REVIEW ON CARDIAC GLYCOSIDES IN CANCER RESEARCH AND CANCER THERAPY

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ABSTRACT

Cardiac glycosides have a long history in the treatment of cardiac disease. The mode of action of cardiac glycosides is inhibition of the ubiquitous plasma membrane Na+, K+-ATPase that leads to increased intracellular Ca2+ ion concentrations, inhibition of IL-8 production, inhibition of DNA topoisomerase II and activation of the Src kinase pathway. Ca2+ ions play pivotal role in many signaling pathways including those regulating apoptosis. Cardiac glycosides can also regulate one of the most potent angiogenesis promoting substances, fibroblast growth factor-2 (FGF-2), and may inhibit activation of the transcription factor NF-κB. FGF-2 and NF-κB are relevant targets for anticancer drugs. To date three cardiac glycosides have been developed for treatment of cancer and were tested in a phase I clinical trial to determine dose-limiting toxicities and maximum tolerated dose. An extensive information pertaining to the biochemical mechanism of cardiac glycosides may give a new hope in the development of semisynthetic derivative of this traditional molecular skeleton.

Keywords
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INTRODUCTION

Cardiac glycosides are organic compounds containing a glycoside that act on the contractile force of the cardiac muscle. Cardiac glycosides contain a common molecular structure comprised of a steroid nucleus, an unsaturated lactone ring at the C-17 position, and one or more glycosidic residues at the C-3 position. Chemically cardiac glycosides are divided into two types. 1. Cardenolides 2. Bufadienolides.

Cardenolides have an unsaturated butyrolactone ring (5-membered unsaturated lactone). Common cardenolides include digoxin, digitoxin, digitoxigenin, lantoside C and ouabain. From a therapeutic point of view, the most important cardiac glycosides are digoxin and digitoxin as they are both used for the treatment of cardiac congestion and some types of cardiac arrhythmias, such as atrial fibrillation. Cardiac glycosides have been used in the treatment of cardiac disease for more than 200 years and were already known to the ancient Egyptians over 3000 Years ago. In the 1960s clear inhibition of malignant cells of cardiac glycosides in-vitro was reported. Almost two decades later, observation of the altered morphology of breast cancer cells from women on digitalis by Stenkvist et al. showed more benign characteristics than cancer cells from control patients not on digitalis. Stenkvist et al. also showed that 5 years after the mastectomy, the recurrence among patients not taking digitalis was 9.6 times that in patients taking digitalis. Bufadienolides are C-24 steroids, its characteristic structural feature is a doubly unsaturated six membered lactone ring having a 2-pyrene group attached at the C-17 β-position of the perhydrophenanthrene nucleus. C-24 Bufadienolide Glycosides (that contain structural groups derived from sugars). These are a type of cardiac glycosides, bufadienolides and their glycosides are toxic specifically and they are heart-arresting.

MODE OF ACTION ON CARDIAC GLYCOSIDES

It is well known that cardiac glycosides, such as digitoxin, inhibit the activity of the Na⁺/K⁺-ATPase (also known as the Na⁺ pump or Na⁺/K⁺ pump). This pump is a transmembrane enzyme that acts as an electrogenic ion transporter in the plasma membrane of all mammalian cells. Each cycle of the Na⁺/K⁺-ATPase activity extrudes three Na⁺ from the cell, moves two K⁺ into the cell and utilizes one ATP. The primary role of the Na⁺/K⁺-ATPase is therefore, to maintain high intracellular K⁺ and low intracellular Na⁺. This pump also has an important role in regulating cell volume, cytoplasmic pH and Ca²⁺ levels through the Na⁺/H⁺ and Na⁺/Ca²⁺ exchangers, respectively, and in driving a variety secondary transport processes.
Such as Na⁺ dependent glucose and amino acid transport. Inhibiting Na⁺/H⁺ -ATPase by cardiac glycosides leads to higher levels of intracellular Ca²⁺, which leads to a decrease in heart rate and an increase in contractility of the heart. However, the decrease in intracellular K⁺ and increase in intracellular Na⁺ and Ca²⁺ following inhibition of the Na⁺/H⁺ -ATPase may also induce apoptosis. Inhibition of the Na⁺/H⁺ -ATPase by digitoxin and subsequent increase in intracellular Ca²⁺ led to the induction of apoptosis of prostate cancer cells. Besides inducing apoptosis by intracellular decrease of K⁺ and of Na⁺ and intracellular Ca²⁺, cytotoxic mechanisms of action include intracellular acidification; inhibition of IL-8 production and the TNF-α/NF-κB pathway; inhibition of DNA topoisomerase II and activation of the Src kinase pathway (Figure 4). Whether the Na⁺/H⁺ -ATPase is the primary target of cardiac glycosides or not is actually a matter of intense debate.⁴
Intracellular decrease of K⁺ and increase of Na⁺ and Ca²⁺

