

Repositioning therapy for thyroid cancer: new insights on established medications

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Abstract

Repositioning of established non-cancer pharmacotherapeutic agents with well-known activity and side-effect profiles is a promising avenue for the development of new treatment modalities for multiple cancer types. We have analyzed some of the medications with mechanism of action that may have relevance to thyroid cancer (TC). Experimental *in vitro* and *in vivo* evidences, as well as results of clinical studies, have indicated that molecular targets for medications currently available for the treatment of mood disorders, sexually transmitted diseases, metabolic disorders, and diabetes may be active and relevant in TC. For instance, the derivatives of cannabis and an anti-diabetic agent, metformin, both are able to inhibit ERK, which is commonly activated in TC cells. We present here several examples of well-known medications that have the potential to become new therapeutics for patients with TC. Repositioning of established medications for the treatment of TC could broaden the scope of current therapeutic strategies. These diverse treatment choices could allow physicians to provide an individualized approach to optimize treatment for patients with TC.

Key Words

- ▶ repositioning therapy
- ▶ thyroid cancer
- ▶ cannabinoids
- ▶ valproic acid
- ▶ lithium
- ▶ nelfinavir
- ▶ chloroquine
- ▶ arsenic
- ▶ statins
- ▶ metformin

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Introduction

Thyroid cancer (TC) is the most common endocrine malignancy. An estimated, 60 220 new cases of TC and 1850 TC-related deaths are expected in 2013 in the USA. Survival of patients with TC depends upon multiple factors, including cancer type and stage. The 5-year survival rate is more than 90% in patients with localized papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC), and only 53.9% in patients with distant metastases (Schlumberger 1998). The prognosis for patients with medullary thyroid cancer (MTC) depends on the stage of tumor progression at the time of diagnosis, with a mean 10-year survival rate of 100, 93, 71, and

21% for stages I, II, III, and IV respectively (Modigliani *et al.* 1998).

An increasing number of phase I and II studies have been conducted to evaluate the efficacy of new molecular targeted drugs, such as tyrosine kinase inhibitors, inhibitors of angiogenesis, radio-immunotherapy, and re-differentiation drugs (Lanzi *et al.* 2009, Liebner & Shah 2011). Several targeted therapies have shown promising biochemical response and have resulted in the inhibition of targeted signaling pathways in TC cells. Most of these therapeutic modalities, however, were associated frequently with high toxicity rates.

The identification of novel drug targets is a significant challenge in drug development. Although the human genome comprises ~30 000 genes, only the proteins encoded by fewer than 400 are used as drug targets (Rask-Andersen *et al.* 2011). Drug discovery is still a very lengthy, as well as an increasingly risky and costly process. It is estimated to cost around \$1 billion per medication and the process takes ~15 years from conception to Food and Drug Administration (FDA) approval (Dickson & Gagnon 2004).

One method to accelerate development and reduce costs is to identify novel indications for already approved medications, also referred to as 'repositioning' (O'Connor & Roth 2005). Repositioning takes advantage of the available pharmacokinetic and toxicity data on existing drugs, limits risks and costs, and could accelerate the implementation of new cancer therapies. There already has been successful repositioning of the existing drugs for the treatment of cancer, including i) cyclooxygenase-2 inhibitors, which are FDA-approved anti-inflammatory drugs and are now approved for cancer chemoprevention in familial adenomatous polyposis patients; and ii) lenalidomide, originally marketed for morning sickness, which is now approved for treatment of myelodysplastic syndromes (Li & Jones 2012). In addition, a well-known and widely used oncology agent, gemcitabine, was developed initially as an antiviral drug, but was later repositioned for the treatment of cancer.

The unexpected side effects observed during clinical trials and off-target toxicity frequently lead to repositioning toward the non-intended use of the drug. For example, Sildenafil (Viagra; Pfizer), was initially developed as an anti-angina medication, but produced the remarkable side effect of inducing prolonged penile erections in human volunteers (Ashburn & Thor 2004). A further approach used to reposition medications is the use of high-throughput screening using drug libraries and computational network analysis. Recent studies have shown that these experimental methods enable the prediction of novel drug targets and the repositioning of known drugs for the treatment of cancer (Shim *et al.* 2012, Emig *et al.* 2013).

Studies of the molecular mechanisms of TC have provided the basis for the development of new diagnostic tools, identification of prognostic markers, and markers of response to the treatment (Ringel 2000, 2009, Xing *et al.* 2005, Nikiforov 2008). Novel insights into the molecular pathology of TC revealed a number of potential cancer drivers that were already explored in the context of non-TC pathology. In TC, a high-throughput screening of 2816

clinically approved drugs demonstrated that medications with non-classic chemotherapy mode of action (cardio- tonic, antilipid, and antiviral medications) inhibited the growth of TC cells (Zhang *et al.* 2012), suggesting their possible repositioning for the treatment of TC.

