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Advances in cancer treatment over the last few decades have resulted in marked improvements in patient survival. Unfortunately, many of these new cancer therapies are also associated with toxic side effects on the heart. Consequently, the use of newer potentially cardiotoxic cancer drugs, combined with an improvement in patient survival from the cancer, has resulted in a sharp rise in the incidence of heart failure in cancer patients. In fact, in many cancer patients the risk of developing heart disease may be higher than the risk of cancer recurrence [1]. Indeed, the American College of Cardiology/American Heart Association guidelines define patients receiving chemotherapy as Stage A heart failure patients, since they have an increased risk of developing heart failure [2]. The cardiotoxicity associated with cancer chemotherapy can range from mild and temporary alterations in left ventricular ejection fraction (LVEF) to severe and irreversible life-threatening heart failure. Because of the importance of cardiotoxicity associated with cancer therapy, this edition of Heart & Metabolism addresses the important issue of cancer and the heart.

Many of the pharmacological approaches to treat cancer involve a mechanism of action that is actually toxic to the cardiomyocyte. This includes approaches to promote free radical production, promote apoptosis, prevent angiogenesis, inhibit metabolism, and promote ischemia. It is not surprising, therefore, that a number of these cancer cytotoxic agents have the potential for cardiotoxicity. This includes the use of agents such as the anthracylines, alkylating agents, microtubule targeting agents, monoclonal antibodies, tyrosine kinase inhibitors, and cytokines to treat cancer. The potential for cardiotoxicity during cancer treatment is not confined to acute use of cancer chemotherapy, but can also manifest in the chronic setting. Chiara Lestuzzi nicely highlights this in the Case Report in this issue of Heart & Metabolism, which describes a case of late left ventricular dysfunction in a long-term survivor of cancer. This case highlights the need to closely monitor survivors of cancer and aggressively treat any risk factors for the development of heart disease. This type of patient should benefit from the many recent advances made in treating heart disease. One of these involves advances made in anti-coagulant therapy. The Hot Topics article by Alda Huqi describes how newer-generation compounds, which includes dabigatran, may replace the use of warfarin as standard anti-coagulant therapy.
The mechanisms by which cancer cytotoxic agents result in cardiotoxicity are multifactorial. The Basic Article by Christian Zuppinger describes potential mechanisms as to how some of these cytotoxic chemotherapies for cancer may exert their cardiotoxic effects. The Main Clinical Article by von Klemperer and Fox also nicely highlights what cardiac manifestations these cancer chemotherapies can exert on the heart. This includes both the acute and chronic cardiac dysfunction that can result from the use of these cancer therapies. In addition, this article also describes how to identify and detect this cardiac dysfunction.

Detecting and monitoring cardiac dysfunction in cancer patients can involve a number of diagnostic methods including the use of echocardiography, radionuclide ventriculography, cardiac magnetic resonance imaging, and the use of biomarkers. Positron emission tomography (PET) has long been an important tool in detecting cancers, particularly by monitoring glucose uptake by the cancer cell. Indeed, as described in the Refresher Corner article by Jagdip Jaswal, cancer cells display a phenomenon called the Warburg effect, in which rapidly proliferating cells have enhanced glycolytic rates, which is uncoupled from the subsequent oxidation of the glucose. This increased uptake of glucose by cancer cells has been successfully exploited using PET to detect tumors. Use of PET to image glucose uptake (using $^{18}$F-deoxyglucose) is an important tool for detecting tumors. In addition, however, PET can also be used to image the heart, and potentially to diagnose cardiac abnormalities. The Metabolic Imaging article by Mukesh Pandey, Aditya Bansal, and Timothy DeGrado describes some novel fatty acid oxidation imaging agents that can be used to assess fatty acid oxidation in the failing heart. These authors also describe how these PET imaging agents may also be useful in detecting tumorigenic cells.

As mentioned above, rapidly proliferating cancer cells have a distinct switch in metabolism, away from mitochondrial oxidative metabolism and towards glycolysis. The decrease in mitochondrial oxidative metabolism may actually decrease apoptosis and promote survival of the tumorigenic tissue. The New Therapeutic Approaches article by Peter Dromparis, Gopinath Sutendra, and Evangelos Michelakis describes a potentially exciting new approach to treating cancer, which involves promoting mitochondrial oxidation of the pyruvate derived from the accelerated glycolysis seen in tumorigenic tissue. These authors have pioneered a new metabolic strategy for treating cancer that involves the stimulation of pyruvate dehydrogenase (PDH), using the metabolic agent dichloroacetate [3]. By stimulating PDH, glycolysis is better coupled to glucose oxidation in the tumor cell, resulting in an effective decrease in tumorigenesis. Another approach to stimulating PDH is to inhibit mitochondrial fatty acid oxidation, which indirectly results in an increase in mitochondrial PDH activity. The Focus on Trimetazidine (Vastarel® MR) article by Alda Huqi describes the potential use of the fatty acid oxidation inhibitor trimetazidine to treat cancer. The potential advantage of this therapeutic approach is that trimetazidine may also decrease the severity of cardiac toxicity in cancer, as a number of studies have shown that trimetazidine has efficacy in treating heart failure.

In summary, cardiac dysfunction is an important consideration when deciding on the best therapeutic approach to treat cancer. This calls for new relationships to be developed between the oncologist and the cardiologist in detecting, treating, and preventing cardiac dysfunction associated with the new cancer treatments. The development of novel therapies to treat cancer that involve metabolic modulation of the tumor cell may be one approach effectively treating cancer, while lessening the possibility of cardiac dysfunction.

References

Cardiotoxicity in cancer therapeutics

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Abstract
It has been increasingly recognized that cardiac disease is a comorbid condition for cancer patients that deserves special attention. Although the primary goal of cancer therapy remains to cure the life-threatening disease, it is also important to maintain the highest possible quality of life, especially since more cancer patients survive either without cancer or with cancer as a manageable, chronic disease. Cardiovascular complications of anticancer therapies have been known for quite some time, particularly with cytotoxic therapies such as anthracyclines used at higher cumulative doses and after mediastinal radiotherapy. Frequently, cancer cells are, or become by mutation, dependent on the activity of specific tyrosine kinases, which promote proliferation and survival. In addition, when tumor size reaches a critical extent, the cancer cells depend on vascularization for further growth and metastasis. The advent of targeted monoclonal antibodies and tyrosine kinase inhibitors has revolutionized the treatment of several types of malignancies. However, many of these targeted growth and survival pathways are also important for the homeostasis of non-cancerous tissue including the myocardium, and modulators may affect protein synthesis and degradation, energy production, calcium handling and tissue integrity.

Keywords: cancer therapy, cardiotoxicity, anthracyclines, cell death, mitochondria

Introduction
Cardiac toxicity associated with cancer therapies can range from asymptomatic subclinical abnormalities, including electrocardiographic changes and temporary left ventricular ejection fraction decline, to life-threatening congestive heart failure or acute coronary syndrome. Delayed toxicities may be less relevant for the acute cancer treatment, but much more so in the neo- and adjuvant setting where the overall duration of treatment can measure several years. Progress has been made in reducing the side effects of oncological treatment, for example by giving an iron chelator for the inhibition of free radicals, by improvements in imaging technologies for radiotherapy, or with different treatment schedules and drug formulations such as liposomes in order to target the therapy more precisely to the tumor [1]. Meanwhile, a large number of molecular targets for new anticancer-therapies have been developed [2].

Cardiotoxicity of cytotoxic chemotherapies
Cytotoxic chemotherapeutic drugs can be divided in anthracyclines, alkylating agents, antimetabolites, microtubule targeting agents, and others. Some of these drugs were discovered as naturally occurring compounds in plants or microorganisms, and they generally lead to growth inhibition and eradication of rapidly dividing cells. Anthracyclines are still widely used for many malignancies, despite their well-described cumulative cardiotoxicity, which is often
irreversible, and can lead to congestive heart failure [1, 3]. While there are a number of discrepancies in studies on anthracycline effects in cardiomyocytes regarding the primary mechanism—which could be explained by differences in drug dosage, animal model, and duration of treatment [4]—the formation of reactive oxygen species (ROS), changes in mitochondrial function [5], and finally mismanagement of cellular calcium handling appears to be a common endpoint [1, 6]. The irreversible loss of cardiomyocytes and hypothetical stem cells might also contribute to the late effects of anthracyclines, which can occur many years after treatment and is especially important for the treatment of pediatric patients [7]. Alkylating agents like cyclophosphamide and cisplatin may cause acute symptoms of chest pain, arrhythmias, and cardiac ischemia with elevated enzymes indicative of myocardial infarction after a single dose [8]. As with the anthracyclines, the formation of ROS is proposed as a cellular mechanism for the cardiotoxicity of alkylating agents. For the class of the antimitabolites, it is mainly 5-fluorouracil (5-FU) that is known for the risk of cancer therapy-associated cardiotoxicity. Coexisting coronary heart disease and hypertension frequently worsens the problem [9]. Microtubule-targeting agents, like the taxanes, with paclitaxel as the best-studied example, can lead to bradycardia and other electrical disorders and to thrombosis [8]. The combined use of paclitaxel with anthracyclines enhances their cardiotoxicity [10]. In contrast to other cytotoxic drugs such as the anthracyclines, the effect of paclitaxel on cardiomyocytes does not comprise immediate cell death [11].

Cardiotoxicity of targeted therapies

So-called targeted therapies are usually designed with a molecular target in mind that has already been identified as a modulator of cell division and growth. Ideally, such a target, or family of target proteins, is more commonly found in those cells that have become, for example by genetic mutation, continuously dividing cancer cells. One of the first therapies of this kind, trastuzumab, is based on humanized antibodies against the receptor tyrosine kinase HER2. This receptor is found overexpressed in some tumors, frequently in breast cancer, making the HER2 receptors (corresponding to ErbB2 in rodents) a seemingly ideal target for cancer therapy. However, the use of trastuzumab in combination with anthracyclines was associated with cardiac dysfunction in up to 28% of patients in a pivotal trial [12]. The cardiac dysfunction observed during adjuvant treatment with trastuzumab for up to 2 years seems to be due to an impairment of contractility, rather than loss of cardiomyocytes, and is reversible in the majority of cases [13]. A mechanism without cardiomyocyte loss is also suggested by results from in vitro studies using adult rodent cardiomyocytes [11, 14]. The experience with trastuzumab and the experiments using neuregulin-1 beta in basic cardiovascular research has led to the hypothesis that survival factors such as the endothelium-derived neuregulin-1 beta, which binds to the heterodimer ErbB2/ErbB4 in cardiomyocytes, play an important role for myofibrillar integrity, contractile function and metabolic homeostasis in the myocardium challenged by cytotoxic cancer therapies and in general in stress conditions [14, 15].

The other and larger group of targeted therapies is tyrosine kinase inhibitors (TKIs), which bind to the ATP pocket of tyrosine kinases, either to receptors or intracellular kinases. Many new TKIs are currently in development and in early clinical trials. Among them are inhibitors of the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathways that are also important in the cardiovascular system.
system. Careful cardiac monitoring seems warranted as these trials proceed [2]. Some TKIs are multi-targeted, i.e., less specific for a single protein kinase. The premise of less selective TKIs is that one or more of these additional targets may also play a role in disease progression and its inhibition will lead to better anticancer efficacy [16].

