Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR Inhibitors: Rationale and Importance to Inhibiting These Pathways in Human Health

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Keywords: *Key Words: Targeted Therapy, Combination Therapy, Drug Resistance, Cancer Stem Cells, Aging, Senescence, Raf, Akt, PI3K, mTOR*

Received: *February 25, 2011,* ***Accepted:*** *March 10, 2011,* ***Published:*** *March 11, 2011*

ABSTRACT:

The Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascades are often activated by genetic alterations in upstream signaling molecules such as receptor tyrosine kinases (RTK). Integral components of these pathways, Ras, B-Raf, PI3K, and PTEN are also activated/inactivated by mutations. These pathways have profound effects on proliferative, apoptotic and differentiation pathways. Dysregulation of these pathways can contribute to chemotherapeutic drug resistance, proliferation of cancer initiating cells (CICs) and premature aging. This review will evaluate more recently described potential uses of MEK, PI3K, Akt and mTOR inhibitors in the proliferation of malignant cells, suppression of CICs, cellular senescence and prevention of aging. Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways play key roles in the regulation of normal and malignant cell growth. Inhibitors targeting these pathways have many potential uses from suppression of cancer, proliferative diseases as well as aging.

INTRODUCTION

The Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR signaling cascades have been extensively studied over the past few decades. In this time there have been breakthroughs in the discovery of pathway components, the mechanisms by which they relay their signals and how mutations of these components can lead to aberrant signaling and uncontrolled proliferative diseases. Research has also lead to the development of inhibitors that specifically target critical elements of these pathways in anticipation of ameliorating patient survival. This review will discuss some of the current inhibitors, their targets and how they are being used to treat cancer and other proliferative diseases including aging.

Signaling through the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways are carefully orchestrated events generally starting from the cell surface and leading to controlled gene expression within the nucleus. Regulation of these pathways is mediated by a series of kinases, phosphatases and various exchange proteins. Mutations occur in many of these pathway elements leading to uncontrolled regulation and aberrant signaling. An overview of the effects of mutations and the activation of these signaling pathways is presented in Figure 1. Deregulated signaling can lead to unrestrained cellular growth and proliferation ultimately resulting in tumor formation or abnormal cellular growth and premature aging. As such, a great deal of research has been aimed to target these mutated proteins to prevent abnormal signaling [1-5].

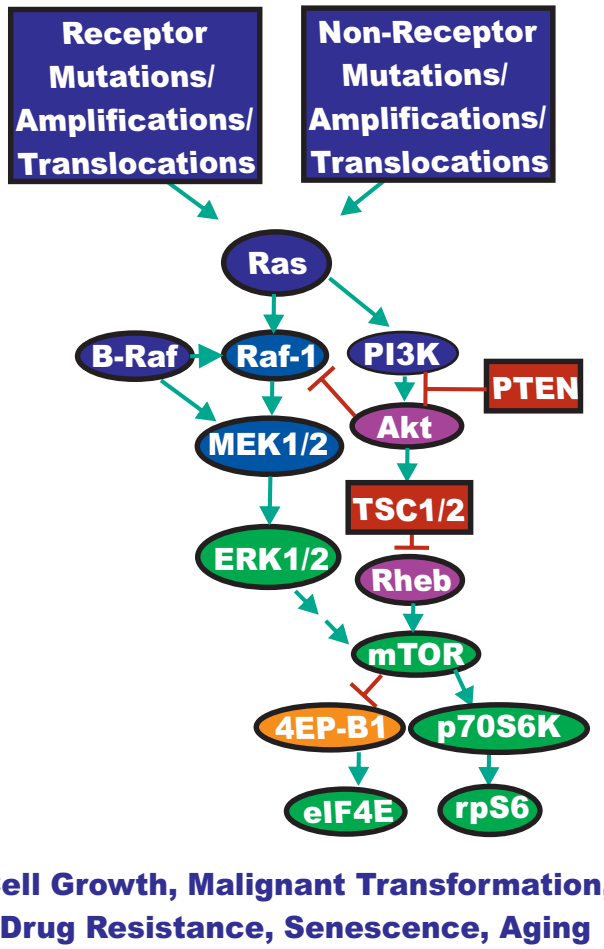

Figure 1: Dysregulated Expression of Upstream Receptors and Kinases Can Result in Activation of the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR Pathway. Sometimes dysregulated expression of growth factor receptors occurs by increased expression, genetic translocations or genomic amplifications which can lead to activation of the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways. Alternatively chromosomal translocations can occur in non-receptor kinases and other genes which result in activation of these pathways. Genes in the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways that have activating mutations detected in human cancer and proliferative diseases are indicated in blue ovals. Genes overexpressed in certain cancers are indicated in purple ovals. Tumor suppressor genes mutated in human cancer are indicated in red rectangles. Other key genes are indicated in green ovals. Genes inactivated by the Ras/PI3K/PTEN/Akt/mTOR pathway are indicated in orange ovals. Green arrows indicate activating events in pathways. Blocked red arrows indicating inactivating events in pathway.

MUTATIONS OR ALTERED EXPRESSION OF THESE PATHWAYS CAN LEAD TO SENSITIVITY TO THERAPY.

Some cancer cells carrying BRAF mutations are highly sensitive to MEK inhibitors, while cells lacking these BRAF mutations or containing RAS or epidermal growth factor receptor (EGFR) mutations are resistant [5, 6]. Increased Akt activity may actually render cells and patients sensitive to Akt as well as downstream mTOR inhibitors. The formation of the rapamycin-sensitive mTORC1 complex (consisting of mTOR, regulatory–associated protein of mTOR [Raptor], DEPTOR and mLST8) in certain cancer cells that overexpress activated Akt may be altered in comparison to cells that do not overexpress Akt. In cells that express activated Akt, Akt may phosphorylate TSC-2 resulting in its inactivation. The mTORC1 complex is formed and downstream $p70^{S6K}$ and 4E-BP1 are phosphorylated, allowing the dissociation of eIF-4E, ribosome biogenesis and protein synthesis. In contrast, in the absence of Akt activation, this complex should not be formed. Rapamycin targets this complex; hence the cells that express elevated levels of activated Akt cells may be more sensitive to rapamycin than the cancer cells that do not express high levels of activated Akt. In the cells that do not express elevated levels of activated Akt, this complex should be transiently assembled after growth factor treatment. In contrast, the assembly of the rapamycin-insensitive mTORC2 complex (consisting of rapamycin insensitive companion of mTOR [Rictor], mTOR, DEPTOR, mLST8) should be lower in the cells that express elevated levels activated Akt than in those cells that do not as there is equilibrium between the mTORC1 and mTORC2 complexes. The significance of these complex biochemical signaling events is that cancer cells that overexpress activated Akt or lack PTEN expression have an Achilles heel with regards to therapeutic intervention as they are highly sensitive to rapamycin treatment. An overview of the interactions between the Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/