In this review, we will discuss several specific examples of repositioning efforts that have been focused on TC, with data suggesting the potential efficacy of psychoactive compounds, medications intended for the treatment of infectious and metabolic diseases, as well as a commonly used anti-diabetic drug for the treatment of TC.

Psychoactive compounds and mood stabilizers

Lithium

The mood stabilizer lithium (Li) is primarily used to treat bipolar disorder, a common mental illness that affects ~1–3% of the population. Accumulating evidence indicates that this agent also holds promise for treating neurologic and/or neurodegenerative diseases via its diverse mechanisms of action.

At therapeutic serum concentrations (0.6–1.2 mM), Li is known to inhibit a group of phosphomonoesterases, the metabolic enzyme phosphoglucomutase, and GSK3b. Moreover, activation of the phosphoinositol cascade, adenylate cyclase, and metabolism of arachidonic acid are also affected by Li. The protein GSK3b is believed to be the main facilitator of Li's mood-stabilizing and neuro-protective effects (Chiu *et al.* 2013).

Thyroid abnormalities such as goiter, hypothyroidism, and autoimmune thyroiditis have been reported to be associated with treatment with Li (Bocchetta *et al.* 2007). Li has been shown to be highly concentrated in thyroid cells, achieving levels 3–4 times more than that observed in the serum. To explain the mechanism of Li action in the thyroid gland, the effects of Li on thyroid function and cell growth were examined in FRTL-5 rat thyroid cells and in primary cultures of porcine thyroid follicles. Li suppressed TSH-induced iodide uptake, reduced iodine organification, and *de novo* thyroid hormone formation. In both FRTL-5 thyroid cells and porcine follicles, Li enhanced cell growth: its growth-promoting effect was mediated through the protein kinase C system (Urabe *et al.* 1991). *In vivo* studies have shown that Li inhibits the release of iodine from the thyroid.

The effects of Li on the accumulation and retention of ¹³¹I in metastatic lesions and thyroid remnants were evaluated in patients with differentiated thyroid

carcinoma (Koong *et al.* 1999). From a series of gamma-camera images, it was found that Li increased ¹³¹I retention in 24 of 31 metastatic lesions and in six of seven thyroid remnants. Li prolonged the effective half-life in responding metastases by more than 50% and increased the estimated ¹³¹I radiation dose in metastatic tumor by 2.29 times. These studies suggest that Li may be a useful adjuvant for ¹³¹I therapy of TC, augmenting both the accumulation and retention of ¹³¹I in lesions. Short-term Li therapy is well tolerated by most individuals, and no serious toxicity in patients with TC was reported in the literature. Among adverse effects, gastrointestinal disturbances (nausea, vomiting, diarrhea) were reported in 10–20% of patients, but were usually mild and did not require discontinuation of the drug (Pons *et al.* 1987).

It has been shown that raf-1-inducible activation of ERK in MTC cells increased phosphorylation of GSK3b (Kunnimalaiyaan *et al.* 2007). Treatment with Li inhibited phosphorylation of GSK3b and led to the inhibition of MTC cell growth. Growth inhibition by GSK3b inactivation was found to be associated with cell cycle arrest due to an increase in the levels of cyclin-dependent kinase inhibitors such as p21, p27, and p15. Furthermore, Li-treated MTC xenografts had a significant reduction in tumor volume compared with those treated with control.

These results showed that inactivation of GSK3b is sufficient to inhibit the growth of MTC cells *in vitro* and *in vivo*, and currently the effects of Li on residual/recurrent MTC are being evaluated in a clinical trial (NCT00582712). There is a clinical trial evaluating the effect of Li on low-dose ¹³¹I therapy in early TC (NCT00251316).

Valproic acid

Valproic acid (VA) is an established agent for the treatment of epileptic seizures and mania in bipolar disorder. In the human brain, VA affects the function of neurotransmitter GABA by inhibition of GABA degradation, increased GABA synthesis, and decreased GABA turnover (Barrueto *et al.* 2002). VA is an effective epigenetic modulator that causes N-terminal tail hyperacetylation of histones and inhibits histone deacetylase (HDAC) enzymatic activity. Chromatin remodeling by acetylation of the N-terminal histone tails is one of the key steps in the regulation of gene expression in cancer cells (Gottlicher *et al.* 2001). HDAC inhibitors were found to upregulate pro-apoptotic (Bim, Bmf, Bax, Bak, and Bik) and downregulate anti-apoptotic (Bcl2 family) proteins in various cancer cell lines and in animal cancer models (Xu *et al.* 2007).

