

c-Myc and Cancer Metabolism

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Abstract

The processes of cellular growth regulation and cellular metabolism are closely interrelated. The c-Myc oncogene is a "master regulator" which controls many aspects of both of these processes. The metabolic changes which occur in transformed cells, many of which are driven by c-Myc overexpression, are necessary to support the increased need for nucleic acids, proteins, and lipids necessary for rapid cellular proliferation. At the same time, c-Myc overexpression results in coordinated changes in level of expression of gene families which result in increased cellular proliferation. This interesting duality of c-Myc effects places it in the mainstream of transformational changes and gives it a very important role in regulating the "transformed phenotype." The effects induced by c-Myc can occur either as a "primary oncogene" which is activated by amplification or translocation or as a downstream effect of other activated oncogenes. In either case, it appears that c-Myc plays a central role in sustaining the changes which occur with transformation. Although efforts to use c-Myc as a therapeutic target have been quite frustrating, it appears that this may change in the next few years. *Clin Cancer Res*; 18(20); 5546–53. ©2012 AACR.

Introduction

It has become increasingly clear over the past 2 decades that the metabolic changes that accompany transformation are intimately related to the growth abnormalities of malignant cells and that these metabolic changes are necessary to provide the energy required for rapid cell division. It has also become clear that the multifaceted oncogene, c-Myc, plays important regulatory roles in many aspects of transformation (1–4). Although c-Myc may play a primary oncogenic role in tumors such as Burkitt lymphoma in which it is translocated under the promoter regions of the heavy- or light-chain immunoglobulin genes (5), it is more commonly a downstream "early-response" gene, which responds to activation of many diverse signaling pathways. It remains unclear whether c-Myc overexpression is primarily responsible for the metabolic changes induced by transformation or whether its common overexpression may be a result of the complex metabolic changes which occur when cells become malignant.

Although our detailed understanding of cancer metabolism and growth regulation have evolved independently (6), these 2 areas of investigation have now merged. For a period of time extending into the 1970s, cellular metabolism was regarded as a collection of metabolic pathways which largely served for energy production. We now know

that malignant transformation induces changes in almost every aspect of metabolism, allowing transformed cells to fill the huge demand for proteins, lipids, and nucleic acids necessary to support rapid cell division. The changes in glycolysis which accompany transformation, known as the Warburg effect (7, 8), which have been recognized for more than 80 years are "the tip of the iceberg" in terms of the many metabolic changes now known to occur. Our current understanding of the Warburg effect and its implications for the regulation of cancer cell growth has been refined by several generations of tumor biologists and has had increasing clarity as the molecular biology of the malignant phenotype has been elucidated. The Warburg effect (9) has been observed in many tumor types and is now being used clinically to detect tumors by fluorodeoxyglucose positron emission tomography (FDG-PET; ref. 10).

Since the discovery of transforming oncogenes in the late 1970s, the biochemical study of cancer metabolism has been overshadowed by efforts to identify the mutations that contribute to cancer initiation and progression. Recently, however, it has been shown that all of the key elements of "metabolic transformation" (the Warburg effect)—increased glucose consumption, decreased oxidative phosphorylation, and accompanying lactate production—are induced by oncogene activation. Induced overexpression of the c-Myc gene is responsible for many of the changes that induce malignant changes (see Fig. 1). These changes support the production of intermediates for cell growth and division and are regulated by both oncogenes and tumor suppressor genes in a number of key cancer-producing pathways. Metabolic transformation is the result of complex interactions between a generally hypoxic tumor microenvironment and multiple oncogenic mutations which drive the alterations in cellular metabolism which occur in transformed cells. The