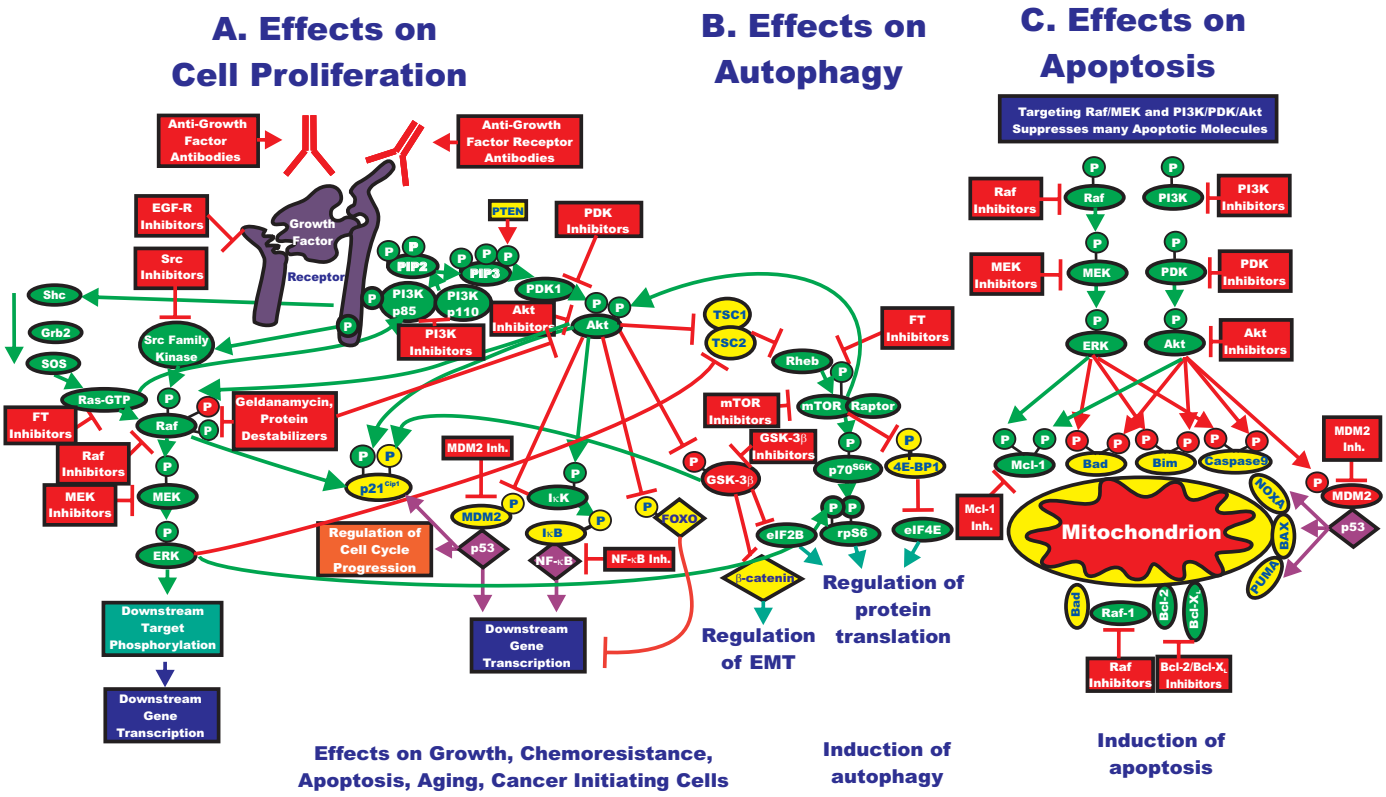

Figure 2: Rationale for Targeting Both the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR Pathways for Suppressing Cancer Growth. A: The Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways are both activated by upstream receptor ligation and frequently co-regulate many downstream targets in parallel. Thus for effective elimination of many cancers or prevention of aging, it may be necessary to target both signaling pathways. Activation of these pathways could also result in increased transcription of many genes that promote cellular growth and malignant transformation. B. Inhibition of mTOR can result in the induction of autophagy, which is a very important mechanism of cell death, especially in solid tumors. C. As described previously, both the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways regulate the activity of apoptotic proteins by post-translational mechanisms. Targeting this pathway may also contribute to the induction of apoptosis. Signaling molecules promoting phosphorylation events are indicated in green. Stimulatory signaling events are indicted in green lines with a green arrow before the target of the phophorylation. Small molecule inhibitors are indicated in red. Inhibitory phosphorylation events are indicated in red lines with a block on the end before the target of the inhibition. Inhibitory signaling or proapoptotic molecules or inactivated molecules are indicated in yellow. A growth factor and a growth factor receptor are indicated in purple. Active transcription factors are indicated in purple diamonds. Inactivated transcription factors are indicated in yellow diamonds.

mTOR pathways and the effects of these pathways on growth, autophagy and apoptosis is presented in Figure 2.

OVERVIEW OF PATHWAY INHIBITORS

Effective inhibitors specific for many of the key components of the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/mTOR pathways have been developed [7-35]. In many cases, these inhibitors have been examined in clinical trials. Furthermore, inhibitors that target the mutant but not the wild type (WT) alleles of various genes (*e.g.*, *BRAF* and *PIK3CA*) either have been or are being characterized. Thus specific inhibitors have been made and some are currently in the clinic. Targeting some components of these pathways has proven clinically effective and in some of the diseases have a very large market with few effective treatments [(*e.g.*, Sorafenib and hepatocellular carcinoma (HCC)] [7].

RAF/MEK INHIBITORS

Raf inhibitors have been developed and some are being used for therapy while others are being evaluated in clinical trials (See Table 1). Some inhibitors (*i.e,* Sorafenib, Bayer) were initially thought to specifically inhibit Raf but have been subsequently shown to have multiple targets (*e.g.*, VEGF-R, Flt-3, PDGF-R). However, that does not preclude their usefulness in cancer therapy. Sorafenib is approved for the treatment of certain cancers (*e.g.,* renal cell carcinoma (RCC) and patients with unresectable HCC and is currently being further evaluated in the Sorafenib Hepatocellular carcinoma Assessment Randomized Protocol (SHARP) trial, which demonstrated that the drug was effective in prolonging median survival and time-to-progression in patients with advanced HCC. Sorafenib is generally well tolerated in HCC patients with a manageable adverse events profile [7]. MEK inhibitors have also been examined for treating HCC in mouse models [8,9] but they do not appear to be as effective as Sorafenib, most likely due to the broad specificity of Sorafenib, which inhibits other targets besides Raf.

PLX-4720 (Plexxikon/Roche) (R7204) is a mutant B-Raf specific inhibitor that has been used for preclinical studies [10]. PLX-4032 is a B-Raf inhibitor that is being evaluated in clinical trials. PLX-4720 was designed using a unique screening platform developed by Plexxikon that involved the use of structural and medicinal chemistry techniques [10]. This more selective screening approach has resulted in a series of B-Raf inhibitors based on the structural implications of BRAF mutation and which discriminate between the mutant and WT protein. PLX-4720 is orally available and is highly selective for the mutant B-Raf protein. PLX-4720 is effective against melanomas, as well as colorectal tumors and other cancers, with the $BRAF^{V600E}$ mutation. $BRAF^{V600E}$ has been associated with more aggressive tumors and lower rates of patient survival [10]. The IC50 value for PLX-4720 is approximately 3-fold lower in in vitro kinase assays with mutant versus WT B-Raf proteins and demonstrates an approximately 60-fold lower IC50 value in vivo when cell lines with mutant and WT BRAF genes are compared [10]. The IC50 value for PLX-4720 was compared with Sorafenib in a panel of melanomas, colon carcinomas and NSCLC. The BRAF gene status was known in all of these cell lines. The IC50 value for PXL-4720 was approximately 100-fold lower (range: 17.5 to 280 nM) than Sorafenib in melanomas and colon carcinomas that had the $BRAF^{V600E}$ mutation; however, the IC50 value for PLX-4720 was approximately the same as Sorafenib in colon carcinomas and NSCLC without BRAF mutations, but with RAS mutations [10]. PLX-4720 arrests mutant but not WT B-Raf melanoma cells at the G0/G1 cell-cycle stage and initiates apoptosis in these cells. The additional B-Raf inhibitor (PLX-4032) developed by Plexxicon shows promising effects [11].

NEED FOR GENETIC SCREENING BEFORE TREATMENT WITH RAF KINASE INHIBITORS.

It has recently become apparent that it will be critical to determine the genetic status at both B-Raf and Ras before treatment with B-Raf selective inhibitors [12]. Class I B-Raf inhibitors (active conformation inhibitors) such as (PLX4720 and 885-A, a close analog of SB590885) will inhibit B-Raf mutants, however these ATP-competitive B-Raf inhibitors will not inhibit WT B-Raf or mutant Ras. In fact, these B-Raf inhibitors can activate Raf-1 in these cells in the presence of active Ras. 885-A could induce B-Raf binding to Raf-1. PLX-4720 can, to a lesser extent, induce B-Raf binding to Raf-1 when the ERK-mediated negative feedback loop on B-Raf was inhibited with a MEK inhibitor. These binding events were determined to require the present of activated Ras (WT or mutant), which may be necessary for the translocation from the cytoplasm to the membrane and assembly into the signaling complex. This has therapeutic implications, as in patients with mutant *RAS*, if they are treated with certain B-Raf inhibitors, B-Raf can bind and activate Raf-1 and promote the oncogenic pathway. In fact, even kinase-dead *BRAF* mutations, which are observed in human cancer, the mutant B-Raf proteins can dimerize with Raf-1, when stimulated by the mutant Ras protein and activate the Raf/MEK/ERK cascade. Clearly for B-Raf-selective inhibitors to be therapeutically useful, prior screening of patients for *RAS* mutations will be mandatory, as well as perhaps additional screening during treatment. Otherwise resistance may develop and lead to further stimulation of the Raf/MEK/ERK cascade.