VA demonstrated a wide range of anticancer *in vitro* effects on prostate, breast, and endometrial cancers, neuroblastoma, glioma, and leukemia, and has been proposed as a re-differentiating agent for hematological malignancy and neuroblastoma (Fortunati *et al.* 2004, Catalano *et al.* 2005). Studies in EBV-related cancer cell lines showed that VA induced expression of EBV lytic-phase gene and increased the efficacy of chemotherapeutic agents in epithelial and lymphoblastoid cell lines, harboring EBV (Feng & Kenney 2006).

TC cells are characterized by elevated expression of HDAC compared with normal thyroid tissue (Nakagawa *et al.* 2007). *In vitro* studies have shown that while treatment with an HDAC inhibitor affects expression of thyroid-specific genes and I-¹³¹I uptake in TC cells (Puppin *et al.* 2005), VA selectively upregulated expression of NIS, but did not alter Tg production and TSHR expression in PTC and ATC cell lines. In PTC-derived cells, induction of NIS expression was associated with its membrane localization and induction of I-¹³¹I uptake. In ATC-derived cells, however, VA-inducible increase in cytoplasmic NIS was not associated with induction of I-¹³¹I uptake (Fortunati *et al.* 2004). VA also was found to inhibit proliferation and induce apoptosis in TC cells. Treatment with VA was associated with increased expression of p21, downregulation of cyclin A, and induction of cell-cycle arrest in G1 phase in PTC-derived cells (Catalano *et al.* 2005). In FTC- and PTC-derived cell lines, treatment with VA was associated with downregulation of anti-apoptotic genes (*BCL2*, *BCL-xl*), upregulation of a pro-apoptotic gene (Perez *et al.* 2004), and induction of apoptosis (Shen *et al.* 2005). VA also decreased invasiveness of FTC and PTC-derived TC cells through inhibition of MMP-2 and MMP-9 and sensitized ATC-derived cells to treatment with doxorubicin (Kim *et al.* 2009, Mitmaker *et al.* 2011). EBV was detected in poorly differentiated TC, and the expression of EBV nuclear antigen and EBV latent membrane protein was proposed to play a role in the progression of PTC to undifferentiated carcinoma (Shimakage *et al.* 2003). Presence of viral genome in poorly differentiated TCs suggests that viruses could serve as a target, and further studies are needed to determine whether VA can be clinically useful in patients with EBV-positive thyroid tumors.

The potential utility of VA for the treatment of patients with MTC was suggested in recent studies. VA upregulated *Notch1* expression in MTC cells, resulting in growth inhibition and induction of apoptosis (Greenblatt *et al.* 2007, Adler *et al.* 2010).

VA acts as a potent HDAC inhibitor at serum concentrations achievable in patients with epilepsy and produces mild adverse effects. There are 62 clinical trials in which VA is currently being tested as an anti-cancer drug for variety of cancers (Clinicaltrials.gov), including advanced TC (NCT01182285).

Cannabis

Cannabis has been used for medicinal purposes for many years. Several types of medicinal cannabinoids are available in the USA and Canada. Dronabinol (schedule III), nabilone (schedule II), and nabiximols (approved in Canada) are cannabis-derived pharmaceuticals. Dronabinol and nabilone are indicated for the treatment of nausea and vomiting associated with cancer chemotherapy, and of anorexia associated with weight loss in patients with acquired immune deficiency syndrome. The hemp plant *Cannabis sativa* produces 70 unique compounds known as cannabinoids, of which D9-tetrahydrocannabinol (THC) is the best characterized, owing to its high potency and abundance. THC exerts a wide variety of biological effects by mimicking endogenous substances – the endocannabinoids – anandamide and 2-arachidonoylglycerol – bind to, and activate, specific cannabinoid receptors.

Studies in neuronal cells demonstrated that the endocannabinoid anandamide inhibits activation of ERK kinase via cannabinoid receptor 1 (CB1). Treatment with anandamide inhibited TrkA-induced Rap1/B-Raf/ERK activation (Rueda *et al.* 2002). In several models, cannabinoid administration has been shown to curb the growth of glioma, astrocytoma, and pancreatic cancer cell lines, and reduced the growth of hepatocellular carcinoma subcutaneous xenografts (Vara *et al.* 2011). The mechanism of cannabinoid anti-tumoral action relies on the ability of these agents to inhibit tumor angiogenesis and inhibit cell-cycle progression. Cannabinoids activate an endoplasmic reticulum (ER) stress-related signaling route that leads to the upregulation of the transcriptional co-activator p8 and its target, the pseudo-kinase tribbles homolog 3 (TRB3; Salazar *et al.* 2009). The stimulation of this pathway promotes autophagy via TRB3-mediated inhibition of the Akt/mammalian target of rapamycin complex 1 (mTORC1) axis and is important for the proapoptotic and anti-tumoral action of THC.