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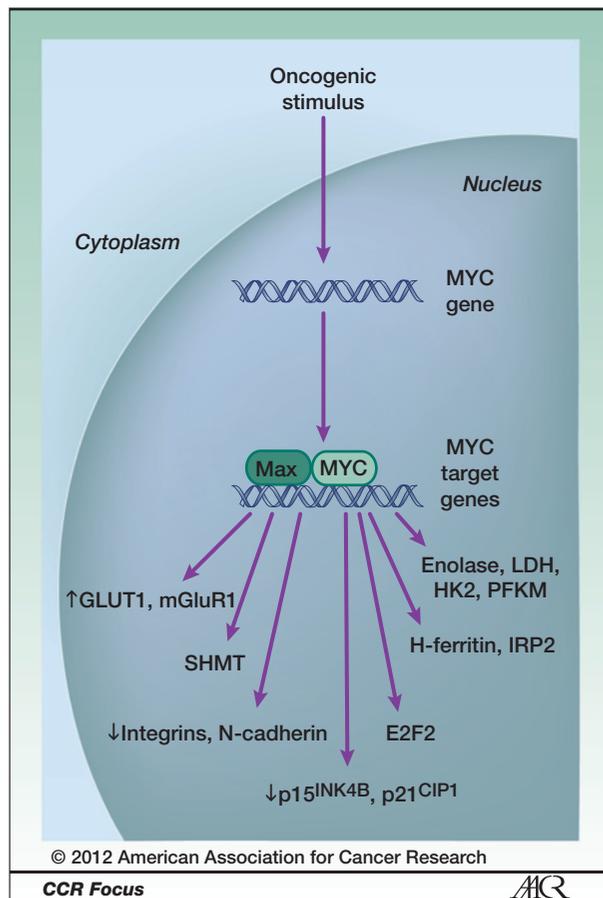


Figure 1. Diagram of Myc effects in transformed cells. There are a wide variety of downstream pathways which are both positively and negatively regulated by Myc expression.

metabolic changes which accompany malignant transformation represent one of a relatively few "hallmarks" of malignancy (11, 12).

Regulation of c-Myc expression

In normal (nontransformed) cells, c-Myc expression and function is tightly regulated by developmental or mitogenic signals. c-Myc mRNA is very short-lived and in the absence of positive regulatory signals, c-Myc transcription decreases and c-Myc protein levels are low, providing no proliferative drive. In tumor cells, on the other hand, c-Myc function is almost always increased, sometimes by mutations in the gene itself but more commonly through the induction of c-Myc expression via upstream oncogenic pathways. The oncogenic properties of c-Myc are counterbalanced by its ability to induce apoptosis through several pathways (13, 14). This dichotomy most likely explains why c-Myc is not commonly the driving oncogene in early tumors.

Another safeguard mechanism by which c-Myc expression is tightly controlled is stability of the c-Myc protein. The short half-life of c-Myc in proliferating cells (~30 minutes; ref. 15) makes this a particularly effective mech-

anism of gene regulation. c-Myc has been shown to undergo ubiquitylation and degradation by the proteasome (16, 17). This includes phosphorylation-dependent degradation of c-Myc (18).

On the other hand, c-Myc overexpression may also occur as a result of posttranslational modifications. For example, mutations in the coding region of c-Myc are commonly found in human lymphomas, particularly in the Thr58 phosphorylation site. This mutation has been shown to enhance the transforming activity of c-Myc by causing inefficient ubiquitination and decreased proteasome-mediated protein turnover (19).

The miRNAs are intertwined in the c-Myc regulatory network, both as targets of c-myc and as regulators of c-Myc expression. The application of microarray technology has revealed both c-Myc-induced and c-Myc-repressed miRNAs. An miRNA which is consistently repressed by c-Myc in multiple tumors is miR-26a (20). On the other hand, overexpression of the let-7a miRNA causes dramatic decreases in c-Myc gene expression and its target genes, as well as antiproliferative activity in lymphoma cells (21). More recent work has also shown that miR-33b (22), miR-143, and miR-145 repress ERK5/c-Myc signaling (23).

c-Myc target genes

Thousands of c-Myc target genes have been identified by one or more differential expression screens including SAGE (24), DNA microarray (25), and subtractive hybridization (3, 26). The list of c-Myc-responsive genes (27) includes genes involved in almost every important cellular function (Table 1). In addition to those genes which are positively regulated by c-Myc, the transcriptional activity of other genes, including *cyclin D1* and *carboxypeptidase D*, is repressed by the c-Myc-Max complex or c-Myc alone (28, 29). Interestingly, it has recently been shown that a network of c-Myc-induced genes accounts for similarities between the transcriptional programs of embryonic stem cells and transformed cells (30).

c-Myc and transformation

The human c-Myc gene was discovered as a result of early studies of very aggressive chicken tumors which led to the identification of the v-myc oncogene as the cause of myelocytomatosis (leukemia and sarcoma; refs. 31, 32). The discovery that human c-Myc is consistently altered by balanced chromosomal translocation in Burkitt lymphoma marked it as a bona fide human oncogene (33). Myc is frequently translocated in multiple myeloma (34) and is one of the most highly amplified oncogenes among many different human cancers (35). Myc is downstream of the deregulated Notch signaling pathways found in T-cell leukemia (36). Hence, c-Myc overexpression may reflect amplification or physiologic overexpression as a downstream member of a signaling pathway.