Inducing apoptosis by excessive K⁺ efflux and intracellular K⁺ depletion are early key steps in apoptosis. Physiological concentration of intracellular K⁺ acts as a repressor of apoptotic effectors. Loss of cellular K⁺, a common event in apoptosis of many cell types, may trigger the apoptotic cascade including caspase cleavage, cytochrome c release, and endonuclease activation. Pro-apoptotic disruption of K⁺ homeostasis can be mediated by over-activated K⁺ channels or ionotropic glutamate receptor channels, and most likely, accompanied by reduced K⁺ uptake due to dysfunction of Na⁺/H⁺-ATPase. Studies indicate that also mitochondrial K⁺ channels and K⁺ homeostasis play important roles in apoptosis. During apoptosis, there is compelling evidence indicating an early increase in intracellular Na⁺ followed by a decrease in both intracellular K⁺ and Na⁺ suggesting a regulatory role for these cations during both the initial signaling, and the execution phase of apoptosis. Studies have shown that the Na⁺/H⁺-ATPase is involved in controlling perturbations of Na⁺ and K⁺ homeostasis during apoptosis. Also cellular Ca²⁺ overload, or perturbation of intracellular Ca²⁺ compartmentalization, can cause cytotoxicity and trigger either apoptotic or necrotic cell death.⁵⁻⁶

Intracellular acidification

Published data suggests that intracellular alkalinisation can produce malignant transformation. It is also suggested that alkalinisation may be required for the development and maintenance of the transformed phenotype cancer cells and may be implicated in key cancer related processes. In contrast, it has been observed that intracellular acidification can induce apoptosis in cancer cells and play an important role in the induction of apoptosis by different stimuli. For example, Rich et al. demonstrated that apoptosis of leukemic cells accompanies reduction of intracellular pH after targeted inhibition of the Na⁺/H⁺ exchanger. Moreover stress-activated protein kinase pathway activation and mitochondrial-derived hydrogen peroxide acts as an effectors mechanism leading to induction of apoptosis by intracellular acidification. These observations indicate that induction of intracellular acidification possesses anticancer effects. Interestingly, cardiac glycosides induce intracellular acidification in cancer cells as the inhibition of the Na⁺/H⁺-ATPase may increase intracellular concentrations of Na⁺, reduce the activity of the Na⁺/H⁺ exchanger and trigger intracellular acidification.⁷⁻⁹

Inhibition of IL-8 production and the TNF-α/NF-κB pathway:

Inhibition of IL-8 production and the TNF-α/NF-κB pathway is another mechanism of cardiac glycosides to produce anticancer effects. As production of IL-8 has been associated with important processes involved in tumor progression such as apoptosis resistance, angiogenesis or metastasis, inhibition of its expression is therefore thought to produce anticancer effects. Juncker et al. demonstrated that the semi-synthetic cardenolide UNBS1450 leads to inhibition of IL-8 synthesis via NF-κB pathway disruption leading to apoptotic cell death. Srivastava et al. showed similar results for digitoxin whereas Yang et al. demonstrated that cardiac glycosides were potent blockers of the TNF-α/NF-κB pathway, which results in apoptosis, as NF-κB induces the expression of genes that are inhibitors of apoptosis.¹⁰

![Figure 1: Transcriptional control of inflammation. Signal transduction of proinflammatory cytokines, for example, TNF-α and/or LPS signals lead to activation of IKK complex to liberate cytosolic NF-κB from inhibition via ubiquitination and degradation of IκBα. These stimuli activate the JNK-AP-1 pathway. Coordinated actions of NF-κB and AP-1 propagate inflammation via promoting transcription of cytokines, chemokines, and other proinflammatory genes.](image-url)
Inhibition of DNA topoisomerase II