Antiangiogenic targeted therapies are currently used in clinical practice in the form of antibodies, such as the anti-vascular endothelial growth factor (VEGF)-A therapy bevacizumab, or as TKIs such as sorafenib and sunitinib. Sunitinib inhibits, among others, VEGF receptor 1–3, c-Kit, platelet-derived growth factor receptor (PDGFR)-A/B, rearranged during transfection (RET), FMS related tyrosine kinase 3 (FLT3), and colony stimulating factor 1 receptor (CSF1R) [16]. The mechanism for the observed cardiotoxicity of sunitinib is probably related to the inhibition of 5’ adenosine mono phosphate-activated protein kinase (AMPK) in cardiomyocytes and can lead to cell death at micromolar concentrations in vitro [17, 18]. We and others have observed changes in

Fig. 2 Some myocardial targets of cancer therapies and potentially cardioprotective signaling pathways in cardiomyocytes and as part of the paracrine signaling of microvascular endothelial cells and cardiomyocytes. Green arrows show therapeutics that potentially interfere with signaling components in the heart, although mentioning a certain therapy does not imply clinically relevant cardiotoxicity. 

Top left: Cytotoxic therapies and other stressors often lead to oxidative stress in cardiomyocytes and can cause protein degradation and cell death.

Top right: Microvascular endothelial cells, among others, release factors that enhance protein synthesis and survival under stress conditions. Neuregulin and VEGF-A are examples, activating the MAPK and PI3K signaling pathways in cardiomyocytes.

Bottom: Gene expression depends on transcription factors, which are also targets. GATA-4 drives the expression of antiapoptotic mitochondrial proteins Bcl-2 and Bcl-XL, but is inhibited by doxorubicin, and HIF-1 alpha is important for VEGF-A production in hypoxia, but is inhibited by new targeted and antiangiogenic therapies.
mitochondrial function by sunitinib in different cell types, as visually demonstrated with the cationic dye JC-1, that selectively enters into mitochondria and reversibly changes fluorescence emission color from red to green as the membrane potential decreases (Fig. 1). Antiangiogenic therapies could also have an impact on the heart by the induction of microvascular dysfunction and/or the reduction of microvesSEL density. However, pathologic changes seen in cultured cells and small rodents do not necessarily translate into clinically significant cardiac toxicity, and the described mitochondrial effect of sunitinib is not a common feature in TKIs with a similar inhibition spectrum [19]. It is important to note, that cardiotoxicity is not a class effect of TKIs, and it is reasonable to assume, that only those therapies targeted at essential kinases in the myocardium and in the vasculature will show cardiotoxicity. Nevertheless, the combined use of therapies, which have not shown significant cardiotoxic potential in single use, could create a potentially harmful and unexpected double-hit on the myocardium.

Conclusions
Cardiovascular side effects of the new signaling inhibitors appear to be particularly relevant in patients with preexisting cardiovascular conditions and risk factors, since they depend on survival pathways that now become targets in modern oncology. Sometimes, it might even appear as oncologists and cardiologists have opposing interests, since most of the cellular factors discussed in recent years for a role in cardioprotection are on the list of new targets under study for their use in anticancer therapy (Fig. 2). On the contrary, given the unique problems presented by the cancer patient with cardiovascular disease, multidisciplinary working among cardiologists, oncologists and cell biologists should be encouraged [20]. Cardiovascular side effects of cancer therapeutics may become a manageable problem in future if we are able to learn more about the role of signaling pathways in the myocardium. Still, this topic will continue to be topical in the years to come.

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References
Assessing and monitoring cardiac function in cancer patients

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Abstract
The use of chemotherapy is associated with improved survival rates for patients with neoplastic disease. Cardiac toxicity is a feature of several therapeutic agents (e.g., anthracyclines) and patients require careful monitoring during treatment. The adverse effects of chemo- and radiotherapy may not be apparent for some time post completion of treatment and patients may require long-term follow-up by oncologists and cardiologists. In this invited article we review the therapies associated with significant cardiac toxicity and provide guidance on the assessment of patients during chemotherapy and during chronic surveillance.

Keywords: cancer, chemotherapy, heart failure, cardiotoxicity

Introduction
Progress made in screening and therapies have greatly reduced morbidity and mortality due to cancer over the past few decades. In 2006, there were 1.13 million cancer survivors in the United Kingdom who were up to 10 years from diagnosis [1]. Some of these therapies do however carry a significant risk of cardiotoxicity and after-cancer recurrence; cardiac events are the most common cause of death in these patients. While coronary ischemia, arrhythmia, thromboembolism, and QT prolongation have all been noted as acute complications of specific chemotherapies (Table 1) [2], in this review we focus on cardiomyopathy due to cancer therapy.

The mechanisms of left ventricular (LV) dysfunction have been extensively studied, yet few prospective clinical studies exist and there is a lack of consensus and guidelines regarding best practice in terms of assessing, monitoring, and managing these patients. The risk-benefit calculations are therefore challenging for the oncologists and cardiologists involved in their care.

Chemotherapy agents

Anthracyclines (doxorubicin, epirubicin, idarubicin)
Anthracyclines are recognized as highly efficacious and are still one of the most commonly used chemotherapies in treating adult solid organ and hematological tumors. They are also renowned for causing a dose-dependent and progressive toxic cardiomyopathy [3].
There are three distinct types of cardiotoxicity associated with anthracyclines:

- Acutely (<1%) or subacutely, they can cause a pericarditis-myocarditis syndrome, or acute left ventricular failure (usually reversible).
- A chronic, progressive cardiomyopathy can present within the first year following the start of treatment.
- Late-onset cardiomyopathy can manifest years to decades after anthracycline treatment. In the pediatric population, the effects are often not seen until up to 25 years post-chemotherapy.

We know from a large experience of endomyocardial biopsies that anthracyclines destroy myocytes. There have been considerable efforts into finding ways to identify those at risk and protect the myocardium from anthracyclines. Identified “risk factors” are: underlying cardiovascular disease, pregnancy, hypertension, pre-existing cardiac disease, age >65 or <18 at time of therapy initiation, combination chemotherapy, radiation therapy, intravenous bolus administration, high cumulative dose [4, 5]. Retrospective analyses suggest that doxorubicin cardiotoxicity occurs at dosages considerably lower than first appreciated: the cumulative dosage that correlated with a 5% incidence of heart failure ranged between 400 and 450 mg/m² [6].

Attempts to minimize the cardiotoxicity of anthracyclines include dose limitation, schedule modification, use of less cardiotoxic analogues, and use of cardioprotective agents. There has been some evidence that adding dextrazoxane (a free radical scavenger) can be cardioprotective [7]. There are, however, concerns that it may interfere with the antitumor effect of the anthracyclines and the American Society of Clinical Oncology currently advises against its routine use [8].

### Alkylating agents (cyclophosphamide, ifosfamide)

Acutely, cyclophosphamide has been associated with a hemorrhagic myocarditis. It has also been associated with heart failure in up to 28% of patients. Cardiotoxicity has been shown to be dose related, irreversible and typically manifests within 2 weeks of therapy [9]. Risk factors for cardiotoxicity include previous anthracycline use and previous radiotherapy.

### Humanized monoclonal antibody based tyrosine kinase inhibitors (bevacizumab, trastuzumab)

Cancer patients receiving trastuzumab (Herceptin®) are a relatively new population (it was first approved by the United States Food and Drug Administration in 1998). It is indicated in combination with paclitaxel for first-line treatment and as a single agent for second- or third-line treatment of metastatic breast cancer. Approximately 25% of women with breast cancer will have a tumor overexpressing human epidermal growth factor 2 (HER2) and addition of trastuzumab to adjuvant

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**Table 1**: Incidence of cardiac complications of commonly used chemotherapies. A summary of the major chemotherapeutic classes and their estimated incidence of cardiac complication based on meta-analysis (modified from Yeh and Bickford [2]).

<table>
<thead>
<tr>
<th>Therapy</th>
<th>LV dysfunction</th>
<th>Ischemia</th>
<th>Prolonged QT</th>
<th>Arrhythmia</th>
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<td><strong>Anthracyclines</strong></td>
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<tr>
<td>Doxorubicin, epirubicin, idarubicin</td>
<td>&gt;5% incidence</td>
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<td><strong>Alkylating agents</strong></td>
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<tr>
<td>Cyclophosphamide, ifosfamide</td>
<td>&gt;5% incidence</td>
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<td><strong>Humanized monoclonal antibodies</strong></td>
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<td>Trastuzumab, bevacizumab</td>
<td>&gt;5% incidence</td>
<td>&lt;2%</td>
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<td><strong>Small molecule tyrosine kinase inhibitors</strong></td>
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<td>Sunitinib, dasatinib, lapatinib</td>
<td>2-5% incidence</td>
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<td>&gt;5% incidence</td>
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<td>Erlotinib, sorafenib</td>
<td>2-5% incidence</td>
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<td>&gt;5% incidence</td>
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<td><strong>Radiotherapy</strong></td>
<td>&gt;5% incidence</td>
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<td><strong>Antimicrotubule</strong></td>
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<td>5-Fluorouracil, capcitabine</td>
<td>&gt;5% incidence</td>
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<td><strong>Antimetabolites</strong></td>
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<td>Paclitaxel</td>
<td>2-5% incidence</td>
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<td>&gt;5% incidence</td>
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<td><strong>Proteosome inhibitors</strong></td>
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<tr>
<td>Botezomib</td>
<td>2-5% incidence</td>
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therapy has been shown to reduce their risk of recurrence at 3 years by half and improve survival by approximately a third [10]. It has also recently been approved for use in HER2-positive metastatic stomach cancer.

The pivotal trials of trastuzumab observed an unexpected risk of cardiac dysfunction. Patients with breast cancer are commonly treated with both trastuzumab and an anthracycline, which poses the problem of isolating effects of either agent from a potential synergistic interaction. Cardiotoxicity has however been shown to be rare in patients on trastuzumab alone.

Endomyocardial biopsy specimens evaluated post-trastuzumab exposure have not shown any changes that suggest significant myocyte destruction [11]. HER2 receptors exist in the heart and it is thought the HER2/erbB2 system may modulate the effects of oxidative stress in the cardiac myocyte. Some postulate that these drugs interfere with myocyte metabolism and, in the patient with impaired contractile reserve due to previous or concomitant anthracycline or alkylating agents, this may manifest as deterioration in cardiac function.

Unlike anthracyclines trastuzumab rarely causes death, is not cumulatively dose related, and the majority of patients who developed trastuzumab-related congestive heart failure and discontinued trastuzumab had recovery of left ventricular ejection fraction (LVEF) with subsequent follow-up [12]. The long-term effects of these drugs remain to be seen.

Small molecule tyrosine kinase inhibitors (lapatinib, imatinib, sumatranib)
The cardiac safety of lapatinib was recently evaluated in the 3,689 patients enrolled in phase I to III lapatinib clinical trials. Of these patients, 1.6% experienced a cardiac event. In patients treated with prior anthracyclines, trastuzumab, or neither, the incidence of cardiac events was 2.2%, 1.7%, and 1.5%, respectively. The mean time to onset of cardiac events was 13 weeks [13].

Radiotherapy
Mediastinal radiation potentially leads to inflammation and progressive fibrosis of all of the heart structures. It is known to cause pericardial disease, accelerated coronary artery disease and has been implicated in conduction disease although causality is difficult to establish. In comparison to the systolic dysfunction seen with chemotherapy these patients have been shown to develop a restrictive cardiomyopathy. The occurrence and manifestation of radiation-related cardiac disease depends mostly on radiation dose, volume of the heart exposed, and specific radiation delivery techniques used. A study of 4,122 patients found the relative risk of cardiac death was 12.5 at radiation doses of 5-15 Gy, and 25.1 at >15 Gy [14]. The Oxford meta-analysis of randomized trials also demonstrated an increased risk of ischemic heart disease [15], however the majority of the patients included in these studies would have been exposed to large volumes of radiotherapy and modern radiation therapy techniques (used since the mid 1980s) with three-dimensional planning, computed tomography guided therapy and lower radiation doses seem to have significantly reduced this risk [16].