The c-Myc oncogene is overexpressed in the majority of human cancers and contributes to the cause of at least 40% of tumors (37). It encodes a helix-loop-helix leucine zipper transcription factor that dimerizes with its partner

Table 1. Selected Myc target genes with relevance to metabolic activity of transformed cells (27, 100)

Target gene	Regulation	Pathway	Functional relevance
<i>Cyclin A2, cyclin D2, cyclin E1</i>	Up	Growth factor response	Response to mitogenic stimuli
<i>Enolase, LDH-A</i>	Up	Glycolysis	Metabolic transformation
<i>Serine hydroxymethyl transferase</i>	Up	C1 metabolism	Anaplerosis
<i>EIF4E, ribosomal proteins L3, L6, S15A</i>	Up	Translation initiation	Global increase in translation
<i>Ornithine decarboxylase, prothymosin-α, HMG1/Y</i>	Up	Transformation	Anchorage-independent growth
<i>Iron-regulatory protein-2 H-ferritin, transferrin receptor</i>	Up	Iron metabolism	Required for Myc-induced proliferation
<i>Nucleolin, NM23, nucleophosmin</i>	Up	Cellular proliferation	Required for RNA and DNA synthesis
<i>p21^{CIP1}</i>	Down	DNA damage response	Differentiation
<i>p15^{INK4B}</i>	Down	TGF β pathway	Resistance to growth arrest
<i>N-cadherin, integrins</i>	Down	Cell adhesion	Metastatic potential

protein, Max, to transactivate gene expression. The c-Myc heterodimer can also repress gene expression via binding to the transcription factor Miz1 (38, 39). The other members of the myc gene family (l-Myc and n-Myc) also encode essential transcription regulators whose expression is altered in a wide variety of tumor types. c-Myc binds to the promoters of thousands of genes, although only a fraction of these respond with transcriptional changes (40, 41). c-Myc regulates several large gene families resulting in coordinated changes in cell proliferation and cellular metabolism. c-Myc stimulates genes involved in protein biosynthesis, cancer metabolism, transcription factors, and cell-cycle genes, and some microRNAs while inhibiting expression of other microRNAs and some tumor suppressor genes (38). The pleiotropic effects of c-Myc expression occur at the molecular and cellular level (Fig. 2) and affect almost every activity of cell life.

c-Myc drives cell proliferation

c-Myc is critically involved in the regulation of many growth-promoting signal transduction pathways and is an immediate early response gene which is downstream of many ligand-membrane receptor complexes (42, 43). c-Myc expression is tightly regulated, its level of expression is influenced at the transcriptional level by a number of transcriptional regulatory motifs within its proximal promoter region (4, 44, 45). Although the c-Myc gene product has been most widely characterized as a driver of cell proliferation (38, 46), it also stimulates glycolysis (47, 48) and has been shown to upregulate expression of a broad variety of metabolic enzymes (49).

Normal proliferating cells express low levels of c-Myc RNA and protein in response to a broad variety of mitogenic stimuli. Almost all cancer-associated genetic changes in c-Myc are associated with noncoding regulatory regions rather than protein-coding sequences. This reflects the fact that deregulation of c-Myc expression, rather than expression of a mutated protein, is what drives the c-Myc effect. Although almost all of the abnormalities of metabolic transformation can be related to c-Myc overexpression, as noted above, it is

not always clear whether c-Myc overexpression is a primary or secondary effect in transformed cells