The inhibitory effects of a metabolically stable analog of anandamide (2-methyl-2'-F-anandamide (Met-F-AEA)) were demonstrated in TC cells. The inhibition of TC cell growth was due to an interaction Met-F-AEA with cannabinoid receptor 1 (CB1), activation of p53 signaling,

and an increased apoptotic rate. It has also been shown that activation of cannabinoid receptor 2 (CB2) triggers apoptosis in anaplastic TC cell lines. A considerable regression of thyroid tumors generated by inoculation of ARO/CB2 cells was observed in nude mice following administration of CB2 agonist JWH133 (Shi *et al.* 2008).

Data indicating that endocannabinoids are effective against cancer cells with activated BRAF/ERK and/or TrkA signaling suggest its potential utility for the treatment of BRAF-positive PTC and TrkA-positive MTC. Expression of CB1 and 2, however, has not yet been examined in BRAF-positive human TC tissue samples, and additional studies are needed to determine the expression of endocannabinoid's molecular targets in human thyroid tumors. Given that cannabinoids have shown anti-tumor effects in many types of cancer models, CB2 may be a viable therapeutic target for the treatment of thyroid carcinomas.

Metabolic and anti-diabetic drugs

Statins

Statins (lovastatin, simvastatin, pravastatin, and atorvastatin) are a widely used group of cholesterol-lowering agents that act by inhibiting the enzyme 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, which catalyzes the rate-limiting step in cholesterol biosynthesis (Evans *et al.* 2004). Statins reduce the concentration of mevalonate, which in turn leads to increased LDL-receptor expression in hepatocytes, and to increased LDL-C uptake from the circulation. Results from large, randomized controlled trials have provided evidence that statin treatment reduces the risks of vascular death, myocardial infarction, and stroke (Baigent *et al.* 2005).

Elevated HMG-CoA reductase activity has been shown in cancer cells, including colorectal and gastric carcinomas and leukemia. Malignant cells appear to be highly dependent on the sustained availability of mevalonate pathway end-products (isoprenoids, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate). These intermediates are critical for the post-translational prenylation of proteins including Ras, Rho, and Rac, which play a role in regulation of cancer cell proliferation, migration, and apoptosis (Zeybek *et al.* 2011). *In vitro* studies demonstrated that statins inhibit cancer cell proliferation via Rac-1 as well as through reduction of Rho geranylgeranylation. Most published data, however, emphasizes the effects of statins on cancer cell migration. It has been shown that the ability of statins to block the isoprenylation of Rho protein represents the primary

mechanism that affects cell motility. Lovastatin-inducible downregulation of the Rho/ROCK signaling led to the inhibition of phosphorylation of focal adhesion complex (FAK) and paxillin, which are involved in the regulation of stress fibers and focal adhesions (Yasunari *et al.* 2001). Growth inhibitory effects of statins were explored in combination with classical chemotherapy. It has been shown that the combination of paclitaxel with lovastatin resulted in antagonism that affected both the cytotoxicity of the compounds and apoptosis (Chung *et al.* 2011), indicating that it is important to examine the compatibility of statins with existing therapy.

Statins were considered for cancer prevention and chemosensitization. Treatment with statins was associated with delayed metastasis of melanoma in a case-control study including 1318 cases and 6786 controls (Koomen *et al.* 2007). Results of this study, as well as data from epidemiological analysis of breast, prostate, and colon cancers in dyslipidemic patients, suggested the potential protective effects of statins on cancer progression.

In PTC- and ATC-derived TC cell lines, Lovastatin induced cell-cycle arrest in G0/G1 phase, inhibited proliferation, promoted formation of autophagic vesicles, and induced apoptosis (Wang *et al.* 2003, Zeybek *et al.* 2011). Pretreatment of ARO cells with a p27, but not p21, anti-sense oligonucleotide prevented the Lovastatin-induced G0/G1 arrest in ARO cells. The Lovastatin-induced growth inhibition was completely prevented by cell treatment with mevalonate and partially by geranylgeranyl pyrophosphate. Lovastatin increased TC cell differentiation and I-131 uptake, and decreased ATC cell invasion through inhibition of RhoA and Rac1. Animal studies have demonstrated that Lovastatin significantly reduced ATC tumor growth in mice treated with 5 or 10 mg/kg per day dosage regimens, but enhanced tumor growth when a dose of 1 mg/kg per day was administered (Wang *et al.* 2010).