Defining and detecting cardiac dysfunction
In order to assess and monitor these patients we have to identify how we recognize and define “cardiac dysfunction”. When the pivotal trials of trastuzumab established that there was associated cardiotoxicity [17], a cardiac review committee established the following criteria to identify cardiac dysfunction:

1. A decrease in cardiac LVEF that was either global or more severe in the septum.
3. Associated signs of heart failure.
4. Decline in LVEF of at least 5% to less than 55% with accompanying signs or symptoms of congestive heart failure, or a decline in LVEF of at least 10% to below 55% without accompanying signs or symptoms.

Initial assessment
Currently in most institutions it is the oncologists who are assessing these patients. Ideally prior to antineoplastic therapy, they will conduct a detailed baseline assessment of functional capacity, known cardiac history as well as risk factors. Notably many of the symptoms of cancer can be difficult to distinguish from cardiac symptoms (chest pain, breathlessness, leg swelling). Oncologists should identify patients at particular risk of cardiotoxicity so that the choice of therapy and risk factors may be optimized and they can be monitored more vigilantly.

Prior to starting therapy, one needs a baseline ECG and study of cardiac function. The most common non-invasive method of monitoring myocardial toxicity has been the assessment of LV systolic function, with either
radionuclide ventriculography or echocardiography. The two measurements are not interchangeable; the same technique should be used for serial measurements. Where possible, this should also include the same operator, machine, and calculation algorithm.

After baseline assessment, patients with LV dysfunction or modifiable cardiac risk factors should be referred for cardiology review. There is some trial evidence for the efficacy of ACE inhibitors in preventing the decrease in LVEF observed in patients after high-dose chemotherapy [18]. The regime of chemotherapy might be modified accordingly.

In the United Kingdom, the National Institute for Health and Clinical Excellence recommends cardiac functional assessments should be repeated every 3 months during trastuzumab treatment. If the LVEF drops by 10% or more from baseline and to below 50%, trastuzumab treatment should be suspended. A decision to resume trastuzumab therapy should be based on a further cardiac assessment and a fully informed discussion of the risks and benefits between the individual patient and his or her clinician [19]. There are consensus guidelines for anthracyclines that adhere to a similar protocol, however they advocate long-term follow-up on a yearly or two-yearly basis depending on the cumulative dose of chemotherapy given and the risk profile of the patient.

Methods of monitoring

MUGA
Radionuclide ventriculography or multiple gated acquisition scan (MUGA) is frequently used. In its favor, it is less subject to observer variability [20] than echocardiography, however it does involve exposure to radiation, is associated with higher cost and does not give us any information other than ejection fraction in comparison to echocardiography, which may identify pericardial or valvular disease as well. Choice between echocardiography and MUGA is based on provider preference or regional practice.

Echocardiography
Echocardiography is widely available and relatively inexpensive and hence is often the initial investigation of choice for evaluating systolic function. While disputed by some, ejection fraction is currently the standard method of screening for cardiotoxicity. Poor quality echo images due to surgery and inter-observer variability may confound these measurements. Contrast or 3D echocardiography might improve reproducibility, and the laboratories performing such studies should have evidence that they can reliably and reproducibly identify a 10% change in EF as a true change [21].

Many studies have aimed to identify more sensitive indicators of early cardiotoxicity, including Tei Index, tissue Doppler, and myocardial strain [22, 23]. Several have suggested that diastolic dysfunction is an early sign of anthracycline-induced cardiac dysfunction. These are generally small studies and further validation is necessary [24–26].

Other methods currently being evaluated
While most of the methods currently used do monitor for deterioration in systolic dysfunction, there is an argument that cardiac dysfunction is not best appreciated by measurements of systolic function. It may be preceded by asymptomatic or sub clinical cardiac dysfunction undetectable at bedside evaluation and/or defined by abnormalities measured by standard non-invasive imaging techniques.

Dobutamine stress echo
It is known that patients with mild and sub-clinical deterioration of systolic LV function can compensate for a decreased cardiac output with a number of adaptive mechanisms, i.e., increasing preload, heart rate, and contractility during stress. Dobutamine stress echo is an established method of assessing contractile reserve in ischemic and valvular heart disease. It is a generally well-tolerated test, which can be repeated. It does however require a less widely available skill set and its role in assessing patients on chemotherapy is less well established [27]. No studies have evaluated the benefits of non-invasive evaluation of asymptomatic cancer survivors for cardiac dysfunction. Yeung et al tested 29 children, 19 of whom had received anthracyclines up to 6 years earlier, with exercise echocardiography. They found an average increase in fractional shortening of 3% in the anthracycline group compared with an average increase of 23% in the control group, although fractional shortening at rest was normal in all participants [28]. This raises the question of how we would interpret and act on such findings.
CMR
Cardiac magnetic resonance imaging (CMR) has the benefits of low intra/inter-observer variability and high test-retest reproducibility. It also has the ability to detect early myocardial damage. Wassmuth et al have demonstrated that CMR may be useful at detecting the sub-clinical effects of chemotherapy. Increased contrast enhancement on day 3 predicted significant decline at 28 days [29]. It could be useful in patients with poor echo pictures and in fact as more CMR services become available, it may serve as a simple non-invasive tool in following up patients post chemotherapy.

Biomarkers
It is well recognized that cardiac dysfunction may become evident weeks, months or years after high-dose chemotherapy. The possibility of developing an early marker of myocardial injury would allow us to appropriately monitor those at higher risk. Troponin and BNP have both been explored in detecting cardiac damage and risk stratifying patients. Cardinale et al demonstrated that troponin I is a sensitive and specific marker for predicting the development and severity of ventricular dysfunction. In one study of 703 patients, elevation of troponin I levels 0–72 hours after chemotherapy administration and at 1 month, predicted a late decline in LVEF and a composite of cardiac events [30]. Cardinale also demonstrated a strong relationship was also found between NT-proBNP value at 72 hours, and LVEF changes at 12 months. While these initial studies of biomarkers as a screening tool are promising the numbers are small and more work in this area is needed for further validation.

Conclusion
Patients with cancer have a substantial risk of developing heart disease as a result of chemo- and radiation therapy. Cardiotoxicity, not only negatively affects the cardiac outcome of patients with cancer, but also largely reduces the range of suitable therapies. The optimal interval and duration as well the cost effectiveness of cardiac monitoring remain undefined. That being said, from the evidence and consensus guidelines we do have it is possible to design sensible monitoring protocols. These need to be clearly defined and regular quality assurance is key. The process of assessing and following up these patients needs to be systematic not only to avoid missing pathology, but also for future research purposes. Most importantly where cardiac dysfunction is identified, there needs to be a clear avenue for communication and collaboration between oncologists and cardiologists within a multidisciplinary cancer team.

References
Fluorine-18 labeled thia fatty acids for PET imaging of fatty acid oxidation in heart and cancer

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Abstract
Myocardial fatty acid oxidation (FAO) imaging is a noninvasive technique that can measure FAO rates in tissues for research applications in animals and humans, as well as clinical applications in managing patients with metabolic disorders. FAO imaging has great potential in diagnosis and monitoring of patients with ischemic heart disease, cardiomyopathies, myocarditis, acute coronary syndrome, and heart failure. Applications of FAO imaging in oncology and endocrinology are also highly anticipated. For over 20 years, our laboratory has investigated fluorine-18 labeled thia-substituted fatty acid analogs as positron emission tomography (PET) probes of myocardial FAO. These FAO probes share a common design motif of metabolic trapping in the myocardium subsequent to their commitment to the mitochondrial FAO pathway, in analogy to the design of 2-[18F]fluoro-2-deoxy-D-glucose (FDG) as a metabolically trapped probe of glucose transport and phosphorylation. This mini-review describes the development of these FAO probes, from the seminal 6-thia substituted analog, 14-[18F]fluoro-6-thia-heptadecanoate (FTHA), to the most recently developed oleate-based FAO analog, 18-[18F]fluoro-4-thia-oleate (FTO). It is shown that small changes in thia fatty acid analog structure can exert profound differences in the biodisposition and specificity of these probes to indicate myocardial FAO, particularly in conditions of oxygen deprivation. The potential of these probes for imaging of FAO in cancer is supported by initial uptake studies in cultured cancer cells. Thus, 18F-labeled thia fatty acid analogs have significant potential to play an important role as clinical PET probes of FAO in cardiovascular diseases, oncology, and future anticipated applications in endocrinology and neurology.

Keywords: PET; fatty acid oxidation; thia fatty acid; FTHA; FTP; FTO

Introduction
There remains a great demand for advancements of molecular imaging techniques to allow noninvasive assessment of biochemical processes for the metabolic characterization of human diseases. Positron emission tomography (PET), and to a lesser extent, single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI) can provide important information on ion and metabolic fluxes in human tissues in a noninvasive and quantitative manner. Energy metabolic pathways in heart and tumors are sensitively regulated and immediately reflect any dietary and hormonal changes or abnormalities. Thus, monitoring of
substrate metabolism provides substantial information about the disease state and therapeutic progress. The major energy producing substrates are fatty acids (derived from plasma nonesterified fatty acids or local lipolysis from circulating lipoproteins), glucose, lactate, and ketone bodies. The glucose-based PET probe, FDG, has already set a benchmark in clinical care by indication of glucose utilization in tissues exhibiting high glycolytic rates, including a broad spectrum of cancer types [1] and ischemic but viable myocardium [2]. Fatty acid-based SPECT probes are currently in routine practice in Japan, but not in the United States. 11C-labeled fatty acid PET radiotracers have been in use for over 35 years, but have been mainly confined to research studies due to their short physical half-life (20.4 m) and the complex nature of the quantitation of the dynamic PET images [3–7]. Quantitation of fatty acid oxidation (FAO) rates is not possible in ischemic myocardium because the diffusion rates of the oxidation product 11CO2 and unoxidized 11C-palmitate are both rapid, thereby confounding the model fit [6, 7]. However, 11C-labeled fatty acid probes have value to indicate accumulation of exogenous fatty acids in the myocardial triglyceride pool, because this component is characterized by a slow turnover. Over the last 20 years, our group has been involved in the development of 18F-labeled thia fatty acids as metabolically trapped probes of FAO. The half-life of 18F (110 minutes) allows for regional distribution of probes, while the presence of the sulfur heteroatom blocks the β-oxidation of the fatty acid and also renders the molecule as a poor substrate for incorporation into complex lipids [8, 9]. In this mini-review, we intend to highlight the incremental development and promises offered by thia fatty acids as potential probe molecules for FAO imaging in heart and cancer.

**14-(R,S)-[18F]fluoro-6-thia-heptadecanoic acid (FTHA)**

FTHA (Fig. 1) was the first-generation thia fatty acid probe synthesized in 1990 and evaluated as a PET probe of fatty acid metabolism in mice [8–9]. After intravenous administration of FTHA, the probe was rapidly taken up by tissues as evident from its rapid blood pool clearance. The heart showed highest uptake of 39.8±3.0%ID/g at 5 minutes and subsequently cleared with a biological half-life of about 2 hours. The heart:blood ratios of uptake were 4.3±0.4, 20±6, 41±6, and 82±16 at 0.25, 1, 5 and 60 minutes respectively. The myocardial trapping of 18F-radioactivity was drastically reduced by pretreatment of mice with the carnitine palmitoyltransferase-1 (CPT-1) inhibitor POCA, showing CPT-1 dependent uptake of FTHA. FTHA has shown high myocardial uptake, longer retention and rapid clearance from the bloodstream in humans also, making it a useful tracer of fatty acid metabolism for PET [9–10]. In a mechanistic study, the net retention of FTHA was depressed in ischemic porcine myocardium, but remained unchanged in hypoxic myocardium [11]. The lower retention in ischemia confirmed the applicability of FTHA for imaging ischemic myocardium [11], but the lack of sensitivity to lower FAO rates in hypoxic myocardium motivated further tracer development to improve specificity to monitor FAO rates. Nevertheless, FTHA continues to be the most-investigated thia fatty acid PET probe, and has been used as a fatty acid uptake probe in human studies in heart [12–15], liver [16], skeletal muscle [12], and brain [17].