Table 1: Inhibitors of Raf/MEK and PI3K/PDK/Akt/mTOR – part 1

Inhibitor	Target(s)	Cancer Examined	Clinical Trials	Company	Ref.
		Ras Inhibitors			
Tipifarnib (Zarnestra™, R115777)	Ras, farnesyl-transferase, Rheb	AML, lymphoma, breast, glioma, melanoma	Phase I, II, III	Johnson & Johnson	121, 122, www.clinicaltrials.gov
		Raf Inhibitors			
BAY 43-9006 (Nexavar®, Sorafenib tosylate)	Raf, VEGFR2, VEGFR3, PDGF-R, c-Kit, c-Fms, Flt-3	renal cell carcinoma, HCC, melanoma, leukemias	Phase I, II, III	Bayer	7, 31, 33, 58, 59, 61, 71, 73, 79, 158, www.clinicaltrials.gov
AAL-881	Raf	thyroid, glioma	Preclinical	Novartis	96, 97
LBT-613	Raf	glioma, thyroid	Preclinical	Novartis	96, 157
RAF265	B-Raf, Raf-1 (c-Raf), A-Raf, B-Raf^{V600E}, VEGFR-2	melanoma	Phase I	Novartis	data on file. Novartis Pharma AG, Basel, Switzerland (Internet)
XL281	B-Raf, c-Raf, B-Raf^{V600E}	colorectal, papillary thyroid, ovarian, prostate, carcinoid tumors, melanoma	Phase I	Exelixis/Bristol Myers Squibb	98
SB-590885	Raf, B-Raf^{V600E}	melanoma	Preclinical	GlaxoSmithKline	99, 155
PLX-4720	Raf, B-Raf^{V600E}	melanoma	Preclinical	Plexxikon/Roche	10
PLX-4032	Raf, B-Raf^{V600E}	melanoma, thyroid, ovarian, solid tumors	Phase I	Plexxikon/Roche	11, www.clinicaltrials.gov
L-779,450	Raf	leukemia	Preclinical	Merck	13, 106
GW5074	Raf-1 (c-Raf)	melanoma, glioblastoma	Preclinical	GlaxoSmithKline	105, 156
SB-699393	Raf		Preclinical	GlaxoSmithKline	106
		MEK inhibitors			
CI-1040 (PD-184352)	MEK1, MKK5	colorectal, NSCLC, pancreatic, kidney, melanoma, breast	Phase I, II (discontinued)	Pfizer	13,17,25,27,29,74,77, 126, www.clinicaltrials.gov
PD0325901	MEK1/2	breast, colon, NSCLC, melanoma	Phase I, II (discontinued)	Pfizer	5,13,28,29,34,35,39, 74,191, www.clinicaltrials.gov
XL518	MEK		Phase I	Exelixis	145, Exelixis (internet), www.clinicaltrials.gov
Selumetinib (AZD6244, ARRY-142886)	MEK	melanoma, HCC, pancreatic, colon, lung, breast	Phase I, II	Astra Zeneca/Array BioPharma	3, 8, 13, 21, 23, 24, 61, 62, 78, 84, ww.clinicaltrials.gov
RDEA119 (BAY 869766)	MAP2K1 (MAPK/ERK kinase 1)	advanced tumors	Phase I, II	Ardea/Bayer	15, www.clinicaltrials.gov
PD098059	MEK1/2	advanced hematological and advanced solid cancers	Preclinical	Parke-Davis/Pfizer	14, 81, 102, 191, 215, 251, 256

Table 1: Inhibitors of Raf/MEK and PI3K/PDK/Akt/mTOR – part 2

Inhibitor	Target(s)	Cancer Examined	Clinical Trials	Company	Ref.
U0126	MEK1/2	advanced hematological and advanced solid cancers	Preclinical	DuPont Pharmaceuticals	14, 81, 83, 104, 215, 256, 267
SL-327	MEK1/2	not evaluated for use in cancer treatment	Preclinical	DuPont Pharmaceuticals	159
		PI3K/Akt/mTOR inhibitors			
UCN-01	PDK-1, Chk1, PKC isoforms	leukemia, lymphoma, ovarian, peritoneal cavity, fallopian tube	Phase I, II	Kyowa Hakko Kogyo Co., Ltd./Keryx Biopharmaceuticals	108, www.clinicaltrials.gov
(NVP)-BAG956	PDK, p110 PI3Ks (except for β isoforms)	leukemia, melanoma	Preclinical	Novartis	111, 112, 153
Celecoxib (Celebrex®)	PDK-1, COX-2	lung, prostate, H&N,	Phase I, II	Pfizer	36,162, 163, www.clinicaltrials.gov
OSU-03012	PDK-1	prostate, glioma, leukemia, HCC, breast	Preclinical	Arno Therapeutics/ The Ohio State University	131-133
BX-795	PDK-1, ERK8, TBK1, IKK-ε	breast, prostate, colon, melanoma, pancreatic, cervical	Preclinical	Berlex/Bayer	124-126
BX-912	PDK-1	breast, prostate, colon, melanoma, pancreatic, cervical	Preclinical	Berlex/Bayer	124-126
BX-320	PDK-1	breast, prostate, colon, melanoma, pancreatic, cervical	Preclinical	Berlex/Bayer	124-126
AR-12	PDK-1, PI3K, Akt	breast, colon, lung, prostate, lymphoma	Phase I	Arno Therapeutics	Arno Therapeutics (internet), www.clinicaltrials.gov
KP372-1	PDK-1, Akt, Flt3	AML, thyroid, glioblastoma	Preclinical	Kinetek Pharmaceuticals	47, 140, 141
LY294002	PI3K, other related kinases	advanced hematological and advanced solid cancers	Preclinical	Lilly	107, 126, 139, 215, 251, 256, 267
PWT-458	PI3K	NSCLC, glioblastoma, renal	Preclinical	Wyeth/Pfizer	136, 137
PX-866	PI3K	glioma, breast, colon, prostate, NSCLC, pancreatic advance solid tumors	Phase I	Oncothyreon Inc.	134, 135, www.clinicaltrials.gov
CAL-101	PI3K (p110δ)	leukemias, lymphomas, myeloma	Phase I	Calistoga Pharmaceuticals	Calistoga Pharmaceuticals (Internet), www.clinicaltrials.gov
XL-147	PI3Ks	NSCLC, solid tumors	Phase I	Exelixis/Sanofi-Aventis	142, Exelixis (internet), www.clinicaltrials.gov
ZSTK474	PI3Ks	NSCLC, melanoma, ovarian, prostate,	Preclinical	Zenyaku Kogyo Co. Ltd	143, 144
GDC-0941	PI3K (p110α), Flt3	lymphoma, NSCLC, breast, solid tumors	Phase I	PIramed Pharma/Roche/ Genetech	146-148, www.clinicaltrials.gov
(NVP)-BEZ235	PI3K, mTOR	breast, glioma, melanoma, pancreatic	Phase I, II	Novartis	54, 149, 150, 170, www.clinicaltrials.gov
AS-252424	PI3Ks (p110γ)		Preclinical	Merck Serona	154

Table 1: Inhibitors of Raf/MEK and PI3K/PDK/Akt/mTOR – part 3

Inhibitor	Target(s)	Cancer Examined	Clinical Trials	Company	Ref.
TGX-221	PI3K (p110β)	treatment for coronary heart disease, not evaluated for use in cancer treatment	Preclinical	Alexis/Enzo Life Sciences, Inc.	160, 161
XL-765	PI3K, mTOR	glioma, NSCLC	Phase I	Exelixis/Sanofi-Aventis	145, Exelixis (internet), www.clinicaltrials.gov
Wortmannin	PI3K, mTOR, DNA-PK, MAPK	advanced hematological and advanced solid cancers	Preclinical		126, 127, 138, 139
PI-103	p110 PI3Ks, mTORC1/2, DNA-PK	glioma, prostate, colon, NSCLC	Preclinical	PIramed Pharma/Roche	44,126-130
Perifosine (KRX-0401)	Akt, MEK 1/2, ERK 1/2, JNK	multiple myeloma, leukemias, NSCLC, advance solid tumors	Phase I, II	Æterna Zentaris Inc./Keryx Biopharmaceuticals	48, 109, 110, 172, 174, 175, www.clinicaltrials.gov
Triciribine (API-2)	Akt 1, 2, 3	AML, advanced hematological cancer	Phase I	VioQuest Pharmaceuticals	45, 55, 166, www.clinicaltrials.gov
SR13668	Akt	breast, prostate, ovarian	Preclinical	SRI International	123, SRI International (internet)
AR-67 (DB-67)	Akt	advanced solid tumors	Phase I, II	Arno Therapeutics	Arno Therapeutics (internet)
AR-42	Akt		Preclinical	Arno Therapeutics	Arno Therapeutics (internet)
GSK690693	Akt1, 2, 3	leukemia, lymphoma	Phase I	GlaxoSmithKline	113, 114, www.clinicaltrials.gov
KP372-1	Akt, PDK-1, Flt3	leukemia, thyroid, H&N, glioma	Preclinical	QLT Inc.	47, 140
VQD-002 (API-2)	Akt	NSCLC, leukemias, lymphomas, prostate	Phase I, II	VioQuest Pharmaceuticals	45, 165
A-443654	Akt	hematological and solid cancers	Preclinical	Abbott Laboratories	46, 164
MK-2206	Akt	solid tumors	Phase I	Merck	Merck (internet), www.clinicaltrials.gov
Rapamycin (Sirolimus)	mTORC1	advanced hematological, advanced solid tumors, HIV, AIDS related malignancies	Phase I, II	Wyeth/Pfizer	2, 50, 51, 53, 63-69, 71, 73, 74, 86-88, 151, 152, 173-185, 191, 212, 215, 216, 255, www.clinicaltrials.gov
CCI-779 (Torisel®, Temsirolimus)	mTORC1	leukemia, lymphoma, NSCLC, prostate, colorectal, renal	Phase I, II	Wyeth/Pfizer	2, 50, 53, 70, 115-118, www.clinicaltrials.gov
RAD001 (Afinitor®, Everolimus)	mTORC1, mTORC2	cervical, renal, HCC, leukemia, lymphoma	Phase I, II	Novartis	2, 50, 52, 53, 93, 115-118, 157, www.clinicaltrials.gov
AP-23573 (Ridaforolimus, Deforolimus)	mTORC1	advanced hematological cancer, prostate, endometrial	Phase I, II	Ariad/Merck	2, 50, 53, 74, 119, 120, www.clinicaltrials.gov
		Active Site mTOR Inhibitors			
AZD-8055	mTORC1/ mTORC2	advanced solid tumors, lymphomas, HCC	Phase I, II	AstraZenica	171, 172, 174-176, www.clinicaltrials.gov
OSI-027	mTORC1/ mTORC2	advanced solid tumors, lymphomas	Phase I	OSI Pharmaceuticals	171, 172, 174-176, www.clinicaltrials.gov
INK-128	mTORC1/ mTORC2	advanced cancers, multiple myeloma, Waldenstrom macroglobulinemia	Phase I	Intellikine	172, 174-176, www.clinicaltrials.gov
PP-242	mTORC1/ mTORC2		Phase I	UCSF	171, 172, 174-176