Studies using FRTL-5 thyroid cells demonstrated a differential response to the treatment with Lovastatin in cells that were transformed with K-ras or H-ras viral oncogenes (Laezza *et al.* 2008). Lovastatin induced apoptosis in K-ras-transformed FRTL-5 cells, but led to cell-cycle arrest in H-ras-transformed FRTL-5 cells. The Lovastatin-induced apoptosis in K-ras-transformed FRTL-5 cells was due to increased intracellular reactive oxygen species, as well as activation of p38 mitogen-activated protein kinase and nuclear factor κ B pathways. Lovastatin anti-tumor efficacy in K-ras-dependent thyroid tumors was further confirmed *in vivo*, therefore suggesting that statins

can represent a novel selective therapeutic tool for tumors harboring K-RAS mutations.

Retrospective analysis of thyroid pathology in dyslipidemic patients showed that use of statins was associated with lower prevalence, lower number, and decreased volume of thyroid nodules, which may be explained by an anti-proliferative and/or pro-apoptotic effect of long-term treatment with statins (Cappelli *et al.* 2008). There are currently no clinical trials using statins in TC patients, but the data suggest that statins merit further investigation as anti-TC agents.

Metformin

Metformin is an oral anti-hyperglycemic agent that has been widely used in the management of non-insulin-dependent diabetes mellitus. Metformin was introduced in 1995 and is the most prescribed anti-diabetic drug in the world. The anti-diabetic effects of metformin are mediated by multiple mechanisms, such as suppression of hepatic gluconeogenesis, increase in peripheral glucose uptake, and reduction in insulin resistance.

The intracellular effects of metformin are mediated by activation of AMP-activated protein kinase AMPK (El-Mir *et al.* 2000). Studies in breast, prostate, ovarian cancer cell lines, as well as in animal models, demonstrated that metformin inhibits cancer cell growth via AMPK-dependent inhibition of the mTOR signaling pathway and inhibition of cyclin D1 expression (Shaw 2009, Ben Sahlra *et al.* 2010, Martin-Castillo *et al.* 2010). In addition to direct growth inhibitory properties, metformin is able to potentiate the cytotoxic effects of chemotherapeutic compounds through AMPK and p53 signaling (Iliopoulos *et al.* 2011, Rocha *et al.* 2011, Petrushev *et al.* 2012).

Recent epidemiological, animal, and *in vitro* studies have demonstrated that metformin also has anti-neoplastic effects in various human cancers. Diabetic patients treated with metformin have a lower risk of cancer than patients not treated with metformin (Evans *et al.* 2005, Buchs & Silverman 2011). Growing evidence has emerged that metformin use may reverse the increased cancer risk associated with administration of insulin (Cazzaniga *et al.* 2009, Lee *et al.* 2011). A recent meta-analysis has identified a 31% reduction in overall cancer incidence or mortality when metformin is used in the treatment of diabetes (Cazzaniga *et al.* 2009). Moreover, observational studies have documented that treatment with metformin is associated with sensitization to neoadjuvant therapy for breast cancer, and prompted phase III clinical trials addressing the role of metformin as

an adjuvant therapy for breast cancer patients (Goodwin *et al.* 2009).

In PTC- and FTC-derived cell lines, metformin inhibited cancer cell growth and downregulated cyclin D1 expression. Interestingly, TC cell treatment with metformin at concentrations that were sufficient for inhibition of cell growth did not affect TC cell migration. Studies of cell signaling activation in metformin-treated cells indicated that activation of AMPK and inhibition of p70S6K/pS6 signaling were common events in all examined TC cells. In an oxidative stress model, metformin potentiated H₂O₂-inducible activation of AMPK and attenuated pERK and p70S6K in PTC- and FTC-derived cells. In addition, metformin antagonized the growth-stimulatory effect of insulin, inhibited clonal cell growth, reduced TC sphere formation, and potentiated the anti-mitogenic effect of chemotherapeutic agents, such as doxorubicin and cisplatin, on undifferentiated thyroid carcinoma cells (Chen *et al.* 2012). In MTC-derived cells, metformin decreased expression of cyclin D1 and inhibited cell growth. Treatment with metformin was associated with inhibition of mTOR/p70S6K/pS6 signaling and downregulation of pERK in MTC-derived TT and MZ-CRC-1 cell lines (Klubo-Gwiedzinska *et al.* 2012).

Clinical studies demonstrated that treatment with metformin was associated with a decrease in nodule size in patients with multinodular goiter as compared with treatment with levothyroxine alone (Rezzonico *et al.* 2011). Moreover, there are studies describing TSH suppression in diabetic patients after treatment with metformin (94), and patients with chronic hypothyroidism managed by levothyroxine therapy (Vigersky *et al.* 2006).

The retrospective analysis of clinicopathological characteristics in diabetic patients with TC showed that tumor size was significantly smaller in metformin-treated compared with the non-metformin groups. A multivariate model revealed that treatment with metformin increased the likelihood of a complete response, and increased progression-free survival in TC patients with diabetes (Klubo-Gwiedzinska *et al.* 2013). Together, these data suggest the potential utility of metformin for treatment of TCs.

