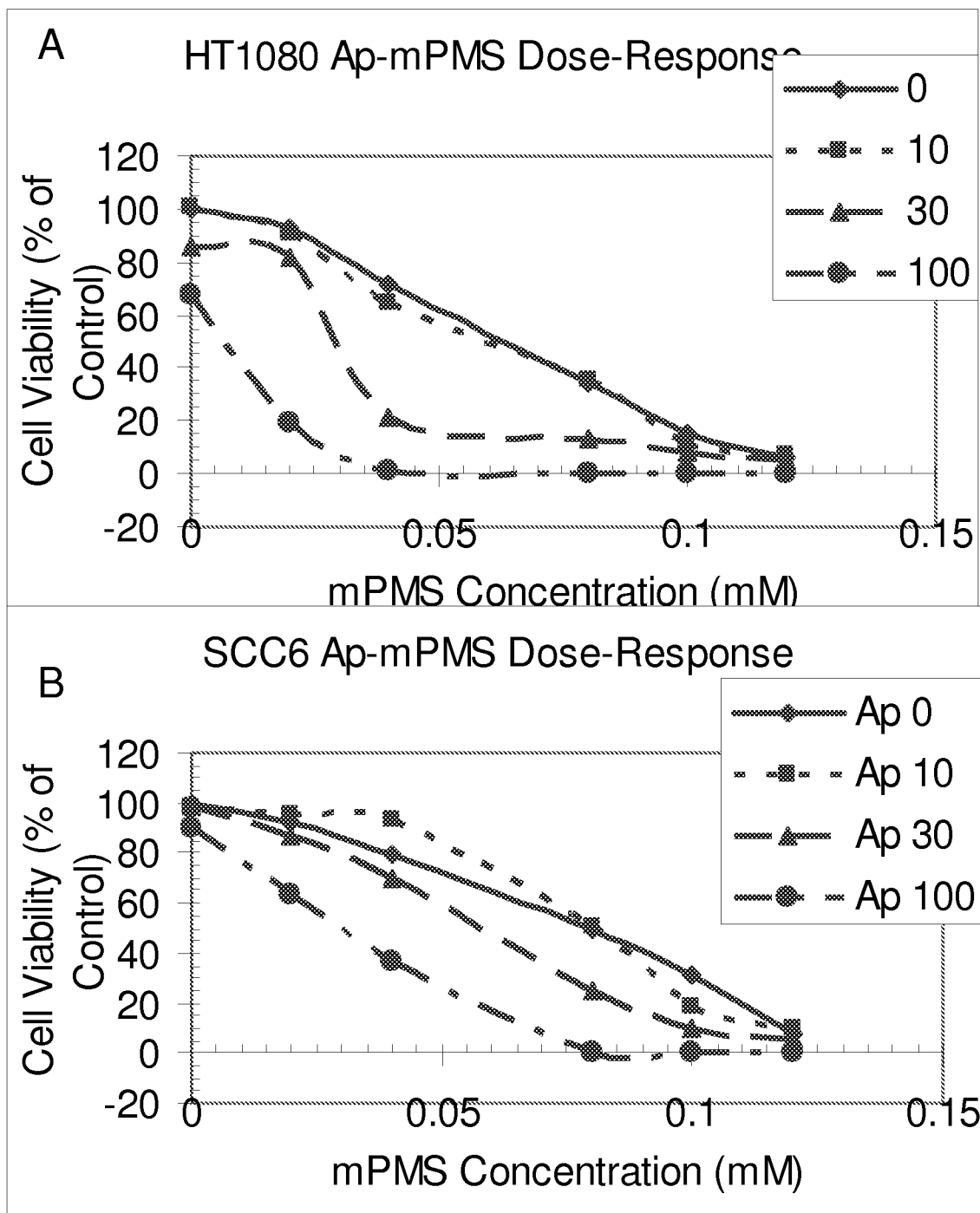


Fig19