**16-[18F] fluoro-4-thia-palmitate (FTP)**

The lack of correspondence of FTHA trapping to FOA rates in hypoxic myocardium was the driving force to develop a second-generation thia fatty acid analog. In 2000, the palmitate-based analog, 16-[18F] fluoro-4-thia-palmitic acid (FTP, Fig. 1), was identified as a FAO probe [18]. FTP was evaluated in a rat model with varying dietary conditions: fed (30 minutes), fasted (30 minutes), fasted (120 minutes), and fasted (30 minutes with CPT-I inhibitor “etomoxir”). The highest uptake was observed in heart, liver and kidneys, regardless of
dietary status [18]. The new tracer FTP was also evaluated in Langendorff perfused rat heart to study the kinetics and relationship of tracer retention to FAO rate in normoxic and hypoxic myocardium. FTP trapping in the rat myocardium correlated well with [9,10-\(^3\)H]palmitate oxidation rates in both normoxic and hypoxic conditions. In the same model, the myocardial accumulation of the 6-thia fatty acid analog, 17-[\(^18\)F]fluoro-6-thia-heptadecanoate, was insensitive to the decrease in palmitate oxidation rate in hypoxic hearts. Thus, the placement of the thia-substituent at the fourth position of the fatty acid analog significantly improved the specificity of the probe for indication of FAO. It was speculated that 4-thia fatty acid analogs have a more rapid turnover in pools of FAO intermediates (i.e., acyl-CoAs and acyl-carnitines) than 6 thia fatty acid analogs that allow these pools to clear the myocardium if they are not further metabolized by mitochondrial \(\beta\)-oxidation. FTP showed high myocardial uptake and retention in porcine heart, consistent with metabolic trapping seen in rats [18]. Detailed mechanistic studies of FTP uptake in isolated rat hearts demonstrated FAO dependent metabolic accumulation that was in proportion to FAO rates as measured by [9,10-\(^3\)H]palmitate oxidation [18]. The ratio of proportionality, which we denoted as the “lumped constant (LC)” in good analogy with FDG modeling methodology, was decreased in hypoxic conditions, suggesting somewhat different affinities of FTP and palmitate at transport and/or metabolic control points of fatty acid disposition within the myocyte [19]. However, changes in the fatty acid composition of the perfusion medium did not influence the LC, which may allow FTP to be a prototypical fatty acid analog for indication of overall FAO [19]. The only major shortcoming observed with FTP during these studies was its lower retention over time in rat myocardium: myocardial clearance was about 70% at 2 hours relative to 30 minutes [18].

18-[\(^18\)F] fluoro-4-thia-oleate (FTO)

The suboptimal myocardial retention of FTP in rat myocardium prompted the development of the oleate-based probe 18-[\(^18\)F]fluoro-4-thia-oleic acid (FTO, Fig. 1) [20]. FTO was recently synthesized and evaluated in rats with and without CPT-1 inhibition [20]. FTO was motivated from oleate’s relative abundance in plasma, and a clinical study showing that dietary oleate had a somewhat higher disposition relative to palmitate toward whole-body FAO [21]. Synthesis of the labeling precursor for FTO required an 11-step synthetic process due to the inclusion of 18-bromo leaving group, 9-cis-double bond and the 4-thia substituent [20]. FTO showed excellent myocardial imaging characteristics and superior myocardial retention than the previously developed tracers FTHA, and FTP. FTO showed three- to four-fold higher heart: background tissue radioactivity ratios than FTP. FTO uptake by heart was approximately reduced to 80% by pretreatment of CPT-1 inhibitor etomoxir indicating high dependence on CPT-1 mediated mitochondrial transport. The microPET images of FTO accumulation in the rat myocardium were clearly superior to those with FTP [20]. The folch-type extraction analysis showed 70–90% of the \(^18\)F-radioactivity to be protein-bound in heart, liver and skeletal muscle. These values were significantly higher than those for FTP and FTHA, suggesting higher specificity of mitochondrial trapping. The preliminary data with FTO indicate this probe to be the most specific myocardial FAO probe to date based on the thia fatty acid concept.

**Mechanism of metabolic trapping of thia fatty acids**

Based on our findings with FTHA, FTP and FTO, we propose a plausible mechanism for the uptake and metabolic trapping in myocardium of terminally \(^18\)F-labeled 4-thia fatty acid analogs, using FTO as an example (Fig. 2). FTO enters into the cardiomyocyte via fatty acid transporters (CD36:FATP (fatty acid transporter protein) [22]. In the cytosol, FTO is esterified to FTO-CoA by fatty acyl-CoA synthase (FACS). FTO-CoA is then transferred to carnitine via CPT-I. The acyl carnitine is then shuttled across the inner
mitochondrial membrane where it gets converted back to FTO-CoA by CPT-2. In the mitochondrial matrix, FTO-CoA may undergo two subsequent steps of β-oxidation, forming the 3-hydroxy acyl-CoA moiety, and then spontaneously decomposes to a long-chain thiol, 14-[18F]fluoro-tetradecane-1-thiol, which in turn covalently or noncovalently binds to various mitochondrial proteins. Differential centrifugation identified the mitochondrial fraction as containing the predominant amount of retained 18F-radioactivity, while native-gel electrophoresis of heart extracts showed that the 18F-radioactivity was associated with a broad spectrum of molecular weights, evidencing the non-specific nature of the protein binding (unpublished data). Thus, the accumulation of protein-bound 18F-radioactivity in tissue is a direct readout for FAO of exogenous fatty acids. Slow clearance processes remain unclarified, particularly for FTHA and FTP. Two likely mechanisms of clearance are slow releases of carnitine esters and/or β-oxidation metabolites (Fig. 2, dotted arrows).

**FAO imaging in cancer**

It is well recognized that not all tumor cell types utilize glucose as primary energy substrate. FAO can provide for a predominant fraction of ATP production in tumors that have sufficient oxygen supply, such as prostate cancer [23]. Indeed, the low rate of glycolysis in early-stage prostate cancer severely limits the applicability of the FDG-PET method to staging of patients with newly diagnosed disease. We have performed a preliminary study of the uptake of [18F]FTP in cultured 9L rat glioma, LNCaP human prostate and PC-3 human prostate cancer cell lines [24] (Fig. 3). FTP was taken up avidly by the cancer cells. Hypoxic incubation resulted in an increase in overall uptake, consistent with AMP-kinase activation of fatty acid transport [22]. Folch-type extractions were performed to indicate the FAO-dependent metabolic incorporation of 18F-radioactivity into protein. The LNCaP cell line, which is derived from well-differentiated, androgen-dependent prostate cancer, showed the highest fractionation of FTP into the protein-bound phase, corroborating the findings of high levels of FAO expressed in early-stage prostate cancer [23]. 18F-labeled straight-chain and β-methylated fatty acids have been shown to be taken up in rat tumor models [25, 26], however these agents do not metabolically trap in a FAO-dependent manner. To our knowledge, PET imaging studies in cancer patients with fatty acid analogs have yet to be done, but we believe there exists considerable potential.
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Abstract
The Warburg effect refers to the cancer’s metabolic shift from mitochondrial oxidation to anaerobic glycolysis, even when oxygen is present. Although this metabolic anomaly was long believed to be due to irreversible mitochondrial damage, it now appears mitochondria in cancer are actively suppressed. Since mitochondria are extensively integrated into the metabolic, signaling and apoptotic biology of the cell, mitochondrial suppression in combination with glycolysis-driven metabolism allows cancer to masterfully inhibit intrinsic cell death mechanisms and promote rapid proliferation even in sub-optimal conditions. In this review, we analyze cancer’s metabolic strategy and describe how active mitochondrial suppression is compatible with both the genetic and evolutionary models of cancer. Furthermore, we discuss several strategies for mitochondrial activation and review current pre-clinical and clinical studies of agents showing promise in effectively and selectively targeting cancer.

Keywords: cancer, metabolic, apoptosis, dichloroacetate, glycolysis

Introduction
We currently perceive cancer as an accumulation of numerous chromosomal, genetic and biochemical abnormalities that result in dysfunctional cells. However, if so extensively damaged, how does cancer escape death, even flourish, in hypoxic and acidic conditions? Perhaps cancer should be viewed as the “healthiest” of cells, developing exquisite mechanisms to suppress cell death (which is mostly regulated by mitochondria) and survive in otherwise uninhabitable microenvironments, essentially achieving what medicine strives for—immortality. One way that cancer could achieve this is by developing strategies to effectively suppress mitochondrial function. Here, we summarize cancer’s metabolic strategies to evade death and discuss potentially effective and selective mitochondria-targeting therapies that induce apoptosis in cancerous but not non-cancerous tissues.

Mitochondria: a central role in cellular biology
Mitochondria produce energy through the oxidation of carbohydrates and lipids. In glucose oxidation (GO), pyruvate is de-carboxylated to acetyl-CoA by the gate-keeping mitochondrial enzyme pyruvate dehydrogenase (PDH). In fatty acid oxidation (FAO), fatty acids enter the mitochondria through carnitine palmitoyl-transferase-1 (CPT-1) and are also converted to acetyl-CoA (Fig. 1). The Krebs’ cycle extracts electrons derived from acetyl-CoA and feeds
Mitochondria are major regulators of apoptosis.

Upon opening of the mitochondrial transition pore (MTP), pro-apoptotic mediators are released into the cytoplasm and ignite the apoptotic process. MTP is a mega-channel that spans the mitochondrial membrane and is both redox- and voltage-sensitive, thus regulated in part by ΔΨm (Fig. 1) [1]. While ΔΨm depolarization promotes MTP opening, hyperpolarization increases its opening threshold, establishing an apoptosis-resistant state. Thus, ΔΨm could also be a surrogate for apoptosis resistance. Furthermore, since ΔΨm depends on metabolism, fuel supply and apoptosis are intrinsically linked. As discussed below, most solid cancers are characterized by hyperpolarized mitochondria compared to non-cancerous tissues, pointing to mitochondria’s critical role in the well-known apoptosis resistance in cancer.

Mitochondria are extensively integrated into cell signaling. In addition to H⁺ extrusion, the ETC also generates superoxide (mitochondria-derived reactive oxygen species – mROS). Unstable mROS, like superoxide, can be dismutated into more stable mROS and leave the mitochondria to regulate redox-sensitive targets like the voltage-gated potassium channels (Kv) in the plasma membrane. As mitochondria are important O₂ sensors, this axis (ETC-mROS-Kv channels) is the basis of hypoxic pulmonary vasoconstriction. mROS also regulate other redox-sensitive targets like the transcription factors p53 [2] and hypoxia inducible factor-1α (HIF1α) [3], important in both vascular diseases and cancer (Fig. 1).