Recently published data suggest that digoxin may inhibit topoisomerase II. Because of their central role in DNA replication, transcription and repair processes, topoisomerase II inhibitors are a category of drugs commonly used in the treatment of malignancies by inducing apoptosis. Lopez-Lazaro et al. demonstrated that a renal adenocarcinoma cancer cell line was hypersensitive to digoxin and died by apoptosis. In vitro experiments showed that digoxin induced levels of DNA-topoisomerase II cleavable complexes comparable to etoposide, a topoisomerase II poison widely used in cancer chemotherapy. Cells exposed to digoxin for 30 min showed low but statistically significant levels of DNA-topoisomerase II cleavable complexes; however these complexes disappeared after 24 h exposure. The same research group also showed that digoxin, at concentrations commonly found in the plasma of cardiac patients, significantly reduced etoposide and idarubicin-induced topoisomerase II cleavable complexes in leukemia cells. Also other cardiac glycosides, such as ouabain, digoxin, proscillaridin and bufalin, have shown to inhibit topoisomerase II. Bielawski et al. demonstrated that digoxin, ouabain and proscillaridin A exerted significant inhibitory effects on the proliferation of breast cancer cells. Of the two cardiac glycosides, proscillaridin A was more effective at inhibiting the proliferation of breast cancer cells than digoxin or ouabain. Hashimoto et al. showed that bufalin caused a marked decrease in the steady-state level of topo II alpha mRNA in human leukemia cells, which led to a decrease in the amount and activity of the enzyme and to the induction of apoptosis.\textsuperscript{11}

![Figure 2: cardiac glycosides, proscillaridin A was more effective at inhibiting the proliferation of breast cancer cells than digoxin or ouabain.](image)

Activation of the Src kinase pathway

Multiple studies have established that the binding of cardiac glycosides to Na\textsuperscript{+}/H\textsuperscript{+}-ATPase not only inhibits the ATPase activity but also stimulates protein tyrosine kinases such as Src. This process is the consequence of an additional function played by Na\textsuperscript{+}/H\textsuperscript{+}-ATPase besides its control of ionic cellular homeostasis, which is already the trigger of complex intracellular signalization pathway forming a signal some. Accordingly, pools of non 120 pumping Na\textsuperscript{+}/H\textsuperscript{+}-ATPase are localized in plasma membrane, where it clusters with other plasma membrane proteins and receptors, including growth factor receptors (i.e., the epidermal growth factor receptor EGFR). Binding of Na\textsuperscript{+}/H\textsuperscript{+}-ATPase by cardiac glycosides may in turn unleash several kinase-dependent cascades, which are implicated in cell proliferation. Activated Src in turn transactivates EGFR, resulting in the assembly and activation of multiple signaling cascades controlled by the extracellular signal-regulated kinase (ERK) 1/2 and phospholipase C-\gamma/protein kinase C pathways. Liang et al. suggested that cells contain a pool of Src-interacting Na\textsuperscript{+}/H\textsuperscript{+}-ATPase that not only regulate Src activity but also serve as receptors for ouabain to activate protein kinases. One year before, in 2005, Kometiana et al. showed in breast cancer cell lines that ouabain-induced cell growth inhibition may be mediated by activation/transactivation of Src/EGFR by Na\textsuperscript{+}/K\textsuperscript{+}-ATPase, which leads to activation of ERK1/2, increase in the levels of the cell cycle inhibitor P21Cip1 and subsequent growth arrest. Kometiana et al. also demonstrated that digoxin and digitoxin concentrations close to or at therapeutic plasma levels had effects both on proliferation and ERK1/2 similar to those of ouabain, supporting the proposed potential value of digitalis drugs for the treatment of breast cancer. The existence of signalosomes where Na\textsuperscript{+}/H\textsuperscript{+}-ATPase plays a non-ionic activity has highlighted an endogenous activity of cardiac glycosides. Ouabain is endogenously produced and circulating in the plasma, it acts in a paracrine/endocrine fashion and its levels are considered critical to determine several physio-pathological responses. Interestingly, these endogenous biological effects correlate with a complex signaling cascade involving kinases. The discovery of these non-canonical functions has very recently suggested a role for Na\textsuperscript{+}/H\textsuperscript{+}-ATPase as hormone receptor. Altogether, these findings suggest in a very next future important hints in the elucidation of anticancer effects ascribed to cardiac glycosides and help in the explanation of preventive effects observed in patients under treatment with digitalis especially towards forms of hormonal cancer.\textsuperscript{12-15}
Cardiac glycosides and apoptosis