AML = acute myeloid leukemia, HCC = hepatocellular carcinoma, NSCLC = non-small cell lung carcinoma, H&N = head and neck cancer

MEK INHIBITORS

Specific inhibitors of MEK have been developed (*e.g.,* PD98059 (Pfizer), U0126 (DuPont), PD184352 [CI-1040] (Pfizer), PD0325901 (Pfizer), Selumetinib (*a.k.a.*, ARRY-142886, AZD6244) (Astra-Zeneca), and RDEA119 (Ardea Biosciences) (See Table 1) [3, 8-9, 13-30]. MEK inhibitors differ from most other kinase inhibitors as they do not compete with ATP binding (non-ATP competitive), which confers a high specificity [17]. Most MEK inhibitors are specific and do not inhibit many different protein kinases [18] although as will be discussed below, certain MEK inhibitors are more specific than others. The crystal structures of MEK1 and MEK2 have been solved as ternary complexes with ATP and PD184352, and have revealed that both MEK1 and MEK2 have unique inhibitor binding sites located on a hydrophobic pocket adjacent to, but not overlapping with, the ATP-binding site [19]. Furthermore, effective targeting of MEK1/MEK2 is highly specific, as ERK1/ERK2 are the only well-described downstream targets. A distinct advantage of inhibiting MEK is that it can be targeted without knowledge of the precise genetic mutation that results in its aberrant activation. This is not true with targeting Raf as certain Raf inhibitors will activate Raf and also certain B-Raf specific inhibitors will not be effective in the presence of Ras mutations as discussed above.

An advantage of targeting MEK is that the Ras/Raf/MEK/ERK pathway is a convergence point where a number of upstream signaling pathways can be blocked with the inhibition of MEK. For example, MEK inhibitors, such as Selumetinib, are also being investigated for the treatment of pancreatic cancers, breast cancers, and other cancers such as hematopoietic malignancies, including multiple myeloma [20-22].

Selumetinib inhibits MEK1 *in vitro* with an IC50 value of 14.1 ± 0.79 nM [23, 24]; it is specific for MEK1 as it did not appear to inhibit any of the approximately 40 other kinases in the panel tested. Selumetinib is not competitive with ATP. Molecular modeling studies indicate that selumetinib binds to an allosteric binding site on MEK1/MEK2. The binding sites on MEK1/MEK2 are relatively unique to these kinases and may explain the high specificity of MEK inhibitors. This binding may lock MEK1/2 in an inactivate conformation that enables binding of ATP and substrate, but prevents the molecular interactions required for catalysis and access to the ERK activation loop. In basic research studies, treatment with the MEK inhibitor results in the detection of activated MEK1/2 when the western blot is probed with an antibody that recognizes active MEK1/2, while downstream ERK1/2 will not appear activated with the activation specific ERK1/2 antibody [24]. Selumetinib inhibited downstream ERK1/ERK2 activation in *in vitro* cell line assays with stimulated and unstimulated cells, and also inhibited activation in tumor-transplant models. Selumetinib did not prevent the activation of the related ERK5 that occurs with some older MEK1 inhibitors, which are not being pursued in clinical trials. Inhibition of ERK1/2 suppresses their ability to phosphorylate and modulate the activity of Raf-1, B-Raf and MEK1 but not MEK2 as MEK2 lacks the ERK1/ERK2 phosphorylation site. In essence, by inhibiting ERK1/2 the negative loop of Raf-1, B-Raf and MEK phosphorylation is suppressed and hence there will be an accumulation of activated Raf-1, B-Raf and MEK [24]. This biochemical feedback loop may provide a rationale for combining Raf and MEK inhibitors in certain therapeutic situations.

In colon, melanoma, pancreatic, liver and some breast cancers, selumetinib inhibited the growth of tumors in tumor xenograft studies performed in mice. The new MEK inhibitors are also at least 10 to 100-fold more effective than earlier MEK inhibitors and hence can be used at lower concentrations [8, 9, 20-24]. Selumetinib also inhibits the growth of human leukemia cells, but does not affect the growth of normal human cells. Selumetinib also suppressed the growth of pancreatic BxPC3 cells, which do not have a known mutation in this pathway, suggesting that this drug may also be useful for treating cancers that lack definable mutations. However, it is likely that BxPC3 cells have some type of upstream gene mutation/amplification or autocrine growth factor loop that results in activation of the Raf/MEK/ERK pathway.

Selumetinib induced G_1/S cell-cycle arrest in colon and melanoma cancer cell lines and activated caspase-3 and -7 in some cell lines (Malme3M and SKMEL2); however, caspase induction was not observed in other melanoma (SKMEL28) or colon cancer cell lines (HT29), demonstrating that further research needs to be performed with this inhibitor to determine if it normally induces apoptosis and whether the induction of apoptosis can be increased with other inhibitors or chemotherapeutic drugs.

Selumetinib suppressed the tumor growth of pancreatic cells, such as BxPC3, in immunocompromised mice more effectively than conventional chemotherapeutic drugs, such as gemcitabine, which is commonly used to treat pancreatic cancer; however, once treatment with selumetinib was discontinued, the tumors regrew [21]. Most likely MEK inhibitors do not induce apoptosis, but rather, they inhibit proliferation. That is, MEK inhibitors are cytostatic.

An additional MEK inhibitor is PD-0325901 (Pfizer) [27-30], which follows on from the earlier MEK inhibitors PD-98059 and PD-184352, both of which have been extensively examined in preclinical investigations to determine the role of MEK in various biochemical processes. PD-184352 was the first MEK inhibitor to enter clinical trials and it demonstrated inhibition of activated ERK and anti-tumor activity in patients [25,26]; however, subsequent multicenter, phase II studies with patients with diverse solid tumors did not demonstrate encouraging results [27]. This was probably due to low

oral bioavailability and high metabolism, which led to plasma drug levels that were inadequate to suppress tumor growth.

The newer PD-0325901 MEK inhibitor is an orally-active, potent, specific, non-ATP competitive inhibitor of MEK. PD-0325901 demonstrated improved pharmacological and pharmaceutical properties compared with PD-184352, including a greater potency for inhibition of MEK, and higher bioavailability and increased metabolic stability. PD-0325901 has a Ki value of 1 nM against MEK1 and MEK2 in *in vitro* kinase assays. PD-0325901 inhibits the growth of cell lines that proliferate in response to elevated signaling of the Raf/MEK/ERK pathways [27]. Clinical trials with PD-0325901 have documented some successes and some adverse side effects [27-29]. Pfizer has suspended it evaluation in clinical trials. This may have resulted in part from the design of the clinical trials as MEK inhibitors may not be appropriate to treat all types of cancer. MEK inhibitors may be appropriate to treat only those cancers that proliferate in response to activation of the Raf/MEK/ERK pathway [30-32]. Furthermore, it may also be important to include a chemotherapeutic drug or radiation treatment to induce death of the cancer cell.