Metformin is well tolerated and common side effects include diarrhea, nausea, and vomiting (Bolen *et al.* 2007). Lactic acidosis is a rare complication, and may occur in patients with liver or kidney comorbidities (Khurana & Malik 2010). Currently, there are 52 clinical trials evaluating the usefulness of metformin as an adjunct treatment for patients with various type of cancer, but no single trial for patients with TC (Clinicaltrials.gov).

Medications for the treatment of infectious diseases

HIV protease inhibitor Nelfinavir (Viracept)

Nelfinavir (NFV) was originally designed as a specific HIV protease inhibitor and, since its introduction in 1997, has served as an effective, reliable, and well-tolerated HIV drug. NFV prevents viral replication by inhibiting the activity of HIV1 protease, an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins, resulting in the formation of immature non-infectious viral particles (Bardsley-Elliott & Plosker 2000). Similarly, NFV was found to inhibit *in vitro* replication of herpes simplex virus and human cytomegalovirus.

Toxicities associated with HIV protease inhibitors include dyslipidemia, insulin resistance, and diabetes. These side effects of NFV were thought to be a consequence of its off-target action on PI3K/AKT signaling pathway, which is frequently activated in cancer. A recent study has shown that NFV acts as an inhibitor of heat shock protein 90 (HSP90) signaling and induces degradation of HSP90 client proteins to include AKT and ERBB2 (Shim *et al.* 2012). Treatment with NFV also was associated with induction of ER stress, and ER stress markers, such as BiP and phospho-eIF-2 expression, increased following treatment with NFV. It was suggested that targeting the ER stress pathway, thereby shifting the balance between survival and apoptosis using a sustained ER stress inducer (NFV), could become an attractive pharmacological strategy for suppressing cancer cells.

It has been shown that NFV displays potent inhibitory activity on Kaposi's sarcoma, the most common HIV-associated cancer. Subsequent experimental *in vitro* and *in vivo* studies have demonstrated inhibitory effects of NFV on the PI3K/AKT signaling pathway in a variety of human cancer cell lines, including breast, lung, and ovarian cancer cells. The average concentration of NFV exhibiting 50% inhibition of cultured cell growth and *ex vivo* ovarian cancer cells was ~5 and ~10 μmol/l respectively. Such concentrations correspond to the C_{max} (~7–9 μmol/l) for NFV in HIV patients. Experiments with animal models of human cancers have demonstrated that at clinically achievable concentrations, NFV inhibited tumor growth (Pore *et al.* 2006, Yang *et al.* 2006, Gills *et al.* 2007, Shim *et al.* 2012). NFV contains a unique *cis*-decahydroisoquinoline-2-carboxamide moiety, which may provide the structural basis for its increased efficacy against cancer compared with other HIV protease inhibitors. Experimental evidence

indicates that NFV is effective against chemotherapy-resistant cancer cells and enhances the sensitivity of tumor cells to radiotherapy *in vitro* and *in vivo*.

Genetic abnormalities in PI3K/AKT signaling pathway are common in TC, especially in anaplastic thyroid carcinomas (Hou *et al.* 2007). NFV, with its anti-apoptotic activity, appears to be a promising agent for the treatment of TC. In MTC cells, a recent study has shown that RET kinase is a client protein for HSP90, and RET mutants rely on HSP90 for protein stability. HSP90 blockade by 17-allylamino-17-demethoxygeldanamycin (17-AAG) induces a 26S proteasome-dependent degradation of RET mutants (Carlomagno *et al.* 2004, Alfano *et al.* 2010), and it is possible that NFV-inducible block of HSP90 could be effective against MTC-derived cells.

NFV is a well-tolerated agent with mild side effects such as diarrhea, nausea, rash, headache, and flatulence (Bruning *et al.* 2010). The anti-cancer effects of NFV are currently under investigation in 21 clinical trials for treating patients with cervical, pancreatic, lung, renal, rectal, head and neck cancers, hematological malignancies, multiple myeloma, liposarcoma, and glioma (Pan *et al.* 2012, Rengan *et al.* 2012). Eleven of these clinical trials are focused on the action of NFV as a radio-sensitizer. There are no active clinical trials examining the effects of NFV on patients with TC.

Anti-malaria drug chloroquine

Chloroquine (CQ) is a 9-aminoquinoline, specifically synthesized for use as an anti-malarial agent early in the 1930s. Because of its weak base properties, CQ can pass through the lipid cell membrane and preferentially concentrate in acidic cytoplasmic vesicles. The resulting slight elevation of pH within these vesicles in macrophages or other antigen-presenting cells may influence the immune response to auto-antigens (Fox 1996). It is believed that this lysosomotropic property of CQ underlines its anti-malarial effects. CQ also demonstrated anti-inflammatory and immunosuppressive effects, which have been attributed to the suppression of antigen presentation, inhibition of prostaglandin and cytokine synthesis, and the modulation of Toll-like receptors. It also has been proposed to work as an antiviral agent by interfering with protein glycosylation necessary for viral function.