Recently, digitalis and other cardiac glycosides in nontoxic concentrations have been shown to induce apoptosis in different malignant cell lines in vitro. Cytotoxicity induced by cardiac steroids includes a series of morphological and biochemical changes that are characteristic for apoptosis, such as phosphatidylserine externalization, internucleosomal DNA fragmentation and mitochondrial membrane potential disruption. Apoptosis, also known as programmed cell death, is distinct from necrosis in that it is an active process that leads to cell death. It plays an important role in embryogenesis, carcinogenesis, regulation of the immune system, and the killing of virally infected cells, serving as a physiological process that regulates cell number and eliminates damaged cells. In light of the pivotal role of apoptosis in cancer development and progression, and this new experimental finding concerning cardiac glycosides, it seems probable that the apoptosis-inducing capability is explained by mechanisms other than just only Na\(^{+}/H^+\) -ATPase inhibition. Intracellular or extracellular Ca\(^{2+}\) chelators, Ca\(^{2+}\) channel blockers, and calmodulin antagonists can all delay or abolish apoptosis in several model systems. Disruption of intracellular Ca\(^{2+}\) homeostasis might result in the induction of apoptosis in diverse type of cells, including tumor cell lines. Furthermore, thapsigargin (Ca\(^{2+}\) ionophore), which selectively inhibits Ca\(^{2+}\)-dependent ATPase pumps in sarcoplasmic and endoplasmic reticulum and directly stimulates an intracellular Ca\(^{2+}\) increase, induces prominently the apoptosis in androgen-sensitive and -insensitive prostate cancer cells. Most prostate cancers are mixtures of androgen-dependent and androgen-independent cells. The major problem in androgen-independent prostate cancer is that, as yet, an effective therapeutic regimen is still lacking. However, it is well known that Ca\(^{2+}\) plays a crucial role in stimulating endonucleases and in cleaving internucleosomal DNA in cells responsive to apoptotic stimuli. Thus, the modulation of intracellular Ca\(^{2+}\) level would be a strategy for the treatment of hormone-resistant prostate cancers. It has been suggested that some forms of cardiac glycosides inhibit proliferation and induce apoptosis in prostate cancer cells in clinically relevant concentrations. These reports suggest that the modulation of intracellular Ca\(^{2+}\) level may induce or enhance the apoptotic response in human cancer cells and provide a new target for therapeutic strategies in cancer chemotherapies. Yehis et al. results have also shown that digitalis inhibits the proliferation of prostate cancer cell lines at least partially through a mechanism of cytotoxicity effects induced by a sustained elevation of intracellular Ca\(^{2+}\). However, they suggest that in addition to sustained intracellular concentration of Ca\(^{2+}\), another possible mechanism involved in the apoptotic effect induced by bufalin or cinobufagin might be the change in intracellular Na\(^+\) concentration. It is worth to note, that malignant cells in general are more susceptible to the effects of cardiac glycosides than normal cells. It may be due to the fact that in many cases, Na\(^{+}/H^+\) -ATPase activity is different in tumor or transformed cells compared to their normal counterparts. One of the cardiac steroids, bufalin, in nontoxic concentrations, was able to induce apoptosis in human leukemia HL60 and ML1 cells but not in normal leukocytes. Therefore, bufalin seems to act as a potent differentiation- and apoptosis-inducing agent in cancer cells. Although the signal transduction pathway from Na\(^{+}/H^+\) -ATPase is unknown, it appears that the signal is transmitted sequentially from Ras, Raf-1, and MAP kinase. Terness et al. found out that cardiac glycosides and their derivatives have a strong antiproliferative action and a tumor-specific, apoptosis-mediated cytotoxic effect. Interestingly, their results show that removal of the chemical groups that are responsible for inhibition of the Na\(^{+}/H^+\) -ATPase weakens but does not abrogate the apoptotic effect of cardiac glycosides. This process involves the classical caspase-dependent pathway with damage of mitochondria and internucleosomal DNA fragmentation. Lopez-Lazaro et al. have also shown the apoptotic activity of extracts obtained from the leaves of Digitalis purpurea on three human cancer cell lines: TK-10 (renal adenocarcinoma), MCF-7 (breast adenocarcinoma) and UACC-62 (melanoma). More recent works have shown that caspase activation and DNA fragmentation are preceded by a drop in intracellular K\(^+\) levels and that inhibition of this drop blocks caspase activation and cell death. Importantly, cardiac glycosides induce both an increase in Ca\(^{2+}\) and a decrease in K\(^+\) ions. In addition, parallel studies have shown that oleandrin suppresses NF-κB (nuclear factor-κB) activation, which could also contribute to cell death induction. However, the cell death-promoting activity of cardiac glycosides appears cell type specific, because other work has shown that they inhibit multiple pathways of apoptosis in vascular smooth muscle cells.
Cardiac glycosides and angiogenesis