Raf is also a key therapeutic target [31-34], which lies upstream of MEK. Hence, targeting MEK is an approach to target tumors containing activated RAF genes. The $BRAF^{V600E}$ mutation is present in approximately 6 to 8% of human cancers (overall). Interestingly, approximately 5% of lung cancers have mutations at BRAF which are not at V600E [35]. The effects of PD-0325901 were examined in conditional $BRAF^{V600E}$ tumor models where genetically modified mice express normal B-Raf prior to Cre-mediated recombination, after which they express $B\text{-}Raf^{V600E}$ at physiological levels [35]. When $B\text{-}Raf^{V600E}$ was induced, the mice developed lung tumors which could be inhibited by PD-0325901 (25 mg/kg/day for approximately two weeks, followed by 12.5 mg/kg/day for an additional two weeks). In contrast, mice treated with vehicle alone developed adenomas. This model indicates that in some cases for MEK inhibitors to yield successful outcomes, the therapy needs to include a cytotoxic drug, as the MEK inhibitors are cytostatic and often as soon as the MEK inhibitors are removed, the tumor may re-emerge.

There are few current effective therapies for HCC [36-39]. Hence targeting signaling pathways activated in HCC has been considered an approach to target HCC. Human HCC tumors have higher expression and enhanced activity of MEK1/2 and ERK1/2 compared with adjacent non-neoplastic liver [37]. Over-expression of activated MEK1 in HCC HepG2 cells resulted in enhanced tumor growth *in vivo* [38]. On the other hand, preclinical studies have demonstrated the potential of MEK inhibition to suppress hepatoma cell proliferation and tumorigenicity [9]. Huynh et al. recently reported that treatment of human HCC xenografts with Selumetinib blocked ERK1/2 activation, reduced *in vivo* tumor growth, and induced apoptosis [9]. Moreover, targeting MEK with PD-0325901 had *in vivo* chemopreventive effects on HCC development in an animal model employing TGF-α-transgenic mice in which liver cancers were induced by diethylnitrosamine treatment [39]. Therefore, MEK represents a potential therapeutic target for HCC.

RDEA119 is a more recently described MEK inhibitor developed by Ardea Biosciences [16]. It is a highly selective MEK inhibitor that displays a >100-fold selectivity in kinase inhibition in a panel of 205 kinases. In contrast, in the same kinase specificity analysis, other recently developed MEK inhibitors (*e.g.,* PD0325901) also inhibited the Src and RON kinases.

There are at least two ERK molecules regulated by the Raf/MEK/ERK cascade, ERK1 and ERK2. Little is known about the differential *in vivo* targets of ERK1 and ERK2. The development of specific ERK1 and ERK2 inhibitors is ongoing and may be useful in the treatment of certain diseases such as those leukemias where elevated ERK activation is associated with a poor prognosis (*e.g.*, AML, ALL) [40, 41].

Some tumors are resistant to MEK inhibitors because they contain *EGFR*, *KRAS, PI3KCA* or *PTEN* mutations [6, 42, 43]. Some cells with *EGFR* or *KRAS* mutations are resistant to MEK inhibitors since they can also activate the Ras/PI3K/Akt/mTOR pathway. These studies, which were performed *in vitro* with cells lines and in vivo using xenografts, also demonstrated that PI3K activation and PTEN inactivation were not always equivalent in terms of inhibitor sensitivity. The authors suggested that a possible reason for this phenomenon could be that PTEN has other functions besides the regulation of Akt (*e.g.*, protein phosphatase activity). Furthermore these studies demonstrated that the combination of MEK and PI3K pathway inhibitors could be an effective approach to treat certain cancers that had activation of both pathways.

Only certain types of breast cancer are sensitive to MEK inhibitors [43]. Breast cancers can be classified into three types: luminal breast cancers which are usually estrogen receptor positive and have a relatively good prognosis and response rate to hormonal based therapies, HER2-positive breast cancers which have a poor prognosis if untreated but are initially responsive to the HER2 targeting monoclonal antibody Herceptin, and basal-like breast cancers which have a poor prognosis and lack expression of HER2, estrogen and progesterone receptors (referred to as "triple-negative"). Many basal breast cancers express high levels of EGFR which results in activation of the Ras/Raf/MEK/ERK cascade. Hoeflich and colleagues [43] found that basal cell breast cancers expressed a Ras-like expression profile and tested their hypothesis that these breast cancers could be sensitive to MEK inhibitors, providing that they do not have *PI3KCA* mutations or *PTEN* deletions. In contrast many luminal and HER2-amplified tumors are resistant to MEK inhibitors. They also determined that PTEN loss was a

negative predictor factor for response to MEK inhibitors. Furthermore, treatment with MEK inhibitors often led to an increase in activated Akt expression, providing the rationale to examine the consequences of co-addition of MEK and PI3K inhibitors. The authors also determined that co-administration of MEK and PI3K inhibitors enhanced killing of the certain breast cancers. Thus the studies by Wee et al, and Hoeflich et al., have shown the concept that elevated PI3K/Akt/mTOR expression will confer resistance to MEK inhibitors. These studies further illustrate a central concept that we have been discussing in this review which is the critical role of genetics in determining the sensitivity to targeted therapy.

Other studies have also indicated that some tumors with *EGFR* mutations are resistant to MEK inhibitors. Mutations at the *BRAF*, *KRAS*, *EGFR* genes or the chromosomal fusion between anaplastic lymphoma kinase (*ALK*) and *ROS* tyrosine kinases are detected in approximately 50% of NSCLC. NSCLC cells with *BRAF* mutations where shown to be more sensitive to MEK inhibitors than NSCLC with mutations in *EGFR*, *KRAS*, or the chimeric fusion between *ALK* and *ROS* [6]. This was determined by screening a large panel of cell lines (n=87) and tumors (n=916). In this study, cells with mutations at *EGFR* were resistant to MEK inhibitors. This may have resulted from the ability of EGFR to activate the PI3K/PTEN/Akt/mTOR pathway which as discussed below has some crucial overlapping targets as the Raf/MEK/ERK pathway. NSCLC patients with *EGFR* mutations should not be treated with MEK (or BRAF) inhibitors as the respective therapies would be ineffectual.

PI3K/AKT/MTOR INHIBITORS

Many PI3K inhibitors have been developed [44, 45]. These include: LY-294002 [Lilly], Wortmannin, PX-866 [Oncothyreon], GDC-0941 [Genentech], CAL-101 [Calistoga Pharmaceuticals], XL-147 and XL-765 [Exelixis and Sanofi-Aventis]. Some PDK1 inhibitors have been described but they are not specific for PDK1 including OSU-03012 [Arno Therapeutics] and Celecoxib [Pfizer]. Various Akt inhibitors have been developed [46-48]. These include: A-443654 [Abbott Laboratories], GSK690693 [GlaxoSmithKline], VQD-002 (a.k.a. API-2, VioQuest Pharmaceuticals), KP372-1 [QLT, Inc] and Perifosine [AEterna Zentaris/Keryx Biopharmaceuticals]. Inhibitors of downstream mTOR have been developed [49-53]. These include: rapamycin [Wyeth-Pfizer, Sirolimus] and modified rapamycins (rapalogs) (CCI-779, [Torisel, Temsirolimus, Wyeth-Pfizer], AP-23573 [Ridaforolimus, Ariad-Merck] and RAD001 [Afinitor, Everolimus, Novartis]). Rapamycin and the modified rapalogs are mTORC1 inhibitors. Some dual PI3K/mTOR inhibitors have also been developed [42, 54]. These include: (NVP-BEZ235 [Novartis] and PI-103).

There may be benefits to treating patients with an inhibitor which can target both PI3K and mTOR as opposed to treating patients with two inhibitors, that is one targeting PI3K and one targeting mTOR. Perhaps the most obvious benefit would be lowered toxicities. Treatment with a single drug could have fewer side effects than treatment with two separate drugs. The effects of unwanted Akt activation by mTOR inhibition might be decreased upon treatment with a dual kinase inhibitor. Furthermore, the negative side effects of mTOR inhibition on the activation of the Raf/MEK/ERK pathway might be alleviated with the PI3K inhibitor activity in the dual inhibitor. There remains, however, considerable uncertainty about potential toxicity of compounds that inhibit both PI3K and mTOR enzymes whose activities are fundamental to a broad range of physiological processes.

Some of the PI3K inhibitors such as Wortmannin and LY294002 have been used extensively to investigate the role of PI3K in various biological properties but these compounds are not being clinically explored for multiple reasons, including insolubility in aqueous solutions and high toxicity. The modified wortmannin PX-866 is undergoing clinical trials for advanced metastatic cancer by Oncothyreon. GDC-0941 is in clinical trial for advanced solid cancers by Genentech. XL-147 and XL-765 are in clinical trials for advanced solid tumors by Exelixis and Sanofi-Aventis. CAL-101, a PI3Kδ specific inhibitor, is in clinical trials for hematological malignancies by Calistoga Pharmaceuticals. NVP-BEZ235 is in Phase I/II clinical trials for advanced cancer patients by Novartis.