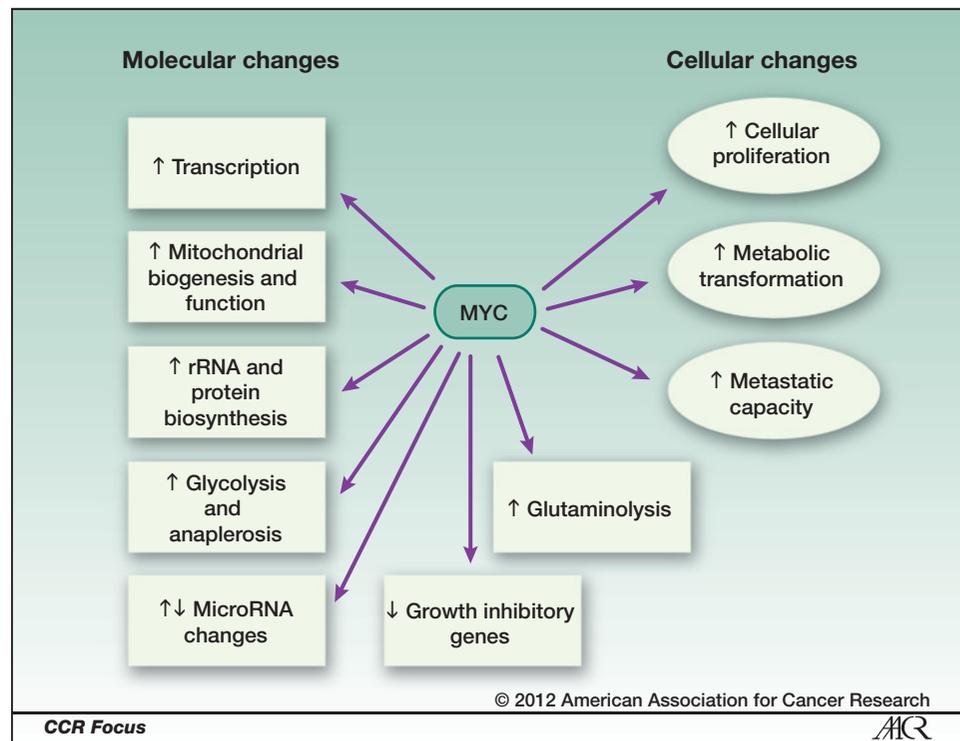
Myc stimulates glycolysis

The first suggestion that c-Myc played an important role in regulation of glycolysis was the observation that lactate dehydrogenase A (LDHA), which converts pyruvate to lactate as part of the glycolytic pathway, was 1 of 20 putative c-Myc target genes (26, 50, 51). Subsequent work has shown that many other glucose metabolism genes are directly regulated by c-Myc, as well (37). These genes include glucose transporter *GLUT1*, hexokinase 2 (*HK2*), phosphofructokinase (*PFKM*), and enolase 1 (*ENO1*; refs. 52–54). Through the upregulation of these genes, c-Myc contributes directly to the Warburg effect (aerobic glycolysis) and the ability of transformed cells to convert glucose to pyruvate even under adequate oxygen tension. Interestingly, *ENO1* has been shown to give rise to an alternative translation initiation product, MBP-1, which is a negative regulator of c-Myc expression (55). This provides a negative feedback loop which is modulated by hypoxia (56).

The direct effects of c-Myc expression on glycolytic activity have been confirmed in studies with transgenic animals (57). Mice which overexpress c-Myc in the liver show increased glycolytic enzyme activity in the liver and overproduce lactic acid. On the other hand, stably transfected rodent fibroblasts overexpressing LDH-A alone, or those transformed by c-Myc, overproduce lactate. This suggests that LDH-A, which is a downstream target of c-Myc, is able to induce the Warburg effect. Soft agar clonogenicity of Burkitt lymphoma cells is markedly decreased by inhibiting expression of LDH-A (50).

Other cancer-related genes also play a role in regulating glycolysis. For example, phosphoinositide 3-kinase (PI3K) and its downstream effector Akt have a direct role in stimulating glucose uptake and metabolism, rendering the transformed cell addicted to glucose for the maintenance of survival. More recent studies have linked Ras, von Hippel-Lindau (VHL), and mutations of isocitrate dehydrogenase 1 (IDH1; ref. 58), succinate dehydrogenase (SDH), and

Figure 2. Pleiotropic effects of c-Myc expression. The c-myc gene has a variety of molecular and cellular effects (which are closely related). These effects result from myc-mediated changes in large gene families which drive cellular functions. Microarray studies have shown that these changes occur in concert and have major effects on cellular function.