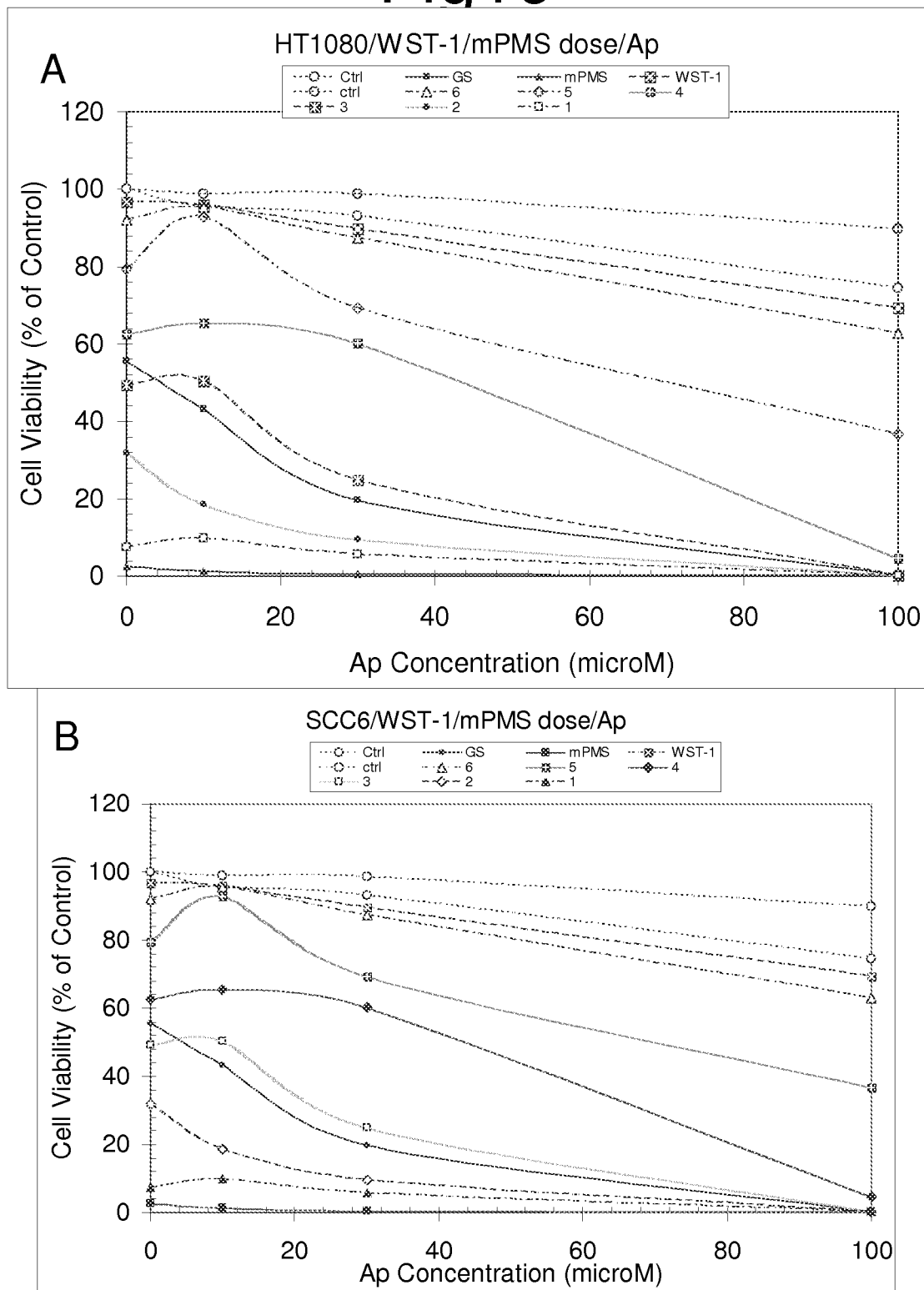


Fig 20

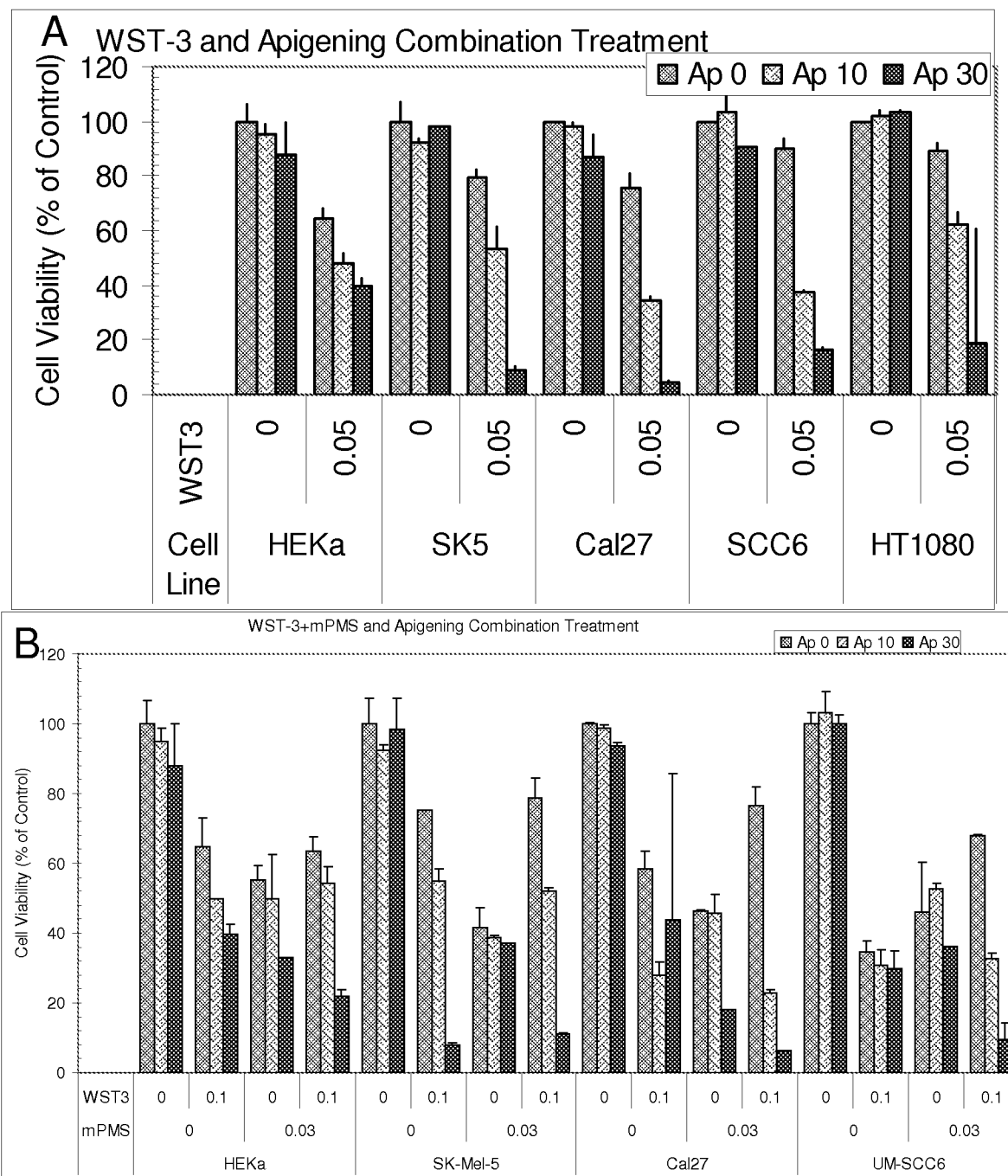