Triciribine (API-2) inhibits phosphorylation in all three Akt isoforms *in vitro* and the growth of tumor cells overexpressing Akt in mouse xenograft models [45]. The mechanism by which triciribine inhibits Akt activity is unknown. Although no studies have been performed with triciribine in preclinical AML models, the drug has been used in a phase I clinical trial in patients with advanced hematologic malignancies, including refractory/relapsed AML. Results from this trial evaluating triciribine administered on a weekly schedule were encouraging and demonstrated that the drug was well-tolerated, with preliminary evidence of pharmacodynamic activity as measured by decreased levels of activated Akt in primary blast cells [55].

The rapalogs have been extensively examined in clinical trials of various cancers including: breast, prostate, pancreatic, brain, leukemia, lymphoma multiple melanoma, HCC, RCC and non small cell lung carcinomas (NSCLC) [49-53]. The rapalogs Torisel and Afinitor are now approved to treat patients with RCC (see below).

mTOR inhibitors initially demonstrated promise, as PTEN is often deleted in various tumors; however, it has been determined that the mTOR pathway has a complicated feedback loop that actually involves suppression of Akt; hence mTOR inhibitors would potentially activate Akt in some cells [2]. When mTORC1 is suppressed by rapamycin, there is increased mTORC2 activity which

is the elusive PDK2 that serves to phosphorylate and activate Akt. mTOR can also be regulated by the Ras/Raf/MEK/ERK pathway and mTOR can activate the Ras/Raf/MEK/ERK pathway. This may be another relevant cross-talk between the Ras/Raf/MEK/ERK and the Ras/PI3K/Akt/mTOR pathways, and might offer a further rationale for treatments combining drugs that inhibit both signaling networks. As mentioned earlier, combination of these novel "dual" inhibitors with either a Raf or MEK inhibitor might lead to more effective suppression of cancer growth.

In addition, it is now emerging that, at least in some cell types, rapamycin does not inhibit 4E-BP1 phosphorylation. Small molecules designed for inhibiting the catalytic site of mTOR have shown promising effects on suppression of signalling downstream of mTOR [56, 57]. The development of mTOR specific kinase ATP-competitive inhibitors is currently under intense investigation.

TREATMENT OF RENAL CELL CARCINOMA (RCC), MELANOMA AND HEPATOCELLULAR CARCINOMA (HCC) WITH SORAFENIB

Due to the broad specificity of Sorafenib (Nexavar™), this drug has been evaluated for the therapy of diverse cancers, including RCC, melanoma and HCC (due to the involvement of the Raf/MEK/ERK cascade, as well as altered VEGR pathway in these cancers) and gastro-intestinal stromal tumors (GIST) (due to the involvement of c-Kit mutations in this cancer) [58-61]. Sorafenib has been approved for the treatment of kidney cancer, including RCC [59]. BRAF is not mutated in RCC, however, VEGFR-2 may be aberrantly expressed as there is dysregulation of its cognate ligand VEGF which can activate VEGFR2 and the Raf/MEK/ERK cascade. Sorafenib is active as a single agent in this disease, probably due to its ability to suppress the activities of multiple signaling pathways activated in RCC, which are required for growth.

As the *BRAF* gene is mutated in approximately 60 to 70% of melanomas, Sorafenib was tested for its ability to suppress melanoma growth in mouse models [60, 61]. The overwhelming majority of *BRAF* mutations occur at V600E. Sorafenib had only modest activity as a single agent in advanced melanoma and it did not appear to be more effective in the treatment of melanomas that are either WT or mutant at the *BRAF* gene, hence it may be targeting a kinase other than B-Raf in these melanomas (*e.g.*, VEGFR). Alternatively, it could be targeting an upstream receptor kinase which signals through the Ras/Raf/MEK/ERK cascade. It is relevant to examine the effects of combining Sorafenib with a MEK inhibitor to treat malignant melanoma and certain other cancers. Sorafenib may target the VEGFR and other membrane receptors expressed on the particular cancer cells, whereas the MEK inhibitor would specifically suppress the Raf/MEK/ERK cascade which is abnormally activated by the *BRAF* oncogene or other mutant upstream signaling molecules. To improve the effectiveness of Sorafenib in the therapy of melanoma, it is being combined with standard chemotherapeutic drugs (see below).

Sorafenib, unlike more novel kinase inhibitors that target the mutant versus WT kinase, binds both the WT and mutant V600E B-Raf proteins and retarded the growth of melanoma xenografts in mice [33, 60, 61]. Other more recently developed Raf kinase inhibitors may show higher selectivity toward the mutant as opposed to WT Raf proteins [10, 11].

TREATMENT OF MELANOMAS, PANCREATIC, COLON, LUNG, BREAST AND HCC WITH SELUMETINIB

Selumetinib is an orally-active MEK1 inhibitor that has undergone phase II clinical trials. It is one of the first MEK1 inhibitors to be evaluated in randomized phase II trials [3, 13, 20-22, 27]. Selumetinib has demonstrated significant tumor suppressive activity in preclinical models of cancer, including melanoma, pancreatic, colon, lung, liver and breast cancer. The effects of Selumetinib are enhanced significantly if the tumor has a mutation that activates the Raf/MEK/ERK signaling pathway. Selumetinib shows great promise in the treatment of pancreatic cancers, which often have mutations in Ras that can lead to downstream Raf/MEK/ERK pathway activation. Due to the frequent detection of pancreatic cancer at advanced stages, it may be necessary to combine signal transduction inhibitor therapy with conventional chemotherapy after surgical removal of the pancreatic cancer if possible.

Selumetinib has undergone several phase I and II clinical trials. A phase I clinical trial to assess the safety, tolerability and pharmacokinetics of selumetinib in patients with various solid malignancies was performed. Phase II clinical trials have compared: (i) the efficacy of selumetinib versus temozolomide in patients with unresectable stage 3 or 4 malignant melanomas, (ii) the efficacy and safety of selumetinib versus capecitabine in patients with advanced or metastatic pancreatic cancer who have failed to respond to gemcitabine therapy, (iii) the efficacy and safety of selumetinib compared with pemetrexed in patients with NSCLC who have previously failed to respond to one or two prior chemotherapy regimens, and (iv) the efficacy and safety of selumetinib versus capectiabine in patients with colorectal cancer who have failed to respond to one or two prior chemotherapy regimens [62]. Initial results from clinical trials have not yielded overwhelming support for the use of MEK inhibitors (see below) as a single therapeutic agent in cancer patients who are not pre-screened for pre-existing

activation of the Raf/MEK/ERK pathway [27, 28]. The proper pre-identification of cancer patients who display activation of the Raf/MEK/ERK pathway may be necessary for prescribing MEK inhibitors as part of their therapy, as we have stated previously that MEK inhibitors are cytostatic and not cytotoxic.

TREATMENT OF RCC AND HCC WITH MTOR INHIBITORS

The modified rapamycins have been approved by the FDA to treat RCC that have been shown to be refractory to other therapies including sunitinib (Sutent) [63]. Recent studies have demonstrated that mTOR inhibition has remarkable activity against a wide range of human cancers in vitro and human tumor xenograft models. The mTOR pathway is known to be up-regulated in a subset of HCC patients [64]. In this study 15% of HCC displayed overexpression of phospho-mTOR, whereas 45% of HCC had increased expression of $p70^{S6K}$, which correlated with tumor nuclear grade. Evidence from in vitro experiments as well as from preclinical in vivo data indicated that mTOR inhibition by rapamycin and its analogues everolimus (RAD001) significantly reduced the growth of HCC cells and improved survival primarily via antiangiogenic effects [64-67]. A pilot study conducted in 21 patients