fumarate hydratase (FH) to the activation of glycolysis through hypoxia-inducible factor (HIF)-1. This results in increased glycolytic enzyme gene expression (50, 59–61). On the other hand, the *Akt* oncogene was shown to stimulate glycolysis posttranscriptionally, and the p53 tumor suppressor emerged as another regulator of mitochondrial function and glycolysis, showing that loss of p53 is associated with enhanced glycolysis (59). The signaling molecule Ras, which becomes a powerful oncogene when mutated, stimulates glycolysis (51, 62, 63). Akt kinase, a well-characterized downstream effector of insulin signaling, reprises its role in glucose uptake and utilization in the cancer setting (64). Other work also suggests that p53-mediated regulation of glucose metabolism may be dependent on the transcription factor NF- κ B (65). The M2 splice isoform of pyruvate kinase (PKM2) is a key regulator of aerobic glycolysis in cancer cells (66).

c-Myc stimulates mitochondrial biogenesis

In addition to its important role in regulating cellular metabolism by altering expression of genes involved in metabolic pathways, c-Myc also plays an important role in mitochondrial biogenesis. Large-scale studies of gene expression in rat and human systems first suggested that c-Myc overexpression can induce nuclearly encoded mitochondrial genes (24, 25, 29). In addition, c-Myc has been shown to bind to the promoters of gene-encoding proteins involved in mitochondrial function (24, 67). Using an inducible c-Myc-dependent human B-cell model of cell proliferation, it was shown that mitochondrial bio-

genesis is completely dependent on c-Myc expression (68). Moreover, the genes involved with mitochondrial biogenesis were among the most highly induced c-Myc target genes.

In addition to its role in generation of functional mitochondria, c-Myc appears to increase mitochondrial function. It has been shown that c-Myc increases mitochondrial synthesis of acetyl-CoA which, in turn, contributes to significant increases in histone acetylation and fatty acid biosynthesis in rapidly dividing cells (69, 70). The ability of c-Myc to induce mitochondrial biogenesis in proliferating cells while inhibiting mitochondrial respiration is very important because mitochondria not only provides a means for efficient production of ATP in the presence of oxygen, but they also play a role in generating substrates for macromolecular synthesis in dividing cells. These components include pyrimidines, whose synthesis is directly linked to the electron transport chain, the carbon backbone for amino acids, as well as citrate which is converted to acetyl-CoA for lipid biosynthesis. These functions complement the stimulation of glucose uptake and metabolism by c-Myc, which provides carbon backbones for critical cellular components, including ribose for nucleotide biosynthesis and NADPH through the pentose phosphate pathway for redox homeostasis, triglycerides and ATP through glycolysis.

c-Myc regulates glutamine metabolism

In addition to their use of glucose, mammalian cells obtain energy for growth and proliferation through the catabolism of glutamine (48, 71, 72). Induced c-Myc

overexpression coordinates the expression of genes necessary for cells to engage in glutamine catabolism that exceeds the cellular requirement for protein and nucleotide biosynthesis. A consequence of this c-Myc-dependent glutaminolysis is the reprogramming of mitochondrial metabolism to depend on glutamine catabolism to sustain cellular viability and tricarboxylic acid (TCA) cycle anapleurosis. Some human tumors have been reported to consume so much glutamine that they decrease circulating plasma glutamine levels (73, 74).

Glutamine is used as a source of energy and nitrogen for biosynthesis and a carbon substrate for anabolic processes in cancer cells (75, 76), but the regulation of glutamine metabolism is not well-understood (75, 77, 78). In contrast to most other metabolites that are taken up by proliferating cells which are not catabolized, but instead are used as substrates for anabolic macromolecular synthesis, glutamine is a very important energy source (72). Glutamine metabolism is an important mitochondrial function in cancer cells, specifically enzymatic glutaminolysis that catabolizes glutamine to generate ATP and lactate (77).

Recent studies have shown that cancer-related alterations in glucose and glutamine metabolism are strongly influenced by c-Myc expression (79). In particular, the demonstration of persistent, c-Myc-dependent hypoxic metabolism of glutamine, even in the absence of glucose, suggests that this is a major influence of c-Myc expression. In fact, in transformed cells, overexpression of c-Myc results in the concurrent conversion of glucose to lactate and the oxidation of glutamine via the TCA cycle (79). Under hypoxic conditions with high c-Myc, a substantial fraction of the glucose consumed was converted to excreted lactate, and glutamine continued to be used by the TCA cycle, which was used for cell survival. This study also found that a glutamine-dependent and glucose-independent TCA cycle may operate under both aerobic and hypoxic conditions under glucose-depleted culture conditions. Moreover, they observed an enhanced conversion of glutamine to glutathione under hypoxia; glutathione is an important reducing agent for controlling the accumulation of mitochondrial reactive oxygen species (ROS). They also showed that inhibition of glutaminase effectively kills hypoxic cancer cells *in vitro* and delays tumor xenograft growth. The essential role of glutamine metabolism in cell survival and proliferation under hypoxia and glucose deficiency makes cells susceptible to the glutaminase inhibitor bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) and hence could be targeted for cancer therapy (as described below).