More recently, the ability of CQ to block autophagy by inhibiting lysosomal proteases and autophagosome-lysosomal fusion has generated further interest in this drug. The autophagy pathway regulates lysosomal degradation of organelles, and can be stimulated by multiple forms of

cellular stress including nutrient deprivation, hypoxia, reactive oxygen species, and DNA damage (Kroemer *et al.* 2010). Initial research demonstrated that autophagy may function as a tumor suppressor with defects in autophagy predisposing to tumor development in certain mouse models. Conversely, autophagy has also been shown to have pro-tumorigenic roles in promoting therapeutic resistance to cytotoxic chemotherapy as well as cancer cell survival (Mancias & Kimmelman 2011). As an inhibitor of autophagy, CQ has been used in combination with diverse chemotherapeutic drugs and radiation, and has been shown to enhance tumor cell killing (Wang *et al.* 2011, Kimura *et al.* 2013).

Recent studies have shown that autophagy plays a protective role against acute kidney injury, and thus, the use of CQ in combination with anticancer drugs may exacerbate kidney damage (Kimura *et al.* 2013). It has to be noted that organs in which autophagy plays a homeostatic role, such as the neurons, liver, hematopoietic stem cells, and heart, may be sensitive to the combined use of CQ and anticancer drugs.

A TC study demonstrated that inhibition of autophagy promotes PTC cell resistance to doxorubicin and radiation, and suggested that autophagy activation may be a useful adjunct treatment for patients with PTC that is refractory to conventional therapy (Lin *et al.* 2009). It also has been shown that an inhibitor of the mTOR signaling (RAD001) induces autophagy, which enhances the therapeutic response to cytotoxic chemotherapy and external beam radiation in PTC (Lin *et al.* 2010). A recent study has shown that inhibition of autophagy decreased the anti-proliferative effects of sunitinib and sorafenib in MTC cells (Lin *et al.* 2012).

It is likely that the function of autophagy in TC is dynamic, with both pro-tumorigenic and tumor suppressive roles that depend on cell type, tumor stage, and treatment context. Recent studies have demonstrated that oncogenic Ras expression leads to an increase in autophagy and that this upregulation is necessary for survival and transformation. These findings suggest Ras-driven thyroid tumors may be particularly sensitive to autophagy inhibition.

Currently, there are ten clinical trials evaluating the usefulness of CQ as an adjunct treatment for patients with various type of cancer, but no single trial for patients with TC (Clinicaltrials.gov).

Arsenic

Arsenic has not only been used extensively to treat infectious diseases such as syphilis, plague, and malaria,

but also been used to commit murder by poisoning. Throughout history, a number of prominent people including Francesco de' Medici, Napoleon Bonaparte, and Simón Bolívar, were poisoned with arsenic compounds.

Arsenic compounds have prominent mitotoxic effects and disrupt ATP production through several mechanisms. At the level of the citric acid cycle, arsenic inhibits pyruvate dehydrogenase, and by competing with phosphate, it uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NAD⁺, mitochondrial respiration, and ATP synthesis (Sun *et al.* 2011). Hydrogen peroxide production is also increased, which might form reactive oxygen species and produce oxidative stress. These metabolic interferences lead to apoptosis and/or necrotic cell death.

The anti-cancer activity of arsenic was demonstrated in hematological malignancies. In the early 1990s, reports from China described impressive results with arsenic trioxide (ATO) in patients with relapsed and refractory acute promyelocytic leukemia (APL) (Zhang *et al.* 2001). Other investigators subsequently confirmed these results, leading to approval of its use for relapsed or refractory APL in the USA. Investigations of this agent have demonstrated that its efficacy in APL and preclinical tumor models is dependent upon a number of mechanisms, including induction of apoptosis, effects on cellular differentiation, cell cycling, and tumor angiogenesis (Emadi & Gore 2010).

The mitotoxic properties of ATO also have been examined in solid tumors. In breast cancer cells, ATO reduced mitochondrial function through the inhibition of cytochrome *c* oxidase (complex IV of the electron transport chain), and its cytotoxicity was correlated with strong suppression of the expression of c-Myc and HIF1 α , and decreased the expression of the survival protein Bcl2 (Sun *et al.* 2011). In cervical cancer, ATO was effective against human papillomavirus (HPV)-infected cancer cell lines. It has been shown that ATO inhibited HPV-E6 protein expression, leading to upregulated p53 levels,

induced S to G2/M arrest, and apoptosis (Wen *et al.* 2012). ATO inhibits the activation of hedgehog signaling and promotes apoptotic cell death in osteosarcoma cells by accumulation of DNA damage, and prevented the growth of osteosarcoma in nude mice (Nakamura *et al.* 2013).