Fig 20

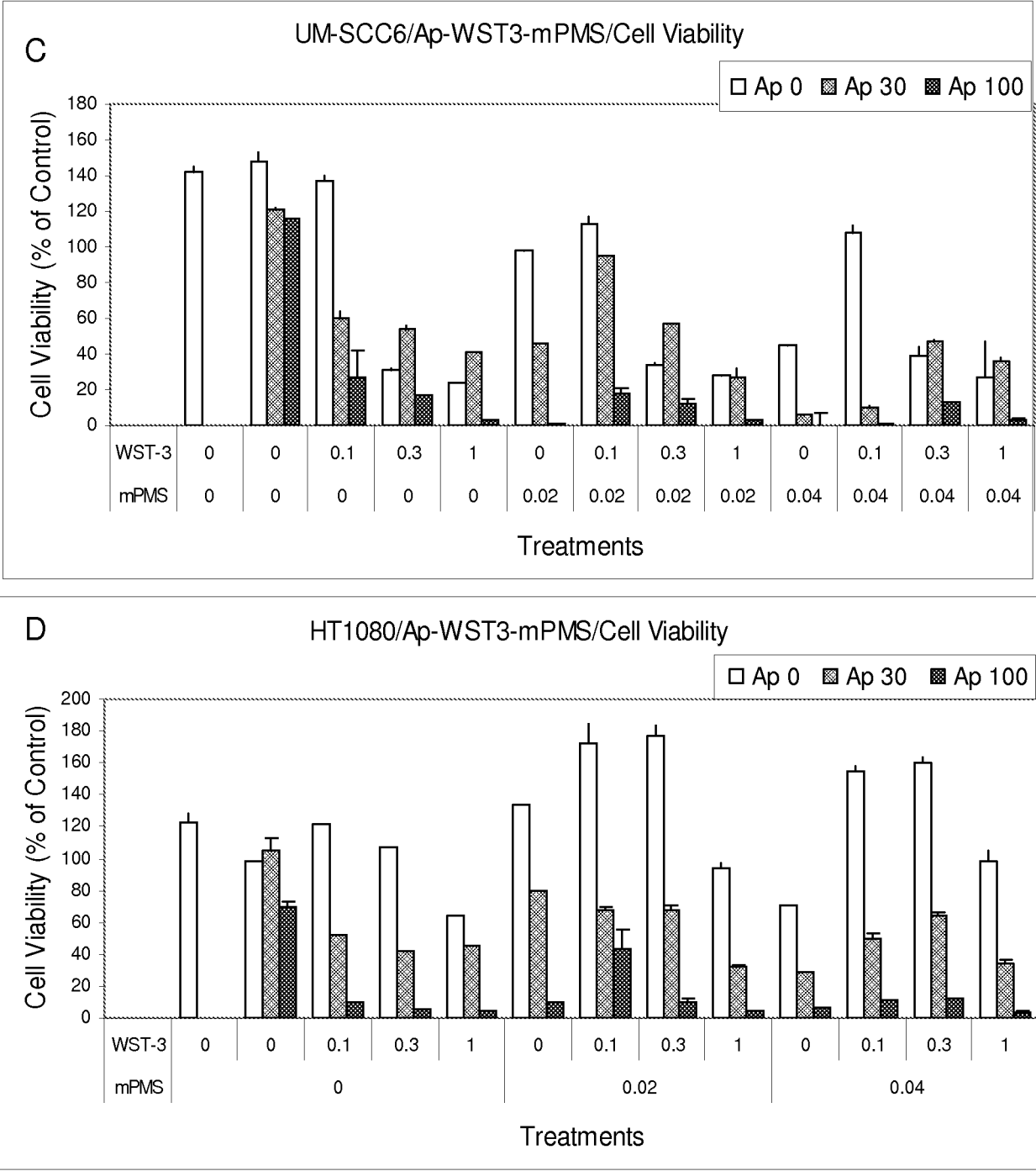