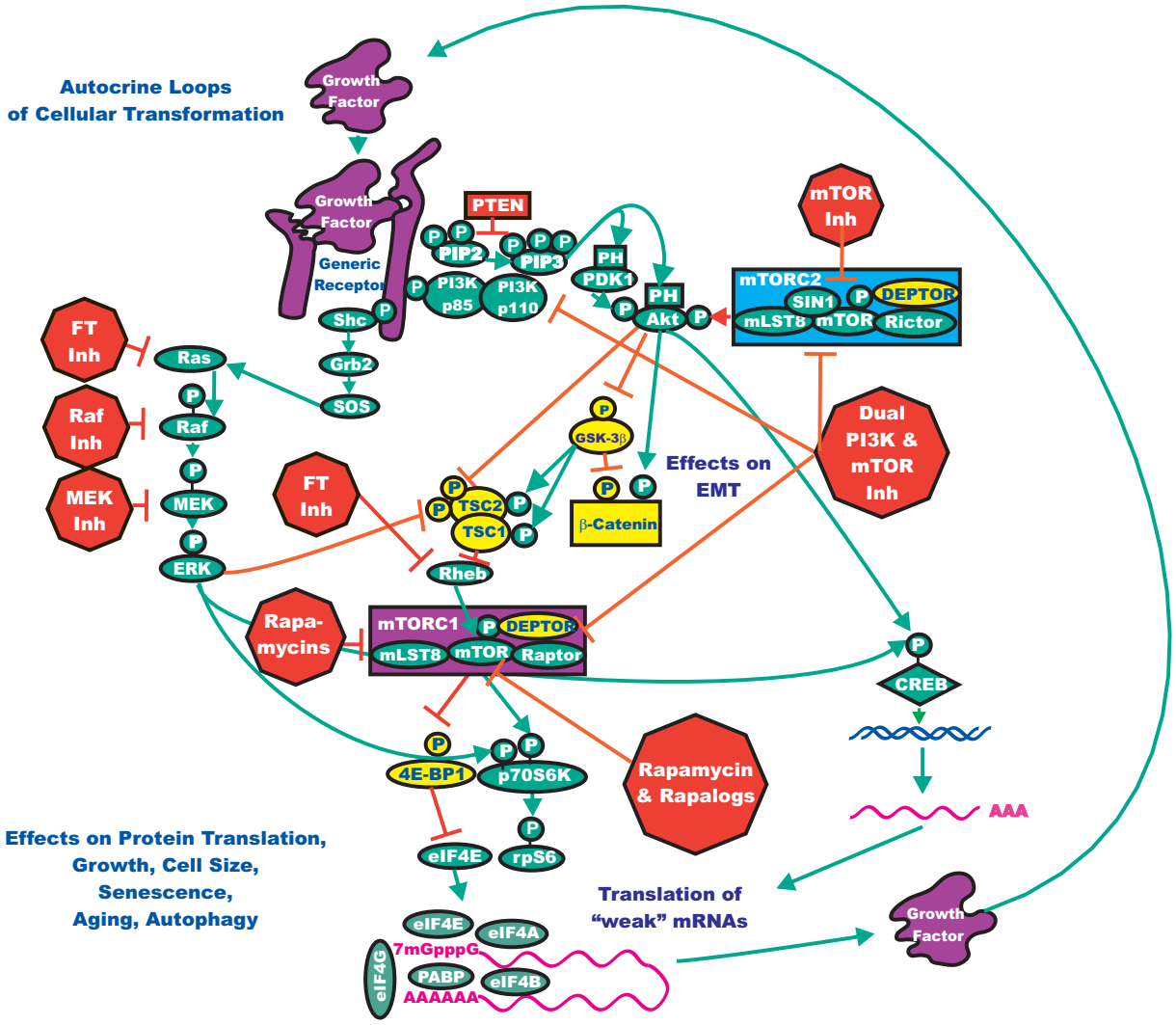

Figure 3: Conceptual Overview of Targeting the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR Pathways to Suppress Malignant Growth. The Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways can interact at many different levels. In this diagram, we have focused on how they interact to regulate mTOR, p70S6K and protein synthesis and autophagy. Targeting both of these pathways may be an effective means to regulate cell growth. Signaling molecules promoting phosphorylation events are indicated in green. Stimulatory signaling events are indicted in green lines with a green arrow before the target of the phosphorylation. Small molecule inhibitors are indicated in red. Inhibitory phosphorylation events are indicated in red lines with a block on the end before the target of the inhibition. More tentative inhibitory phosphorylation events are indicated in dotted red lines with a block on the end before the target of the inhibition. Inhibitory signaling or proapoptotic molecules or inactivated molecules are indicated in yellow. A growth factor and a growth factor receptor are indicated in purple. Active transcription factors are indicated in purple diamonds. Inactivated transcription factors are indicated in yellow diamonds.

with advanced HCC indicated that sirolimus (rapamycin) was a promising drug for the treatment of HCC, and currently, a phase I/II trial evaluating the rapamycin analog RAD001 for advanced HCC is recruiting patients (http://clinicaltrials.gov/ct2/show/NCT00390195).

A topic of considerable current interest concerns the signal transduction pathways and the molecular mechanisms linked to chemoresistance of tumor cells to conventional anticancer drugs. In this context, combination of rapamycin with the conventional cytostatic drugs doxorubicin and vinblastine enhances the antineoplastic activity of the respective monotherapeutic HCC treatment with either doxorubicin or vinblastine alone [68, 69]. Taken together, the *in vitro* and preclinical *in vivo* data as well as the clinical trials conducted so far demonstrate that mTOR inhibitors are promising agents for HCC treatment, particularly in combination with conventional chemotherapeutic drug therapy.

INCREASING THE EFFECTIVENESS OF TARGETING THE RAF/MEK/ERK AND PI3K/PTEN/AKT/MTOR PATHWAYS BY SIMULTANEOUS TREATMENT WITH TWO PATHWAY INHIBITORS

The obvious goal of current inhibitor development is to improve the effectiveness of treatment of cancer patients with small molecule signal transduction inhibitors. This has proven to be difficult for multiple reasons: first, as previously discussed, there tends to be a distinct genetic susceptibility for the success of a signal transduction inhibitor in suppressing growth, second, many of the small molecule signal transduction inhibitors are cytostatic as opposed to being cytotoxic and therefore will need to be combined with a therapeutic modality that induces cell death and will be discussed below and third, more than one signal transduction pathway may be activated in the cancer cells, which will be discussed in detail below.

Previously, we have predominantly discussed studies that employed a single Raf or MEK inhibitor, sometimes in combination with a chemotherapeutic drug. In the following section, we discuss the potential of combining inhibitors that target two pathways to more effectively limit cancer growth. In addition to the *BRAF* mutations present in melanomas that we have previously discussed, the *PTEN* phosphatase tumor suppressor gene is also deleted in approximately 45% of melanomas and the downstream *AKT* gene is amplified in approximately 45%. Both of these mutations result in increased expression/activity of Akt which is often associated with a poor prognosis in human cancer. Increased Akt expression will lead to mTOR activation and increased efficiency of protein translation. The targeting of mTOR has been examined in melanoma therapy as well as in the treatment options for many diverse cancers. Administration of mTOR inhibitors to melanoma patients as monotherapy resulted in 1 partial remission out of 33 patients [70]. Preclinical studies performed in human melanoma cell lines have highlighted that co-targeting of the Raf and PI3K/PTEN/Akt/mTOR pathways with Raf and Akt/mTOR inhibitors resulted in synergistic inhibition [71]. Treatment of inducible murine lung cancers containing *KRAS* and *PIK3CA* mutations with PI3K/mTOR (NVP-BEZ235) and MEK (selumetinib) inhibitors led to an enhanced response [72]. Recent reports have also indicated synergistic responses between sorafenib and mTOR inhibitors in xenografts of a highly metastatic human HCC tumor [73]. An illustration documenting the rationale for the targeting of both pathways is presented in Figure 3.

The combined effects of inhibiting MEK with PD-0329501 and mTOR with rapamycin or its analog AP-23573 (ARIAD Pharmaceuticals/Merck) were examined in human NSCLC cell lines, as well as in animal models of human lung cancer [74]. PD-0325901 and rapamycin demonstrated synergistic inhibition of proliferation and protein translation. Suppression of both MEK and mTOR inhibited ribosomal biogenesis and was associated with a block in the initiation phase of translation. These preclinical results support suppression of both the MEK and mTOR pathways in lung cancer therapy and indicate that both pathways converge to regulate the initiation of protein translation. ERK phosphorylates MAPK signal integrating kinases (Mnk1/2) and p90 ribosomal S6 kinase $p90^{Rsk}$, which regulate the activity of the eukaryotic translation initiation factor eIF4E. The phosphorylation of 4EBP1 is altered in cells with the BRAF mutation. It should also be pointed out that the 4EBP1 is also regulated by Akt, mTOR and p70S6K. This may result in the efficient translation of certain mRNAs in BRAF-mutant cells. This could explain how co-inhibition of MEK and mTOR synergize to inhibit protein translation and growth in certain lung cancer cells.