Cardiac glycosides can also regulate one of the most potent angiogenesis promoting substances, fibroblast growth factor-2 (FGF-2), and may inhibit activation of the transcription factor NF-κB (nuclear factor-κB). FGF-2 and NF-κB are relevant targets for anticancer drugs. FGF-2, a regulatory peptide secreted from cells, is involved in a variety of biological processes including cell differentiation, cell growth and migration, angiogenesis, and tumor formation. Unlike other proteins, FGF-2 lacks the signal peptide sequence required for export from the cell by the endoplasmic reticulum for protein secretion. Hence, the mechanism of FGF-2 release from the cell was previously believed to require disruption of the cell membrane. It was, however, reported that FGF-2 export occurs through an ATP-dependent pathway. Furthermore, investigators have demonstrated that FGF-2 binds to the α1-subunit of Na'/H' - ATPase and have hypothesized that inhibition of this enzyme activity would decrease FGF-2 release from the cell. Therefore, cardiac glycosides that inhibit Na'/H' - ATPase activity may also inhibit FGF-2 release from the cell. The findings of Newmanis study support the hypothesis that cardiac glycosides inhibit FGF-2 export from the cell in a concentration- and time-dependent manner. NF-κB regulates the expression of various genes that play critical roles in apoptosis, viral replication, tumorgenesis, various autoimmune diseases, and inflammation. NF-κB seems to be an ideal target for novel anticancer drugs. Activation of NF-κB has been shown to block apoptosis, promote proliferation, and to induce resistance to chemotherapeutic agents. More recently, it was found out that the Na'/H' -ATPase might also play a role in the regulation of cell growth and expression of various genes. It was only newly discovered that cardiac glycosides might affect cells at the concentrations lower than that required for the inhibition of the sodium pump. Dmitrieva suggested that the Na'/H' -ATPase might act as a cell signaling receptor activated by a cardiac glycosides binding. It is thought that this signaling may influence cytoskeleton reorganization as well as cell survival, its growth and differentiation. This pathway is still unresolved form of cardiac glycosides action. Interestingly, studies have recently shown that the Na'/H' -ATPase pump is also involved in membrane transport of selected cellular proteins and cationic substances important to tumor cell growth. These all findings provide novel insight into the mechanism of action of cardiac glycosides and raise new questions regarding functions of these compounds in the cell.

Figure 4: Differentiate between Tumor and endothelial cells.

Importance of cardiac glycosides on cancer cells:

Cardiac glycosides exert anti-proliferative and cytocidal effects on different cancer cell models. Their ability to impair cancer cell viability represents a main hallmark of their anticancer activities. Nevertheless, multiple types of cell death are triggered by cardenolides and bufadienolides. The induction of apoptosis has been frequently reported. Both extrinsic and intrinsic apoptosis pathways were triggered. Moreover, the sensitization to other therapeutic agents has been also described. In a consistent number of reports, cardiac glycosides led to the accumulation of cells essentially in the S phase and G2/M phase. This event has been correlated to the elicitation of intracellular reactive oxygen species.
Besides, in adherent cancer cell models, cardiac glycosides have been shown able to activate an autophagic cell death. This dual cytocidal ability underlines the promising use of cardiac glycosides especially for the treatment of those forms of cancer that are resistant to agents inducing apoptosis. Nevertheless, the mechanisms determining the kind of cell death accomplished upon treatment with cardiac glycosides remain still unclear and debated. One possibility is that sustained autophagy may be commonly activated as a first response by the cells followed by a switch to apoptosis in cancer cells prone to activate programmed forms of cell death. In contrast, autophagic cell death may be undertaken as a kind of final backup cell death modality whenever apoptosis cannot take place. This hypothesis implies that cardiac glycosides may induce stress conditions that potentially lead to alterations of metabolic activities. Finally, very recently clinically used cardiac glycosides, as digoxin and digitoxin, have been shown to induce immunogenic cell death. Interestingly, among the parameters determining immunogenic cell death is the autophagy-dependent secretion of ATP.

**Phase I trials:**

To date, there are three cardiac glycosides or derivatives that have been developed for treatment of cancer and were assessed in a phase I clinical trial. The initial product was Anvirzel™, an aqueous extract of Nerium oleander, the second was PBI-02504, a super critical CO₂ extract of Nerium oleander and the third UNBS1450, a semisynthetic cardenolide derivate of 2''-oxovoruscharin extracted from Calotrops procer, a desert shrub. In 2000, Manna et al. demonstrated that oleandrin inhibits the activation of NF-xB and AP-1 and their associated kinases. Smith et al. showed that Anvirzel™, like oleandrin, inhibits fibroblast growth factor (FGF)-2 export in vitro from prostate cancer cells in a concentration and time-dependent fashion and may, therefore, contribute to the antitumor activity of this treatment for cancer. Based on these preclinical data, a phase I study started and Mekhail et al. reported in 2006 the results of this study of Anvirzel™. The study reported a phase I trial to determine the maximum tolerated dose (MTD) and safety of Anvirzel™ in 18 patients with advanced, refractory solid tumors. Patients were randomized to receive this agent by intramuscular injection at doses of 0.1, 0.2 and 0.4 ml/m²/day with subsequent patients receiving 0.8 or 1.2 ml/m²/day sequentially. Eighteen patients were enrolled and completed at least one treatment cycle of 3 weeks. Most patients developed mild injection site pain (78%). Other toxicities included fatigue, nausea, and dyspnea. Traditional dose-limiting toxicity was not seen, but the MTD was defined by injection volume as 0.8 ml/m²/day. No objective antitumor responses were seen. They concluded that Anvirzel™ can be safely administered at doses up to 1.2 ml/m²/day, with the amount administered intramuscularly limited by volume. The recommended phase II dose level is 0.8 ml/m²/day. PBI-05204 has recently completed testing for safety in Phase I clinical trial. The publication of conclusions is in process and the initial findings were presented at the annual meeting of the American Society of Clinical Oncology (ASCO) in June 2011. PBI-05204 (Oleandrin), inhibits the α-3 subunit Na⁺/H⁺-ATPase pump. Relative expression of the α-3 subunit in tumor cells correlates with proliferation. Oleandrin inhibits FGF-2 export, activation of NF-xB, phosphorylation of Akt, p70S6K and decreases mTOR activity. In this first-in-human study, the authors sought to determine the MTD/recommended phase II dose and to define the pharmacokinetics (PK) and pharmacodynamics (PD) of PBI-05204 in advanced cancer patients. Forty-six patients were dosed at 8 dose levels (DL) of PBI-05204 (0.6 to 10.2 mg/day). Two dose-limiting toxicities occurred at DL 8 (grade 3 proteinuria, fatigue) thus the MTD was DL 7. Most common adverse events (AEs) were fatigue (56.1%), abdominal pain (41.5%), constipation (41.5%), nausea (41.5%), and diarrhea (39.0%). Cardiac disorders were reported in 10 patients (24.4%), all grade 1, except for one patient with grade 2 supraventricular tachycardias (SVT). Of the 45 evaluable patients, 7 showed a stable disease for >4 months, with bladder, colorectal, fallopian tube, breast, appendical and pancreatic carcinoma (2 patients). They concluded that PBI-05204 is well tolerated up to 10.2 mg/day with very little AEs or cardiotoxicity. UNBS1450, has also been tested in an open-label dose escalation study to evaluate the safety, tolerability and pharmacokinetics of this single agent, administered once every 3 weeks in separate cohorts of patients with advanced solid tumors or lymphoma. Chemical modifications of 2''-oxovoruscharin (a novel cardenolide extracted from Calotrops procer) has led to the identification of UNBS1450.