Fig 21

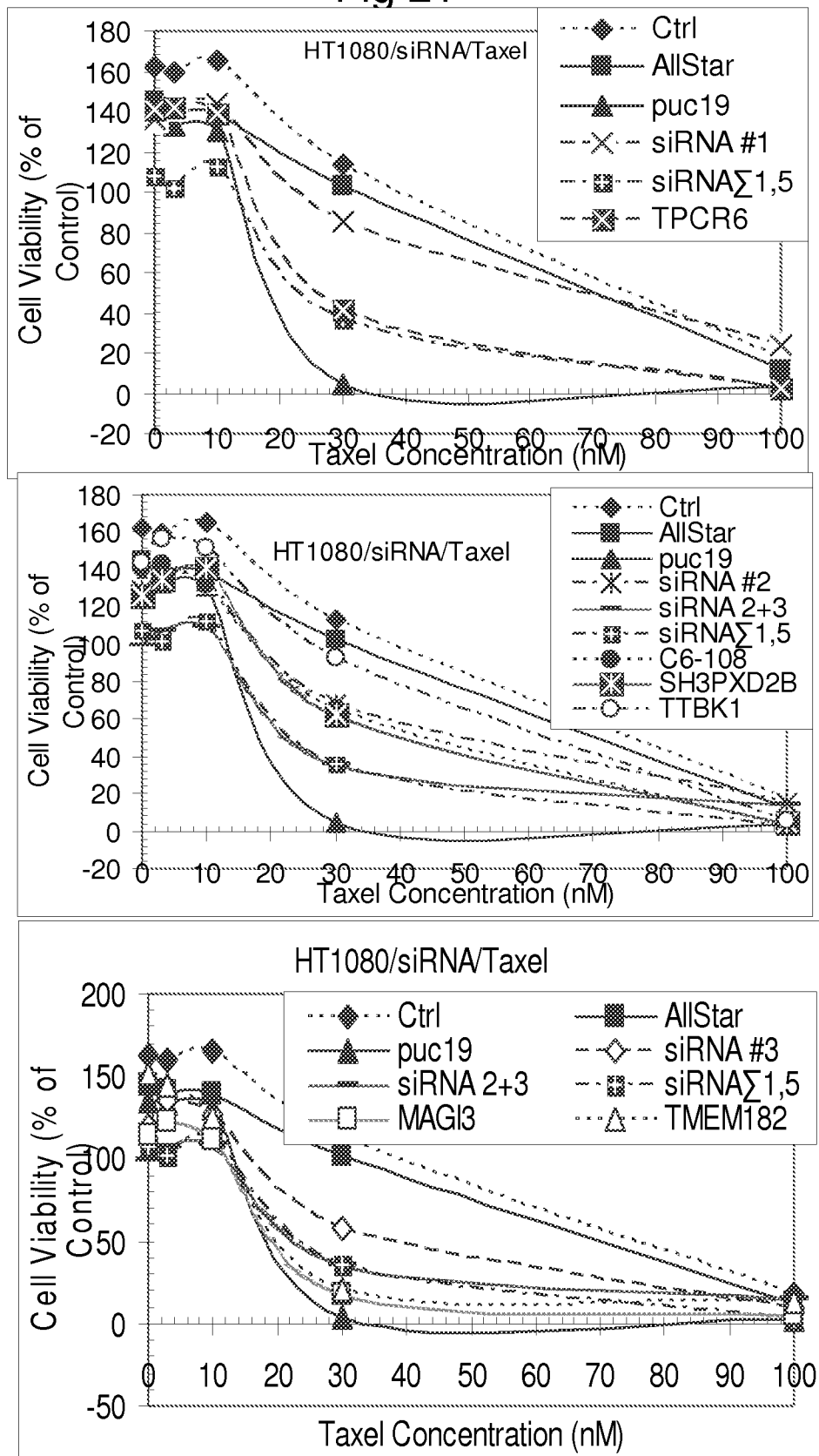