Mitochondria may also signal by releasing metabolic substrates. Alpha-ketoglutarate (αKG) is a Krebs’ cycle intermediate that, once in the cytosol, acts as a co-factor for prolyl-hydroxylases which degrade HIF1α [3]. In other words, this critical transcription factor that drives angiogenesis in both cancer and vascular diseases, is regulated by at least two mitochondria “signals”, mROS and αKG, both of which are linked to fuel processing and mitochondrial respiration.

Mitochondria can also sequester calcium. Being the most negatively charged organelles, they function as Ca²⁺ sinks, in a ΔΨm-dependent manner. Among the myriad of Ca²⁺-dependent signaling processes are transcription factors that are integral in both vascular disease and cancer, like the nuclear factor of activated T-cells (NFAT) [3].

Therefore, suppression of mitochondrial function has the potential to influence a myriad of cellular processes critical in cancer biology. Suppressed...
Mitochondria would increase MTP opening thresholds, suppress mROS, inhibit Kv-channels, and activate HIF1α and NFAT, all promoting a proliferative and anti-apoptotic state.

Mitochondria as integrators of early genetic and environmental signals in cancer

A number of common molecular abnormalities described in cancer have direct metabolic and mitochondrial effects that result in suppressed oxidative phosphorylation and mitochondrial function. For example, p53 loss-of-function, cancer’s most common genetic anomaly, strongly promotes a glycolytic phenotype by upregulating glycolytic enzymes including phosphoglycerate mutase and the rate-limiting enzyme hexokinase (HK) [4]. p53 also regulates Tp53-induced glycolysis and apoptosis regulator (TIGAR), an enzyme with fructose-2,6-bisphosphatase activity [2]. When suppressed, fructose-2,6-bisphosphatase accumulates and potently simulates the rate-limiting glycolytic enzyme phosphofructokinase-1. Moreover p53 regulates the expression of the ETC protein cytochrome-c oxidase and suppresses the expression of glucose transporters (GLUTs) [2]. Thus, as a result of p53 loss-of-function, ATP supply is maintained by elevated cytoplasmic glycolysis and glucose uptake, compatible with the increased 18-fluorodeoxyglucose positron emission tomography (PET) signal in most tumors compared to non-cancerous tissues.

Similarly, activation of Akt (or PTEN loss-of-function) not only enhances the activity of rate limiting glycolytic enzymes but also translocates GLUTs to the cell membrane. c-myc upregulates nearly all glycolytic enzymes including HK [4, 5]. Intriguingly, in addition to regulating carbohydrate metabolism, several cytoplasmic glycolytic enzymes directly suppress apoptosis [5] or even modulate mitochondrial function. For example, activated HK translocates to the outer mitochondrial membrane and binds the voltage-dependent anion channel (VDAC) [6]. This prevents anion efflux contributing to the ΔΨm hyperpolarization that characterizes most cancers [7] and prevents MTP opening [6].

Mitochondrial enzyme mutations also impair mitochondrial function and promote a glycolytic phenotype. The Krebs’ cycle enzymes succinate dehydrogenase (SDH) [8], fumarate hydratase (FH) [9] and isocitrate dehydrogenase (IDH) [10, 11] are all associated with cancers like renal cell carcinoma, paragangliomas, or glioblastomas.

Thus, mitochondria appear to integrate a large number of diverse genetic signals that all result in a common phenotype (i.e., hyperpolarized mitochondria with suppressed function, upregulated glycolysis and secondary signaling consequences) promoting proliferation and suppressing apoptosis. The evolving metabolic theory of cancer that suggests that the mitochondrial and metabolic remodeling promotes a proliferative and apoptosis-resistant state is compatible with the genetic theory of cancer that has dominated the field for forty years but has failed to deliver effective and selective cancer therapies (except few and isolated examples, such as imatinib mesylate).

Actively suppressed mitochondria are also compatible with the evolutionary model of cancer, which suggests that early carcinogenesis is hypoxia-driven [12]. Here, decreased oxygen suppresses respiration and limits proton influx through ATP synthase. This would result in mitochondrial hyperpolarization and subsequent mitochondria-dependent metabolic signaling and apoptosis suppression. HIF1α facilitates a shift from mitochondrial oxidation to glycolysis, which ensures ATP production even in sustained hypoxia [12]. In addition to upregulating GLUTs, HIF1α upregulates pyruvate dehydrogenase kinase (PDK), the enzyme that tonically inhibits PDH, limiting pyruvate flux into the mitochondria (Fig. 1). When angiogenesis occurs and tumor oxygen delivery is restored, the upregulated PDK maintains mitochondrial suppression, and at this point, loss of secondary mitochondrial signals (mROS, αKG) sustains HIF1α even in the absence of hypoxia, maintaining a glycolytic state.

Mitochondrial suppression in cancer may have pro-proliferative effects in addition to the apoptosis inhibition and HIF1α driven angiogenesis. Metabolites that are now not oxidized in the mitochondria are shifted toward biosynthetic pathways important for rapidly proliferating cells [13]. For instance, glucose-6-phosphate (the product of HK) can be shunted into the pentose phosphate pathway (PPP) whereby ribulose-5-phosphate is produced for nucleotide synthesis (Fig. 1). This pathway also produces NADPH, which protects against oxidative stress and is used in de novo lipid and amino acid synthesis [13]. Thus the beneficial effects of an overall mitochondrial suppression in cancer may extend to multiple levels.
Mitochondrial activators in cancer

Not surprisingly, therapies targeting single genetic or molecular abnormalities are ineffective in most cancers, which are commonly heterogeneous in nature. For example, there is significant diversity in glioblastoma multiform (GBM), where a given tumor includes multiple abnormalities and therefore is unlikely to respond to single target strategies [14]. A common feature among all GBM (and most solid tumor) cells is metabolic remodeling. In the 1920s, Warburg suggested that this feature of most cancers is caused by abnormal mitochondria. However, his theory was dismissed and most considered mitochondria inhibition as a secondary effect, possibly a result of oxidative damage.

DCA and preclinical studies

Recently, we showed that cancer cells treated with dichloroacetate (DCA), a PDK inhibitor (and thus a PDH activator), acutely increased GO [15]. Because GO occurs exclusively in the mitochondria, these data suggested that cancer mitochondria are perhaps functionally and reversibly suppressed, not permanently damaged, providing strong rationale for the development of similar drugs as novel cancer therapies. We first published the preclinical effects of DCA in cancer in 2007. We found that non-small cell lung, breast and GBM cancer cells had hyperpolarized ΔΨm compared to non-cancerous cells and that DCA rapidly depolarized mitochondria to normal levels, suggesting restoration of mitochondrial function [15]. In keeping with this, DCA decreased the glycolysis to GO ratio by enhancing GO and lowering lactate production. DCA induced mitochondria-dependent apoptosis and reduced proliferation both in vitro and in vivo [15]. DCA-treated cancer cells had increased mROS, activated Kv-channels and inhibited NFAT. Importantly, DCA did not affect mitochondria and their downstream targets in non-cancerous cells. DCA also decreased tumor size in a xenotransplant model (Fig. 2). Since then, many independent studies have confirmed our findings (reviewed in [16]) in colon [17], prostate [18], endometrial [19] or metastatic breast cancer [20]. In vivo, DCA was effective in attenuating aggressive metastatic breast cancer [20] (Fig. 2). In another study, DCA was chemically linked to cisplatin, a commonly used cancer drug that induces DNA cross-linking [21]. This new molecule, mitaplatin, was cancer selective, inducing mitochondrial-dependent apoptosis and reversing the apoptosis resistance that limits cisplatin’s effectiveness.

DCA and early clinical experience

DCA has been used clinically for decades to symptomatically treat congenital mitochondrial diseases including PDH deficiencies and their resulting lactic acidosis. This, along with promising pre-clinical findings provided the rationale for early phase clinical trials in cancer. The first such study was recently published examining DCA’s effects in GBM, a highly vascular and deadly brain cancer [22]. Examination of 49 freshly excised tumors showed mitochondrial hyperpolarization compared to non-cancerous brain tissue, which was reversed with acute DCA, supporting reversible mitochondrial inactivation in this tumor. The tumors showed high PDK2 expression, the most DCA-sensitive PDK isoform [23] (Fig. 3a). DCA was orally administered to five (previously treated) patients at a dose of 6.25mg/kg bid giving plasma concentrations of 0.266-0.626mM after at least three months, well within the range for PDK2 inhibition (K_{i}=0.2mM [23]). Importantly, none of the patients developed hematologic, hepatic, renal or cardiac toxicity. Peripheral neuropathy was the only apparent toxicity, which
was reversed at lower doses. This study’s major strength was comparing tumor tissue obtained before and after DCA therapy. Post-DCA, tumor tissues exhibited higher PDH activity, confirming PDK2 inhibition in vivo. Further examination revealed p53 activation, HIF1α inactivation and decreased vascularity. Moreover, the tumors were less proliferative, more apoptotic (Fig. 3a) and some, but not all, showed evidence of regression or stability (Fig. 3b) [22]. Even more exciting was the apparent effects on putative GBM stem cells (GBM-SC; CD133+/Nestin+), which are the most apoptosis-resistant cells and the cause of recurrence after remission. In line with this, the GBM-SC had the highest ΔΨm within the tumor in vivo. DCA induced apoptosis in these cells in vitro (Fig. 3c) and in vivo, suggesting that this traditionally resilient population is also metabolically vulnerable [22].

**Lactate dehydrogenase**

LDH-A, a downstream target of HIF1α[3], converts pyruvate into lactate, restoring NAD+ to sustain glycolysis and preventing pyruvate oxidation. Inhibition of LDH-A may restore mitochondrial function by promoting mitochondrial pyruvate metabolism, in a sense mimicking DCA (Fig. 1). LDH-A inhibition decreases lactate, depolarizes mitochondria, increases respiration and
decreases proliferation in cancer cells [24, 25]. Animals injected with LDH-A-knockdown cancer cells had smaller tumors and improved survival [24], and inhibition of LDH-A reduced tumor volumes in lymphoma and pancreatic xenotransplant models without kidney, liver or blood toxicity [25]. These promising preclinical results and the relative selectivity for cancer suggest LDH-A inhibitors may be a viable therapeutic intervention.

Pyruvate kinase M2 (PKM2)
Conversion of phosphoenol-pyruvate to pyruvate occurs as the final rate-limiting glycolytic step. This reaction is catalyzed by pyruvate kinase, which exists in high (PKM1) and low activity (PKM2) isoforms. The recent identification of PKM2 has generated excitement since this isoform is selective to rapidly proliferating cells [13]. Although it appears paradoxical, this low-activity isofrom may actually promote proliferation by reducing ATP production (preventing allosteric inhibition of prior glycolytic reactions) and shunting metabolites into biosynthetic pathways [13, 26]. PKM2 would also reduce mitochondrial pyruvate influx (Fig. 1). Thus, targeting PKM2 not only reduces biosynthetic processes, but may also promote GO, similar to DCA. Indeed, inhibition of PKM2 increases oxygen consumption in some cancers and limits tumor growth in xenotransplant models [27]. The PKM2 data are compatible with the PDH/DCA work and the idea of an overall suppression of mitochondrial function in cancer.

Fatty acid metabolism inhibitors
Cancer’s highly proliferative nature creates a large demand for fatty acids for membrane synthesis. Rather than extracellular sources, cancer obtains fatty acids via de novo synthesis from the Krebs’ cycle intermediate citrate [28]. Citrate is upstream of many Krebs’ cycle enzyme mutations commonly associated with cancer as described above. Mutations in these enzymes would lead to accumulation of upstream metabolites (i.e., citrate) for biosynthetic purposes (Fig. 1). Inhibition of several fatty acid biosynthetic steps has shown efficacy in preclinical cancer models [29, 30]. Intriguingly, fatty acid oxidation (FAO) inhibition also inhibits cancer growth [31], as it indirectly promotes GO through the Randle cycle (i.e. FAO-generated acetyl-CoA inhibits PDH) [32]. In other words, this strategy is similar to DCA in terms of “refueling” mitochondria with pyruvate and reversing the mitochondrial suppression and the secondary upregulation of glycolysis.