In TC cells, ATO was investigated for its potential to increase iodide uptake. In transformed thyrocytes, ATO increased iodide uptake, inhibited proliferation, and induced apoptosis, therefore suggesting its potential usefulness for the treatment of radioiodine non-responsive differentiated thyroid carcinomas (Frohlich *et al.* 2008). Moreover, ATO affected metabolic activity and decreased glucose uptake in transformed thyrocytes. A recent study has demonstrated that a mitochondria-targeted drug was synergized with an inhibitor of glycolysis (2-deoxyglucose (2-DG)) to trigger breast cancer cell death (Cheng *et al.* 2012). In anaplastic TC cell lines, treatment with 2-deoxyglucose resulted in cytostatic effects, but did not induce apoptosis (Sandulache *et al.* 2012). Experiments in an animal model of anaplastic TC showed that 2-DG affected cancer cell metabolism. The magnitude of 2-DG effects on tumor metabolism, however, was small and its effective duration was limited. It is tempting to speculate that dual targeting of energy metabolism with mitochondrial drugs (ATO) and glycolytic inhibitors (2-DG) could be effective against anaplastic TC cells.

Currently, there are more than 100 clinical trials evaluating the usefulness of ATO for patients with various type of cancer, but no single trial for patients with TC (Clinicaltrials.gov).

Summary

The role of activating mutations in mitogenic RAS/RAF/MEK/ERK and anti-apoptotic PI3K/AKT signaling pathways is well documented in TC. Efforts over the past decade to design targeted chemotherapy for BRAF, RET, and PI3K/AKT have shown narrow therapeutic effects, and

Table 1 List of pharmacotherapeutic agents with their described mechanism of action and cellular effects

Agent	Molecular targets	Cellular effect	Evidence in thyroid
Lithium	Notch, GSK3	Radio-sensitization, cell growth arrest	<i>In vitro</i> , <i>in vivo</i> , and human data
Valproic acid	HDAC	Re-differentiation	<i>In vitro</i> , <i>in vivo</i> , and human data
Cannabinoids	CB, ERK/TrkA	Cell growth arrest	<i>In vitro</i>
Statins	HMG-CoA reductase	Inhibition of cell growth and migration, re-differentiation	<i>In vitro</i> , <i>in vivo</i> , and human data
Metformin	AMPK, mTOR, ERK	Cell growth arrest, radio-sensitization	<i>In vitro</i> , <i>in vivo</i> , and human data
Nelfinavir	AKT, HSP90	ER stress, autophagy apoptosis	Not tested
Chloroquine	Lysosomal proteases	Autophagy, chemosensitization	<i>In vitro</i>
Arsenic	Mitochondrial complex IV	Mitochondrial toxicity, apoptosis, necrosis	Not tested

have not yet been proven to have widespread benefit in the treatment of TC patients. It has become evident that agents targeting signaling molecules often exhibit limited clinical activities. Biological redundancies and alternative pathways can often bypass the inhibition of a single target, suggesting that in some cases, broad-specificity compounds or multi-target drug therapies may be more effective than individual high-affinity, high-specificity therapies.

Experimental and clinical evidence shows that a number of widely used drugs for conditions other than cancer hit the same targets of known anti-cancer drugs with limited toxicity. We present herein the mechanistic allure of several therapies in the treatment of TC. In Table 1, we have summarized these agents with their proposed mechanism of action in relation to cellular effects in TC, as supported by both *in vitro* and *in vivo* evidence, as well as by studies in patients with thyroid disorders.

Several of these medications currently are being examined in clinical trials involving TC patients and can be quickly translated to the clinical practice. The results of studies demonstrating the anti-cancer effects of the metformin in patients with various types of cancer as well as data indicating the usefulness of metformin in diabetic patients with TC strongly suggest that this anti-diabetic drug with a well-established safety profile could be quickly repositioned for the treatment of TC.

The provocative *in vitro* results with other medications need to be further confirmed in animal models of TC. The retrospective analysis of pathological data from patients with TC and concomitant pathologies requiring medical therapy (mood disorders, HIV, malaria, arsenic poison, and dyslipidemia) could provide important information regarding the potential utility of non-cancer medications for the management of TC.

Repositioning of established pharmacotherapeutic agents shows promise to open new treatment modalities leading to a broadened scope beyond current cancer therapeutic strategy. These diverse treatment choices could allow physicians to provide an individualized approach to optimize treatment for patients with TC.

Declaration of interest

The authors have nothing to disclose. The views presented in this manuscript are those of the authors; no endorsement by the USUHS or Department of Defense has been given or should be inferred.

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