Fig 22

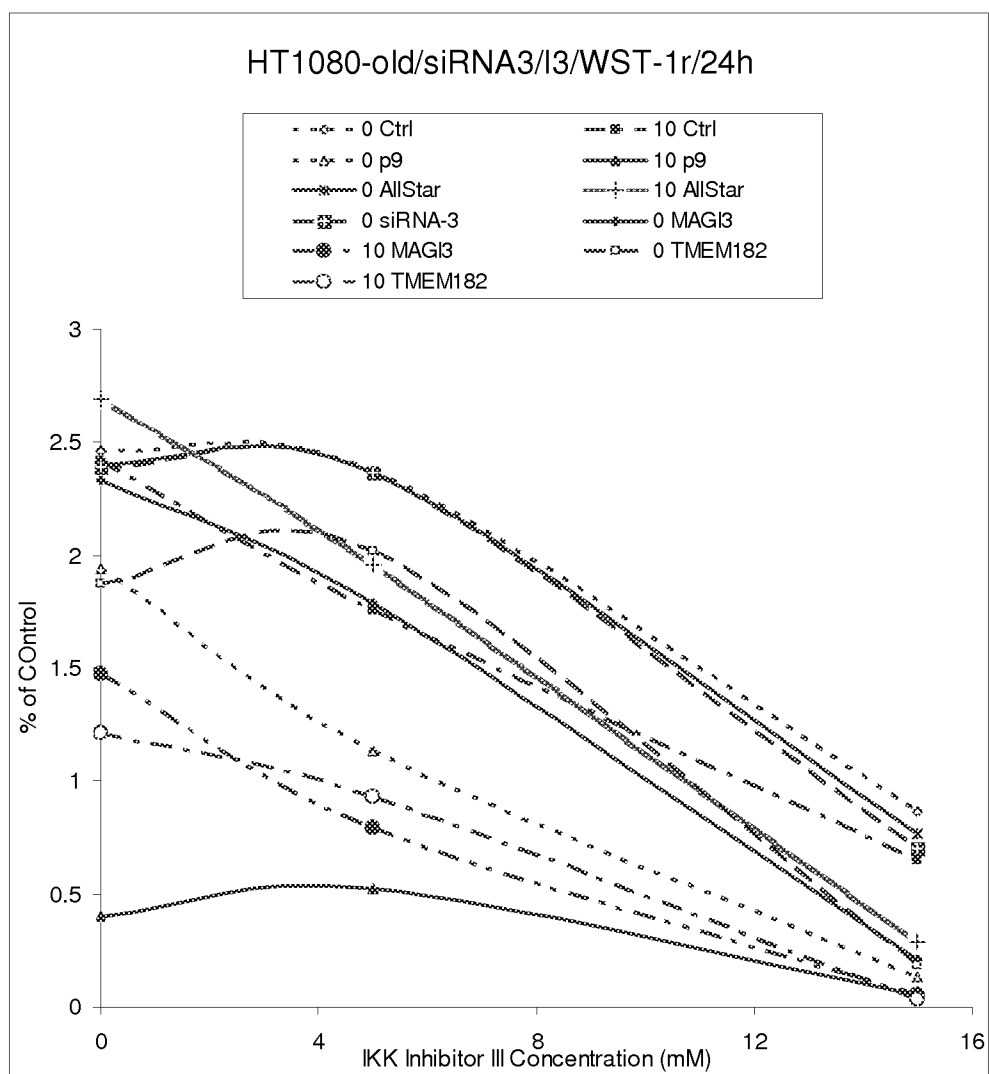


Fig 23