Glycolysis inhibitors
Cancer’s glycolytic environment provides sufficient energy and building blocks for proliferation while simultaneously suppressing apoptosis. Although direct glycolysis inhibition seems logical, this strategy rapidly (and predictably) depletes ATP causing ATP-independent (necrotic) cell death, which is likely to damage non-cancerous tissues as well. Inhibiting glycolysis may not be selective since non-cancerous tissues (i.e., skeletal muscle, brain, etc.) also rely on glycolysis. In fact, several recent clinical trials of glycolysis inhibitors have not been successfully completed. Clinical trials for 2-deoxyglucose (Hk inhibitor) for prostate cancer (NCT00633087) and intracranial neoplasms (NCT00247403) have been suspended. Phase II/III trials for the Hk-inhibitor lonidamine in benign prostate hyperplasia have also been terminated (NCT00435448, NCT00237536). Another Hk inhibitor, 3-bromopyruvate, which pre-clinical work suggests induces necrosis in cancer, has also demonstrated significant toxicities in animal models at doses only slightly higher than therapeutic doses[33]. In other words, the non-selectivity of these drugs may limit their translational potential. In summary, the metabolic modulators that improve coupling between glycolysis and GO, “refueling” mitochondria and “normalizing” remodeled metabolism should not be confused with glycolysis inhibitors, which rather than normalize metabolism, cause energy starvation (Fig. 1).

Conclusions
Revisiting Warburg’s original hypothesis that abnormal metabolism causes cancer has provided newfound optimism. Once thought to be a secondary result of upstream abnormalities, it now appears that cancer’s metabolic remodeling is integral for survival and exposes many therapeutic targets. Various strategies that restore mitochondrial metabolism have shown selectivity and efficacy in pre-clinical studies and need to efficiently be moved into clinical trials.

References


Cancer and inhibition of fatty acid oxidation

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Abstract
Recently, a “metabolic transformation” property with increased glycolytic rates (Warburg effect) was described for cancer cells. It has been shown that there is an active interaction between mutations of crucial enzymes involved in metabolic pathways and of oncogenes, whose effector proteins also exert metabolic functions. Interestingly, because many of these metabolic changes are confined to the mutant clones, targeted therapy appears very promising in terms of both safety and efficacy. Pharmacological inhibition of glycolysis by dichloroacetate (DCA) significantly inhibits proliferation of cancer cells. However, because of the pharmacokinetic characteristics, the plasma concentration levels, and, therefore, toxic effects (i.e., hepatotoxicity and neurotoxicity) of DCA are difficult to predict. Similar metabolic modulation and antiproliferative effects can be obtained through inhibition of fatty acid oxidation. Such agents have been safely used in other clinical conditions such as heart disease and, as such, deserve further testing in cancer therapy.

Keywords: cancer, Warburg effect, metabolic modulation therapy, fatty acid oxidation

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Cancer continues to ferociously strike humanity. This has led to a major effort to develop new therapies to treat cancers. While major advances have been made in the treatment of this debilitating disease, it still remains a major cause of mortality in our society. As a result, new therapies to treat and prevent cancer are being sought.

Over the past decades, cancer studies have mainly focused on identifying gene and protein networks involved in the regulation of cell growth, differentiation, and death. However, although the genetic mutations may be specific to the tumor, the downstream effectors are also found in normal tissues, therefore resulting in a narrow range of effective and safe molecular targets. Recently, a “metabolic transformation” property, amenable to metabolic modulation therapy, was described for cancer cells. Importantly, because many of these metabolic changes are confined to the mutant clones, targeted therapy appears very promising in terms of both safety and efficacy.

Aerobic glycolysis (Warburg effect) [1] constitutes a common feature of cancer cells and, as such, appears to confer resistance to cell death. Importantly, increases in glycolytic rates in cancer cells indirectly reflect also a decrease in the “mitochondrial energy production state” of cancer cells. Similar to normal growth, apoptosis is an encoded cellular phase that implies adequate mitochondrial energy production and signaling, which ultimately causes the release
of pro-apoptotic factors. Alterations in cellular metabolic pathways appear therefore to play an important role in conferring cancer cells their distinctive “immortality status”. In accordance with these observations, genetic analyses have shown that cancer cells displaying these features have mutations of crucial enzymes involved in metabolic pathways (i.e., phosphoinositol-3-kinase [PI3K], Akt, AMP-activated protein kinase [AMPK] and its upstream kinase LKB1, etc.) [2]. In addition, oncogenes whose effector proteins also exert metabolic functions (i.e., p53 target TP53-induced glycolysis and apoptosis regulator [TIGAR], anti-apoptotic protein bcl-2, etc.) also show mutations [3]. Such findings indicate that metabolic and apoptotic pathways are more than two associated phenomenon and therefore represent the scientific basis for considering metabolic modulation as a treatment strategy for cancer.

With regard to metabolic transformation, the key events that cause cancer cells to display the characteristic altered metabolic features are the “pseudo-hypoxic” mitochondrial oxygen sensing with abnormal activation of the hypoxia-induced factor 1-alpha (HIF1-alpha). Increased HIF1-alpha expression activates a series of glycolytic genes, and also suppresses the glucose oxidation activity of the mitochondria by trans-activating the pyruvate dehydrogenase (PDH) kinase enzyme (PDK), which phosphorylates and inhibits the PDH complex [4]. PDH is a key enzyme in controlling the rate of glucose oxidative metabolism in that it catalyzes the irreversible oxidation of pyruvate, yielding acetyl-CoA and CO2 and, therefore its inhibition creates a glycolytic shift of glucose metabolism (Fig. 1). This phenomenon is associated with other mitochondrial-metabolic abnormalities that include: mitochondrial hyperpolarization, decreased superoxide dismutase-2 (SOD2) with reduced production of reactive oxygen species (ROS), and decreased Kv (voltage gated) 1.5 expression. Loss of Kv 1.5 depolarizes the membrane and elevates cytosolic K+ and Ca2+, leading to Ca2+-calcineurin dependent activation of the proliferative transcription factor (NFAT) and inhibition of caspases through elevation of cytosolic K+.

The relevance of these changes in the coupling of glycolysis to glucose oxidation on cellular proliferation capacity is confirmed by the inhibitory effects on cell proliferation observed through pharmacological inhibition of PDK. Dichloroacetate (DCA) is the most frequently tested pharmacological agent for this purpose (Fig. 2). Besides metabolic changes, DCA restores mitochondrial ROS production capacity, releases...
pro-apoptotic factors [5] and, ultimately, decreases cell survival and increases apoptosis rates [4]. However, despite these promising cytological effects, the potential use of DCA in cancer patients is regarded with some degree of skepticism. In fact, when administered for other medical conditions (such as lactic acidosis, sepsis, burns, etc.), DCA has been reported to cause important adverse effects such as hepatotoxicity and neurotoxicity (although to some degree, these toxic effects are reversible with short-term administration) [6]. Such effects would be particularly relevant in cancer patients who are frequently on concomitant neurotoxic chemotherapy. Moreover, since multiple administrations of DCA leads to inhibition of hepatic enzymes responsible for its own metabolism, plasma concentration levels and therefore toxic effects of the drug are difficult to predict [7].

DCA is not the only available agent to stimulate PDH activity. The effects of DCA to increase glucose oxidation rates can be achieved by inhibiting mitochondrial fatty acid oxidation rates [8]. The reciprocal relationship between glucose and fatty acid metabolism is part of a phenomenon known as the “Randle cycle.” Since glucose oxidation and fatty acid oxidation both produce mitochondrial acetyl-CoA, the rate of each other’s activity has a direct reciprocal effect on the rates of others pathway. Therefore stimulation of glucose oxidation through direct inhibition of PDK activity can similarly be obtained by inhibiting fatty acid oxidation. This has been the rationale for the development and use of fatty acid oxidation inhibitors in other conditions such heart disease. However, at this point one may argue: how can this approach be transferred to cancer cells when the latter have been defined by relying on glycolysis rather than oxidative metabolism?

Nevertheless, although still recognizing glycolysis as the main source for energy production of cancer cells, increasing the coupling of glycolysis to glucose oxidation by switching existing mitochondrial oxidative metabolism from fatty acids to glucose, rather than a reduced overall oxidative capacity of the mitochondria, has been proposed [9]. Therefore, similar to cardiac disease, stimulation of glucose oxidation through inhibition of fatty acid oxidation may be a reasonable approach for cancer therapy (Fig. 3). In support of this concept, fatty acid inhibitors such as etomoxir and ranolazine have been shown to have beneficial effects in leukemia cells [10]. Trimetazidine, another fatty acid oxidation inhibitor, was recently shown to dose dependently induce cancer cell apoptosis in experimental conditions [11]. Similarly, Zhou et al showed that increasing malonyl-CoA levels (a key enzyme that inhibits the transfer of fatty acids for oxidation into the mitochondria) through inhibition of malonyl-CoA decarboxylase (MCD), can decrease proliferation of human breast cancer cells [12]. However, the pro-apoptotic effects in the latter two studies were mainly attributed to modifications in fatty acid synthesis (FA) that occurred in addition to trimetazidine and the MCD inhibitor. Indeed, besides increased glycolysis, cancer cells have been also shown to display increased de novo FA synthesis [13] with high levels of the lipogenic enzymes ATP-citrate lyase (ACL), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS). However, modulation of FA synthesis has yielded contrasting results. Likewise, in the study by Andela et al [11], trimetazidine was tested in addition to a peroxisome proliferator receptor gamma (PPARγ) agonist, which, among other effects, causes an increase in FA synthesis. On the contrary, in the study by Zhou et al,
the MCD inhibitor was tested with cerulin, a FAS inhibitor [12]. These contrasting results in the modulation of FA synthesis may, to some degree, reflect the tissue-specific (in this case cancer cell type) expression of enzymes involved in the regulation of FA. However, both studies showed clear reduction of cellular proliferative capacity, in this way highlighting the potential relevance of fatty acid inhibition and therefore, as mentioned, relief of glucose oxidation, as a therapeutic approach to treat cancer. This is ultimately in line with the beneficial results obtained from direct stimulation of glucose oxidation with DCA. Importantly, the increased apoptotic rates are confined only to cancer and not to normal control cellular lines. Moreover, trimetazidine has been widely tested in human clinical trials, and shows a high safety profile and no major adverse events. In this particular setting, the beneficial results were observed in experimental conditions and do not represent the current indication of the product (Vastarel®, MR), which is an established therapy for the management of patients with myocardial ischemia. However, trimetazidine has been shown to have cardioprotective effects on cancer patients with anthracycline-induced acute cardiotoxicity [14, 15]. Alternatively, MCD inhibitors, another drug class that inhibit fatty acid oxidation, can be further studied in cancer setting.

In conclusion, altering glucose and fatty acid oxidation in cancer cells provides a potential new approach to treat cancer. The appealing potential of controlling cell proliferation by metabolic modulation therapy has engendered new hopes on the horizon. Increasing glucose oxidation rates, through either a direct induction of glucose oxidation or, a reduction in the fatty acid oxidation rates appears to be a logical approach to treat cancer. DCA, the main direct glucose oxidation stimulator available, has shown to have adverse effects that may be particularly relevant in patients who receive concomitant chemotherapeutic agents. On the other hand, inhibitory agents of fatty acid oxidation appear to have a safer therapeutic profile and may therefore represent a valid alternative to DCA.