The activity of the compound in preclinical cancer models, independent of cell type, has been tested in vitro on 57 human cancer models from 11 distinct histological types. In aggressive and metastatic orthotopic NSCLC refractory prostate cancer and glioma models, UNBS1450 was more potent than tested reference compounds, including paclitaxel, irinotecan, oxaliplatin, mitoxantrone and temozolomide. UNBS1450 was the most potent inhibitor of all three isozymes (α3β1, α2β1 and α1β1) with a potency ~6 to >200 times greater than ouabain (another cardenolide) and digoxin. The general mechanism of action associated with UNBS1450-mediated anticancer effects relates to the compound’s propensity in disorganizing the actin cytoskeleton and thus non-ATPase-mediated effects. UNBS1450 can thus be considered both anti-proliferative (cytotoxic) and anti-migratory, given that the actin cytoskeleton is essential to cytokinesis and to cancer cell migration. In sharp contrast to digitalis-like cardenolides, UNBS1450 does not induce intracellular Ca²⁺ or Na⁺ increase at concentrations at which it induces potent antitumor effects. UNBS1450 induces both apoptotic and non-apoptotic cell death processes depending on the cellular environment.
Non-apoptotic cell death mechanisms such as lysosome membrane permeabilisation and autophagy were observed in solid tumors and thus may overcome major apoptosis resistance pathways responsible in part for the failure of therapeutics in certain cancers. Canonical intrinsic apoptosis was demonstrated by Juncker et al. in leukemia and lymphoma cellular models with an early degradation of anti-apoptotic Mcl-1, Bak and Bax activation leading to cytochrome C release, caspase-9, -7 and -3.36 Experimental data involving NF-κB inhibition/ deactivation evidenced it as an important new approach to the treatment of various malignancies was shown by the same authors. UNBS1450 deactivates the cytoprotective NF-κB pathways at several points, in sharp contrast to specifically designed NF-κB inhibitors acting at one precise point. In leukemia cells, UNBS1450 inhibits degradation of the IκB inhibitor of p50/p65 NF-κB heterodimers thus preventing transcription factor translocation in the nucleus. Using genomic and proteomic approaches, it was possible to evidence UNBS1450-mediated down-regulation of c-MYC gene, MYC oncoprotein-related genes, and genes with nucleolar functions.15 UNBS1450-induced marked down-regulation of c-MYC expression in a number of human cancer cell lines lead to nucleolar disorganization resulting in impairment of cancer cell survival. Unfortunately the phase I study was closed in 2011 by the sponsor because of bankruptcy before reaching the MTD after including 23 patients. Preliminary data will be published elsewhere.

CONCLUSION
Cancer remains a life-threatening disease that is typically characterized by frequently related to dysregulated cell growth and resistance to apoptosis. Within the past decade, cancer research has provided interesting insights with the potential to define the exact causes of cancer and to aid in the development of anticancer agents with enhanced effectiveness against and selectivity for cancer. Numerous studies have screened medicinal plants for compounds with anticancer activity, including cardiac glycosides. These compounds have been reported to be therapeutically beneficial for the treatment of various tumor types because of their anti-proliferative effects, ability to induce apoptosis, and ability to sensitize cells to chemo/radiotherapy-induced cell death. As already discuss, cardiac glycosides have a narrow therapeutic index, which could cause serious cardiovascular toxicity. Interestingly, it has been observed that the concentration required to treat cancer was lower than of that used to treat cardiac disorders. However, the expression pattern of the enzyme subunits and the target specificity of cardiac glycosides must be optimized. Synthetic cardiac glycosides have been designed to achieve the desired effects; these compounds include UNBS-1450 and D6-MA. Although cardiac glycosides have potential effects on cancer, at present, evidence supporting their usefulness is still needed, and the safety profile of cardiac glycosides as anticancer agents must be determined. For the proper exploration of the functional details of cardiac glycosides further review and research is recommended.

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