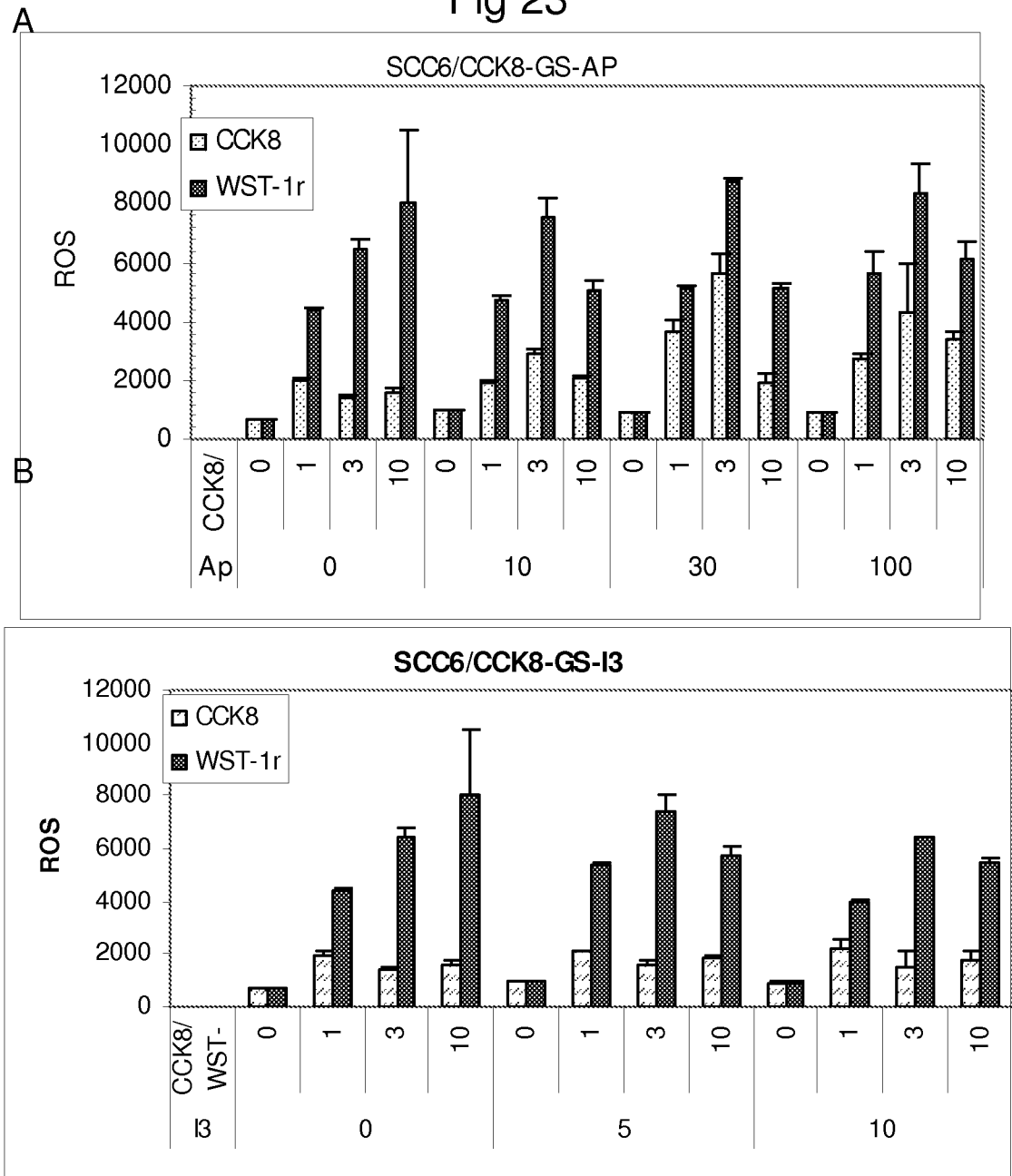


Fig 24 IFN substitute pUC19 transfection to enhance IKK inhibitor III and WST-1 effect