Given the quickly fatal natural history of the disease and the availability of these agents, efforts to translate these findings in clinical practice are urgently needed.

References

A case of multifactorial late left ventricular dysfunction after cancer treatment

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Abstract
A case of hypokinetic cardiomyopathy in a young lady previously treated with anthracyclines and mediastinal radiotherapy (RT) is described. The dysfunction was first considered due to anthracyclines cardiomyopathy, possibly with superimposed myocarditis, and treated with enalapril and bet blockers obtaining an improvement in left ventricular function. After a nine-year follow-up, left ventricular dysfunction worsened and inducible silent myocardial ischemia was detected. When coronary artery disease (CAD) was diagnosed and cured by angioplasty and stenting, left ventricular function improved. In this patient, CAD was likely secondary to both RT and dyslipidemia, and was probably a co-factor of left ventricular dysfunction. Patients treated with chest radiotherapy are at increased risk of CAD, and any other risk factors (with particular attention to metabolic factors) should be thoughtfully searched and promptly treated. This is mostly important in long-term survivors, and in patients treated more than 30 years ago, when RT techniques were not yet planned to reduce radiation-induced heart disease.

Keywords: cardiotoxicity; radiotherapy; left ventricular dysfunction; Hodgkin’s disease; coronary artery disease; dyslipidemia

Case report
A 40-year-old woman, a former smoker with family history of hypertension and diabetes, was first seen in our outpatient clinic in June 2001. Her past clinical history included Hodgkin disease stage III (supraclavear and mediastinal lymph nodes, spleen) at age 14 (in 1974), treated with splenectomy, six courses of chemotherapy with adriamycin (ADM), bleomycin, vinblastine, dacarbazine (ABVD) up to a total dose of 300 mg/sm of ADM, and mantle field radiotherapy (RT) (30 Gy). In January 2001, after traveling in Thailand, she was admitted to a general hospital with fever and worsening dyspnoea. Chest x-rays revealed bilateral pneumonitis, immunological tests resulted positive to mycoplasma pneumonia. At that time electrocardiography (ECG) revealed complete left bundle branch block (LBBB).

In June 2001, the patient was asymptomatic, without cardiac murmurs or other pathologic findings. The ECG showed sinus rhythm, with LBBB. At echocardiography the left ventricle (LV) showed normal dimensions, with asynchronous contraction, septal hypokinesis and slightly reduced ejection fraction (EF): 43%. Cytomegalovirus antibodies were elevated; other routine blood tests were negative. Enalapril 5 mg/day was started. The patient took a treadmill stress test in August 2001 and attained a workload of 9.3 metabolic equivalent units (METS), without symptoms (ECG not evaluable for LBBB). The patient refused coronary angiography
and myocardial biopsy (to rule out coronary artery disease and myocarditis), and nuclear imaging tests.

Up to 2008, the patient remained asymptomatic, with EF ranging from 51% to 56% and mild diastolic dysfunction (mitral E/A <1 at pulsed Doppler) on yearly echocardiograms. Treadmill stress tests, repeated every 3 years, were always clinically negative, with the patient able to perform >9 METS. Therapy included enalapril and beta-blockers at the highest tolerated dose (enalapril 7.5 mg, bisoprolol 2.5 mg: up-titration was difficult because of hypotension). In 2004, blood tests revealed dyslipidemia (total cholesterol 350 mg/dL), and we prescribed atorvastatin 20 mg, which was changed to rosuvastatin 10 mg/day in 2006. In November 2007, the patient underwent mastectomy for left breast carcinoma, followed by hormone therapy (tamoxifen and triptorelin). After surgery, the patient spontaneously stopped rosuvastatin. In November 2009, she was still asymptomatic, with echocardiographic EF at 52%, but further impairment of diastolic function (pseudonormal pulsed Doppler mitral flow pattern, E/E' = 22). A myocardial perfusion stress test showed reduced septal perfusion, partially reversible at rest. The patient finally accepted coronary angiography in June 2010: a 95% ostial stenosis of the right coronary artery was detected and treated with angioplasty and stenting. At echocardiography, one month later both systolic and diastolic functions were improved (EF 57%, mitral E/A <1).

Comments

The cardiotoxicity of both ADM and RT may become clinically evident years after treatment, mostly in patients treated at age <18 (as our patient was) and in those who received both treatments [1, 2]. The intracellular accumulation of doxorubicinol, an ADM metabolite, may act as a toxic reservoir potentiating the damage of further cardiac insults [3]. The heart was formerly considered resistant to radiation, but from the late 1970s more and more reports about the late sequelae of chest RT have been published [4–6]. Radiation-induced damage of normal tissues depends on dose and volume: according to predictive algorithms, new RT techniques have been developed in the past 30 years in order to concentrate the radiation beam on the tumor, sparing the surrounding structures [7–10]. Since therapeutic plans have changed over time, the risk of cardiac damage is obviously higher for patients treated earlier and lower for those treated more recently [11]. Long-term adverse cardiac effects of RT include dilated and/or restrictive cardiomyopathy, pericarditis, valvular disease, arrhythmias and coronary artery disease (CAD) [12, 13]. The pathophysiological link between RT and accelerated atherosclerosis is the radiation-induced inflammatory reaction followed by endothelial and fibroblast proliferation, lipid deposition, fibrosis, and formation of inflammatory plaque [14–16]. The clinical incidence of CAD is relevant after 10 years, and increases thereafter. A study of 1998 reports an increased prevalence (2.9% after 10 years, 13.7% after 20 years, 24.7% after 25 years) in the subgroup of patients with additional classic cardiovascular risk factors only; in a study of 2003 hypertension and hypercholesterolemia were significant risk co-factors; in a study of 2007 stress-induced ischemia was found in 18.4% of patients, and among them CAD was detected in 40% of those examined <10 years and in 58% of those examined >10 years after RT, without any difference between the patients with and those without additional risk factors; in all studies the risk was higher in males than in females [17–19].

Since the initial stress test in 2001 did not induce clinical ischemia in our patient, and she had no additional risk factors, we considered LV dysfunction probably due to late ADM cardiomyopathy, possibly with myocarditis as co-factor. On enalapril and beta-blockers the patient remained asymptomatic and had clinically negative stress tests for years. When dyslipidemia was first detected, we prescribed a statin, which was later discontinued by the patient. This decision could have accelerated the progression of CAD. Hormonal therapy for breast cancer, on the contrary, had probably no role on coronary pathology: tamoxifen has a beneficial effect on lipid profiles and on cardiac risk; for triptorelin no effect is reported [20, 21].

A peculiarity of radiation-induced CAD is the frequency of ostial lesions and the high prevalence of silent ischemia, and this was also the case for our patient, who was asymptomatic but whose LV diastolic function had worsened over time, suggesting a progression of CAD. In fact, when the ostial lesion was detected and cured with angioplasty, an improvement in systolic and diastolic function was obtained. Thus, cardiac dysfunction, in this particular patient, was probably multifactorial and included ADM myocardial toxicity, radiation-induced myocardial damage (both as direct insult and as increased sensitivity to other stress factors) and coronary artery subclinical damage by RT.
leading to early development of CAD (with dyslipidemia as co-factor), which further impaired LV function.

**Conclusion**

The late cardiotoxicity of anticancer therapies is relevant in long-term survivors, mostly in case of curable malignancies of young patients, leading to a large cohort of subjects at increased risk of cardiac disease in early adulthood. Antracyclines irreversibly damage the myocites, and prevention of late cardiac disease implies the prevention of other factors potentially affecting cardiac function, as hypertension, diabetes and ischemic heart disease. RT may cause myocardial, valvular, pericardial and coronary artery damage. In patients having undergone mediastinal or left chest wall RT, prevention should also include the control of risk factors such as dyslipidemia. The highest risk for radiation-induced heart disease in general and of CAD in particular regards subjects treated before 1980–1985, for several reasons: at that time radiation techniques were not yet planned to prevent the possible long-term adverse cardiac effects, it was possible that dyslipidemia was not promptly treated (its role as CAD risk factor was still under debate and powerful therapeutic agents like statines were not available yet), and the incidence of heart disease increases anyway over time.

All the patients who had been cured by RT for mediastinal lymphomas (or other thoracic tumors) and for left breast carcinomas should absolutely avoid smoking and should be frequently screened, and promptly treated, for any other CAD risk factor, with particular attention to diabetes and dyslipidemia. Moreover, a regular follow-up with echocardiography and stress test is recommended.

**References**

The Warburg effect – aerobic glycolysis in proliferating cells

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Abstract
The metabolic phenotype of proliferating cells is characterized by enhanced glycolysis uncoupled from pyruvate oxidation, but instead coupled to increased lactate release even in the presence of oxygen (Warburg effect/aerobic glycolysis). Alterations in the pathways regulating glycolysis and the oxidation of glucose-derived carbon are responsible for maintaining this phenotype. This article reviews the molecular mechanisms inherent to the intermediary metabolism of glucose contributing to the Warburg effect.

Keywords: cellular proliferation; glycolysis; glucose oxidation; cellular biosynthesis

Introduction
In the 1920s Otto Warburg demonstrated that rapidly proliferating mouse ascites tumor cells preferentially converted glucose to lactate even in the presence of oxygen (Warburg effect/aerobic glycolysis) at a much greater rate than that observed in quiescent, differentiated cells [1]. These observations were later extrapolated to proliferating primary lymphocytes [2], demonstrating that the metabolic phenotype of cell proliferation is attended by specific alterations in the intermediary metabolism of glucose. Although glycolysis generates only 7–10% of the adenosine-5'-triphosphate (ATP) compared to the complete mitochondrial oxidation of glucose-derived carbon, it does, nonetheless, efficiently generate substrates that meet the biosynthetic requirements of cellular proliferation [3]. This article provides an overview of mechanisms intrinsic to the intermediary metabolism of glucose that contribute to the Warburg effect.

Cellular glucose metabolism in differentiated versus proliferative tissues
Differentiated tissue such as cardiac muscle derives greater than 90% of its ATP requirements via mitochondrial oxidative phosphorylation [4]. The reduced forms of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂) generated during the catabolic degradation of glucose and fatty acids, as well as in the tricarboxylic acid (TCA) cycle deliver protons and electrons to the electron transport chain, reducing molecular oxygen to water, and generating ATP. With regards to carbohydrate metabolism, the catabolism of glucose in differentiated tissues occurs via two separate, but coupled processes—glycolysis and subsequent glucose (i.e., pyruvate) oxidation. In sharp contrast, cellular metabolism in proliferative cells is characterized by accelerated rates of glycolysis that are uncoupled from the glucose oxidation.
Glycolysis, localized to the cytosolic compartment, converts glucose to pyruvate, or lactate, in the presence or absence of oxygen, respectively, and generates 2 mol ATP/1 mol glucose. Glycolysis consists of an ATP utilizing stage and a subsequent ATP generating stage (Fig. 1). The first enzymatic reaction committing glucose to catabolism by glycolysis, catalyzed by 6-phosphofructo-1-kinase (PFK-1), is subject to allosteric inhibition by ATP, citrate, and protons [4]. The first reaction of the ATP generating stage of glycolysis, catalyzed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH), is coupled to the reduction of NAD⁺ to NADH. ATP itself is generated by reactions catalyzed by phosphoglycerate kinase and pyruvate kinase. The continual regeneration of NAD⁺ is required to ensure that glycolysis is not limited. In differentiated cells and tissues, under aerobic conditions, NAD⁺ is re-generated from NADH via the malate-aspartate shuttle and the mitochondrial electron transport chain; whereas, under anaerobic conditions lactate dehydrogenase (LDH) couples the reduction of pyruvate to lactate with the regeneration of NAD⁺.