Interestingly, c-Myc appears to alter glutamine metabolism by transcriptionally repressing the miRNAs, miR-23a, and miR-23b (80, 81). This results in increased expression of their target protein, mitochondrial glutaminase (GLS). This leads, in turn, to upregulation of glutamine catabolism (82), resulting in increased glutamate which is further metabolized through the TCA cycle or serves as a substrate for glutathione synthesis. This unexpected mechanism of

gene regulation connects myc regulation of miRNAs, glutamine metabolism, and ROS homeostasis.

c-Myc is a therapeutic target

Because of its ubiquitous role in human tumors, c-Myc is an attractive therapeutic target. Blocking the metabolic pathways which are "driven" by c-Myc or restoring these altered pathways could lead to a new approach in cancer treatment. Inhibition of LDH-A has been shown to inhibit tumor progression (83). Other groups have targeted c-Myc transcription by interfering with chromatin-dependent signal transduction (84). Using a potent, selective small-molecule inhibitor of (BET) bromodomain proteins, proteins which associate with acetylated chromatin and activate transcription by increasing the concentration of attracted transcriptional activators (85, 86). Using this approach, growth-inhibitory activity was seen in 3 murine models of multiple myeloma (87). Histone deacetylase inhibitors, including several which are in early-phase clinical trials, have also shown marked downregulation of c-Myc (88–91). Another interesting approach has been the development of a dominant negative c-Myc construct, Omomyc, which is a c-Myc-derived bHLHZip domain which forms heterodimers with wild-type c-Myc but interferes with the formation of c-Myc–Max dimers and suppresses binding to E-box elements (92–94). More recent work (94) has shown that this interesting c-Myc inhibitor effects some, but not all, c-Myc functions. In addition, work which targets closely related pathways, such as the HIF-1 pathway, may have dramatic effects on c-Myc function (95).

Other groups have focused on transcriptional inhibition of the c-Myc gene. Preliminary evidence from experiments using c-Myc antisense oligonucleotides has been encouraging but has not translated into effective clinical treatments (96). Using the Pu27 quadruplex-forming sequence present in the c-Myc promoter, quadruplex-stabilizing compounds have been shown to decrease c-Myc expression levels (97). Recent work has shown that treating cells with oligonucleotides encoding the genomic c-Myc promoter quadruplex-forming sequence, Pu27, results in leukemic cell death (98). New approaches, such as genome scale metabolic modeling, hold promise for the identification of novel drug targets and biomarkers (99)

Summary

The c-Myc gene serves as a "master regulator" of cellular metabolism and proliferation. Because it is activated by a large number of oncogenic pathways and, in turn, stimulates many of the metabolic changes that result in malignant transformation, it is truly "both the chicken and the egg." Under normal circumstances, c-Myc is dependent on mitogenic stimulation for its expression and function. c-Myc is a multifunctional transcription factor which drives the multiple synthetic functions necessary for rapid cell division while at the same time, inhibiting expression of genes with antiproliferative functions. Because of its propensity to induce apoptosis, its expression is tightly regulated. It

influences expression of a wide variety of gene families which contribute to the abnormal growth abilities of transformed cells. It is quite clear that this central role in regulating cell function represents a unique opportunity to develop novel cancer therapies.

Disclosure of Potential Conflicts of Interest

D.M. Miller is on the Board of Directors of Advanced Cancer Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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Development of methodology: D.M. Miller, S.D. Thomas, A. Islam

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D.M. Miller, S.D. Thomas, A. Islam, D. Muench, K. Sedoris

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.D. Thomas, A. Islam, D. Muench, K. Sedoris

Writing, review, and/or revision of the manuscript: D.M. Miller, S.D. Thomas, K. Sedoris

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