The oxidation of glucose-derived carbon requires the transport of pyruvate into the mitochondrial matrix via a monocarboxylate (MCT) transporter [5], and its subsequent oxidative decarboxylation, catalyzed by the pyruvate dehydrogenase (PDH) complex yielding acetyl-CoA [6]. The PDH complex consists of PDH, PDH kinase (PDK), and PDH phosphatase (PDHP). The flux of pyruvate through PDH is subjected to regulation by both substrate/product ratios and covalent mechanisms. The increased generation of pyruvate, decreased ratios of NADH/NAD⁺ and acetyl-CoA/CoA, as well as PDHP-mediated dephosphorylation increase flux through PDH. Conversely, increased ratios of NADH/NAD⁺ and acetyl-CoA/CoA, as well as PDK-mediated phosphorylation decreases flux through PDH, thereby restricting the oxidation of glucose-derived carbon units [6].

Underlying molecular mechanisms

The Warburg effect is a predominant metabolic phenotype observed in proliferating cells. Detailed studies in a variety of cancer cell types have revealed that this metabolic phenotype may arise from a number of cellular alterations and occur downstream of growth promoting signal transduction pathways (PI3K-Akt-mTOR), mutations in proto-oncogenes (Myc), in response to loss of function mutations of tumor suppressor genes (p53), and transcriptional responses to hypoxia-inducible factor-1α (HIF1α) [3]. These alterations in cellular signaling elicit concerted effects on the intermediary metabolism of glucose in both the ATP utilizing and ATP generating stages of glycolysis, and facilitate enhanced glycolysis characteristic of the Warburg effect.
Growth factor-mediated activation of the PI3K-Akt-mTOR pathway via transcriptional and post-transcriptional effects increases the expression and cell surface localization of GLUT1 glucose transporters [7]. Similarly, loss of function mutations of the p53 tumor suppressor de-represses the transcription of GLUT1 and GLUT4 [8]. These alterations can increase cellular glucose uptake. Glucose entering the cytosolic compartment is efficiently trapped as glucose-6-phosphate (G6P) by hexokinase II, a HIF1α- and Myc-target gene up-regulated in a variety of cancer cells [9]. Loss of p53 function also enhances flux through the ATP utilizing stage of glycolysis, secondary to the loss of TP53-induced glycolysis and apoptosis regulator (TIGAR) [10]. TIGAR is functionally similar to the fructose-2,6-bisphosphatase (FBPase) domain of the bifunctional enzyme, 6-phosphofructo-2-kinase (PFK-2), which converts fructose-6-phosphate to fructose-2,6-bisphosphate, a potent allosteric stimulator of PFK-1. Thus, p53 can restrain flux through PFK-1 via TIGAR-mediated degradation of fructose-2,6-bisphosphate [10], whereas loss of p53 function via loss of TIGAR can promote flux through PFK-1 [11].

ATP is generated by the phosphoglycerate kinase and pyruvate kinase reactions of glycolysis and both are of central importance in mediating the Warburg effect. HIF1α and Myc increase the expression of phosphoglycerate kinase in a cooperative manner [12], which contributes to increasing glycolytic flux. Pyruvate kinase (PK) catalyzes the final reaction of glycolysis, coupling the production of pyruvate with the production of ATP. Proliferating cells, including cancer cells predominantly express the PKM2 isoenzyme [13]. Interestingly, the affinity of dimeric PKM2 for its substrate PEP is low, and PKM2 is therefore nearly inactive at physiological/pathophysiological intracellular PEP concentrations [14]. These effects are associated with increased cellular ADP/ATP ratios, which enhance flux through the PFK-1 reaction of glycolysis. These effects may appear paradoxical, as the generation of the glycolytically derived pyruvate via the PK reaction is limited, which would be expected to decrease lactate release and hence decrease an important component of the Warburg effect. However, a recent report identifies a novel/alternative pathway of glycolysis in proliferating cells that circumvents this reaction and generates pyruvate from PEP by transferring the phosphate from PEP to the catalytic histidine residue of the glycolytic enzyme phosphoglycerate mutase [15]. The generated pyruvate can subsequently be reduced to lactate and hence maintain the Warburg effect.

Mechanisms affecting pyruvate/lactate metabolism and lactate release are also required to support the Warburg effect. The expression of the LDH-A isoform is under the transcriptional control of both HIF1α and Myc [16]. LDH-A effectively converts glycolytically derived pyruvate to lactate. HIF1α also upregulates the expression of the plasma membrane MCT4 isoform [17], as well as PDK1 in proliferating cells [9]. Taken together, these metabolic alterations provide a means to regenerate NAD+ (LDH-A) required for glycolysis, facilitate lactate release (MCT4), and restrict glucose oxidation (PDK1) (Fig. 2).

**The Warburg effect and anabolic metabolism**

The Warburg effect is of central importance in supporting the requirements of cell division which require a doubling of total biomass (including cellular nucleic acid, lipid, and protein contents) [3]. The non-oxidative arm of the pentose phosphate pathway (PPP) utilizes the glycolytic intermediate, fructose-6-phosphate for the synthesis of ribose-5-phosphate required for nucleotide biosynthesis [18]. Proliferating cancer cells
in particular also have high requirements for de novo lipid synthesis, evidenced from the elevated expression of lipogenic enzymes including ATP citrate lyase (ACL), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS) [19]. ACL can contribute to maintaining the Warburg effect as it cleaves cytosolic citrate (originating from the mitochondrial matrix), yielding oxaloacetate and acetyl-CoA. To ensure that the requirements for de novo lipid synthesis do not deplete the TCA cycle, the cycle is replenished via the anaplerotic flux of glutamine [20]. These effects of ACL decrease the content of cytosolic citrate, enhancing PFK-1 activity, as well as providing substrate (i.e., acetyl-CoA) for de novo lipid synthesis via ACC and FAS. The metabolism of cytosolic oxaloacetate can also contribute to supporting the Warburg effect as malate dehydrogenase couples the conversion of oxaloacetate to malate with the formation of NADH. Malic enzyme subsequently couples the conversion of malate to pyruvate (which can subsequently be converted to lactate and exported) with the production of nicotinamide adenine dinucleotide phosphate (NADPH), which serves as a cofactor in both the PPP and de novo lipid synthesis [18]. These complimentary activities of the Warburg effect and several pathways of anabolic cellular metabolism (Fig. 3) meet the requirements of cellular proliferation, and in cancer cells support tumor development and progression.

Conclusions
Alterations in the intermediary metabolism of glucose, exemplified by the Warburg effect are critical in supporting the biosynthetic requirements of cellular proliferation. The Warburg effect adapts cellular metabolic phenotype, such that enhanced flux through glycolysis provides a number of critically important substrates for the macromolecular synthesis of cellular biomass. As such a greater understanding of molecular mechanisms regulating the intermediary metabolism of glucose is relevant for the identification novel pharmacological targets that can influence cellular proliferation. With regards to cancer cells, this may lead to the development of promising therapeutic agents to limit tumor development and progression.

References
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It was in the early 1943 that Karl Paul Link first lectured on the anticoagulant properties of dicumarol. Since then, warfarin and other vitamin K antagonists have been used for prevention and treatment of thrombosis for almost 70 years. Because atrial fibrillation (AF) is the most common heart rhythm disorder [1], and its presence increases the chance of stroke by five-fold [2], AF patients constitute the vast majority of patients with indications for anticoagulation therapy. However, anticoagulant therapy is often a source of frustration to both patients and physicians. Although it has been shown to reduce the incidence of stroke by 61%, only half of eligible patients receive appropriate treatment [3]. While on warfarin, patients are required to complete an international normalized ratio (INR) monthly check and, if they are above or below the recommended range, have to go back the following week. Nonetheless, despite the frequent check up and the institution of anticoagulation centers, patients on warfarin are <60% of the time in the therapeutic INR range [4]. In addition, many elderly patients, which represent the vast majority of patients with indications to anticoagulation therapy, often present with limited mobility and autonomy and therefore have additional difficulties for attending hospital-located monitoring facilities. Moreover, since warfarin effects are affected by factors such as food and drug-drug interactions, patients are also equipped with a long list of “things to avoid while on warfarin”, which is commonly a life-long therapy. Therefore, fairly enough, when implementing such a treatment, patients tend to be very reluctant and regard it as a kind of a “punishment”, especially the asymptomatic ones, in whom AF diagnosis was based on an accidental electrocardiogram (ECG) finding.

Although researchers have been looking for replacing warfarin for several decades, until recently no drug presented as a suitable substitute. However, after a long quiescence period, we are perhaps witnessing an historical event. Indeed, as reported in the 2010 European Society of Cardiology guidelines for the management of patients with AF, dabigatran received guidance for use as an alternative to warfarin [5]. Moreover, in the more recently published, 2011 American College of Cardiology/American Heart Association homologue guidelines, dabigatran obtained a “Class I, Level of Evidence B” insertion as an alternative to warfarin for AF patients [1].

Dabigatran is a “classmate” of ximelagatran, the first direct thrombin inhibitor which, despite showing clinical efficacy for prevention of thromboembolic events, led to fatal hepatotoxicity and therefore was withdrawn from clinical use. The first credentials for safety and efficacy issues arose from phase III clinical studies that compared dabigatran to enoxaparin or
warfarin in patients undergoing orthopedic surgery [6]. However, the conclusive results are based on the RE-LY trial, which assessed the two-year outcomes of some 18,113 AF patients receiving dabigatran (110 or 150 mg twice daily) or warfarin. In this study, the rate of the primary outcome (systemic embolism) was lower with dabigatran at a dosage of 150 mg twice daily. Importantly, although gastrointestinal bleeding in elderly patients slightly increased, a 70% reduction in intracerebral bleeding was observed in patients receiving both doses of dabigatran as compared to warfarin. Moreover, continuous monitoring revealed no significant increase in hepatic enzymes associated with dabigatran. Nonetheless, there was a very small but statistically significant increase in the risk of myocardial infarction with dabigatran (reaching statistical significance in the 110 mg regime), which may temper some of the enthusiasm for the use of this drug.

Another important issue with dabigatran is the lack of an antidote for reversal of anticoagulation, although, in extreme cases, can be controlled by fresh plasma infusion.

Dabigatran etexilate is an oral pro-drug rapidly converted by serum esterases to dabigatran, a competitive direct thrombin inhibitor. It reaches peak plasma concentrations within 1–2 hours after administration and has a half-life of 12–17 hours. It necessitates twice-daily administration, does not interact with CYP450 system and is excreted by kidneys. There are, however, other "attention" labels to its use. Dabigatran is a substrate of p-glycoprotein, a protein involved in active transportation of drugs through membranes. Therefore, drugs that strongly inhibit (amiodarone, verapamil, and quinidine) or induce (rifampicin, pantoprazole) p-glycoprotein activity should be used with caution or avoided. Nonetheless, contrary to expectances, such interactions did not reveal relevant in the RE-LY study.

From a practical point of view, besides evaluating the above-mentioned medical conditions, when considering a switch from warfarin to dabigatran we should ask whether patients have plain access to anticoagulation centers, can fully comply with the twice-daily dosing, or prefer one to another treatment. On the other hand, cost issues will also have a great impact in determining treatment choices. However, a preliminary cost-effective study that based the estimates on the United Kingdom prices of dabigatran showed promising results [7].

In conclusion, provided the brand new, guideline-recommended dabigatran fulfill current promises, we may be witnessing an historical warfarin-to-dabigatran handover.

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