ENHANCING EFFECTIVENESS OF RAF/MEK AND PI3K/MTOR INHIBITORS WITH CHEMOTHERAPY

Classical chemotherapy often remains the most prescribed anti-cancer therapy for many different types of cancer treatment [75]. Drugs such as doxorubicin and taxol are effective in the treatment of many cancers, even though in some cases drug resistance develops after prolonged treatment. Doxorubicin and taxol target cellular events, such as DNA replication and cell division, which are often downstream of the targets of signal transduction pathway inhibitors. Chemotherapeutic drugs can activate the Ras/Raf/MEK/ERK pathway by diverse mechanisms (See Figure 4). Drugs such as doxorubicin can activate p53 which can lead to increased expression of the discoidin domain receptor (DDR), which in turn can result in Raf/MEK/ERK pathway activation. Activated ERK can

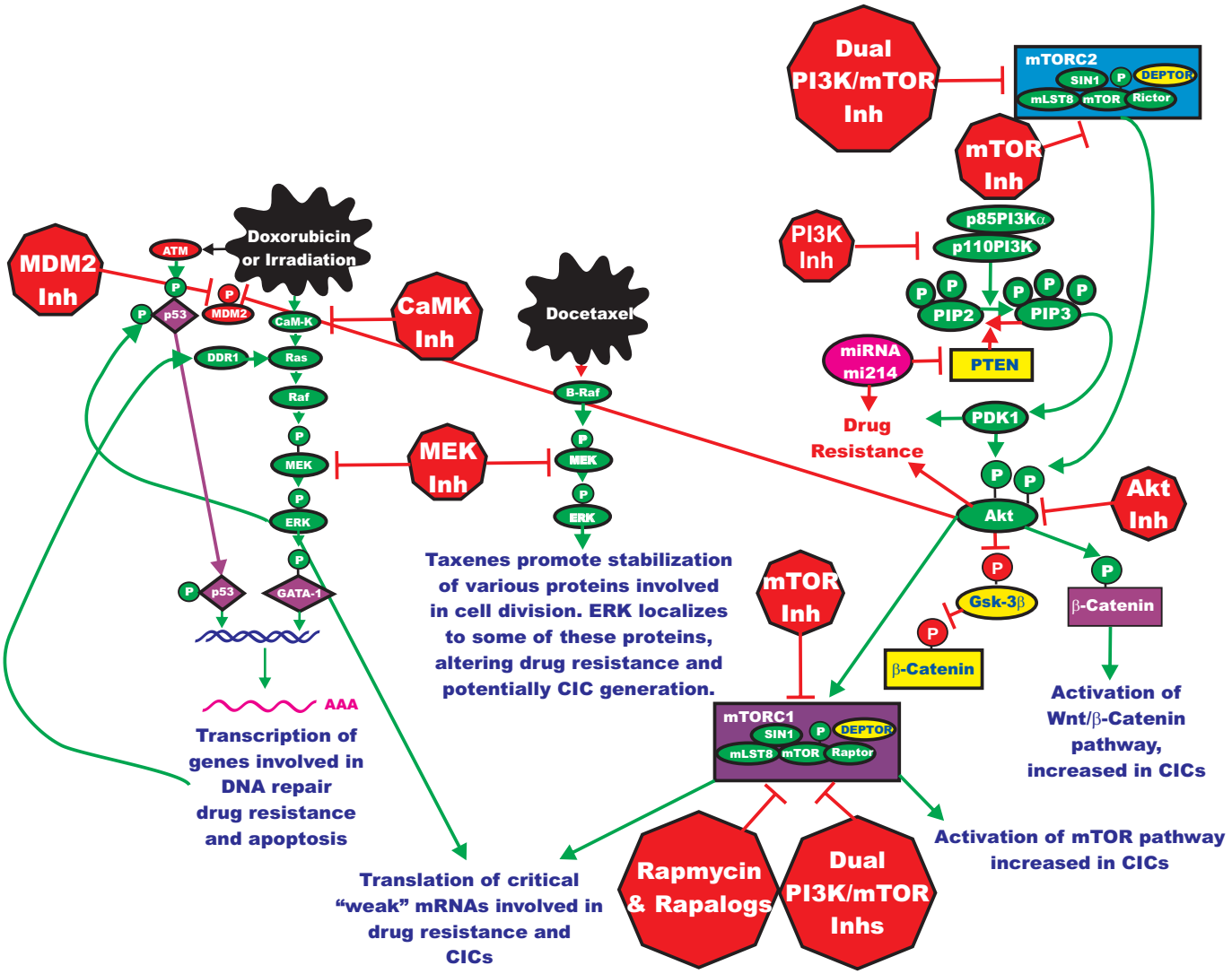

Figure 4: Targeting Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR Pathways May Prevent Drug Resistance and Reemergence of Cancer Initiating Cells. Chemotherapeutic drugs such as Doxorubicin and Docetaxel may also induce the Raf/MEK/ERK pathway which may contribute to emergence of drug resistant clones. The Raf/MEK/ERK pathway may regulate downstream transcription factors such as GATA-1 which control the transcription of genes such as XRCC1 and ERCC1 which are involved in DNA repair and their aberrant expression may contribute to drug resistance. Treatment of drug resistant cells with MEK inhibitors, or combined treatments consisting of a chemotherapeutic drug and a MEK inhibitor, may be an effective approach to prevent drug resistance. Treatment of certain cancer initiating cells with Akt or mTOR inhibitors may prevent their reemergence. Various components of the Ras/PI3K/PTEN/Akt/mTOR pathway are implicated in drug resistance. Changes in Akt expression may occur to mutations at PI3K or PTEN. Furthermore, altered expression of microRNAs may be involved in decreasing PTEN expression which results in drug resistance. The roles of these various genetic changes in cancer initiating cells are beginning to become apparent. Chemotherapeutic drugs are indicated in irregular black elipses. Treatment of certain cancer initiating cells with Akt or mTOR inhibitors may prevent their reemergence. Signaling molecules promoting phosphorylation events are indicated in green. Stimulatory signaling events are indicted in green lines with a green arrow before the target of the phosphorylation. Small molecule inhibitors are indicated in red. Inhibitory phosphorylation events are indicated in red lines with a block on the end before the target of the inhibition. More tentative inhibitory phosphorylation events are indicated in dotted red lines with a block on the end before the target of the inhibition. Inhibitory signaling or proapoptotic molecules or inactivated molecules are indicated in yellow. A growth factor and a growth factor receptor are indicated in purple. Active transcription factors are indicated in purple diamonds. Inactivated transcription factors are indicated in yellow diamonds.

phosphorylate p53 and regulate its activity. Doxorubicin can also activate the calcium calmodulin dependent kinase (CaM-K) cascade via reactive oxygen species (ROS) [4]. Activation of this cascade can also result in activation of the Raf/MEK/ERK cascade. Activation of this cascade can result in the transcription of genes such as XRCC1 and ERCC1 which are involved in DNA repair and lead to drug resistance [75, 76]. Taxols can also stimulate activation of the Raf/MEK/ERK cascade and lead to their increased association with proteins involved in cell division [77, 78]. Thus, by combining classical chemotherapy with targeted therapy, it may be possible to enhance toxicity, while lowering the prescribed concentrations of classical chemotherapeutics necessary for effective elimination of the tumor [78]. As we have previously discussed, activation of the Raf/MEK/ERK cascade can alter the activity and subcellular localization of many proteins that play critical roles in apoptotic cascades. Also the Raf/MEK/ERK cascade can regulate the transcription of many critical genes involved in cell cycle progression, growth and differentiation.

A phase II trial demonstrated that the combination of sorafenib and doxorubicin improves progression-free and overall survival of patients with advanced HCC [79]. Moreover, a phase II trial is currently recruiting patients to determine the progression-free survival of sorafenib plus tegafur/uracil (UFUR) for the treatment of advanced or metastatic HCC.

As mentioned previously, a side effect of some chemotherapeutic drugs, such as paclitaxel, is the induction of the Raf/MEK/ERK pathways. Activation of this pathway can under certain circumstances promote proliferation and prevent apoptosis. Also the PI3K/PTEN/Akt/mTOR pathway can modulate the Raf/MEK/ERK pathway and altering MEK activity can have opposing effects on different cell types [80, 81]. Combining paclitaxel treatment with PI3K inhibitors enhances apoptosis and inhibits growth of ovarian carcinoma cell lines, and this may have been mediated in part by suppression of inhibitory phosphorylation of Raf by Akt [80]. In addition, the effects of combined treatment with MEK inhibitors and paclitaxel have been examined. The synergistic effects of paclitaxel and MEK inhibitors are complex and have not been fully elucidated, but may be in part mediated by inhibition of Bad phosphorylation at S112 by ERK in UM-SCC-23 squamous carcinoma cell line [82]. This is just one documented interaction that may be suppressed by MEK inhibitors. Obviously many other key phosphorylation events mediated by ERK may be suppressed which play critical roles in cell growth.

The cytotoxic effects of combinations of MEK inhibitors and paclitaxel may be specific for cells of certain origins and may depend on the levels of endogenous activated MEK/ERK present in those cells. In a study with NSCLC cells which constitutively-expressed activated MEK/ERK, no increase in paclitaxel-induced apoptosis was observed when the cells were treated with a MEK inhibitor [81]. In contrast, addition of a dominant negative (DN) MEK gene to these cells potentiated paclitaxel-induced apoptosis.

Cisplatin-induced apoptosis was associated with increased levels of both p53 and the downstream Bax protein in a study with neuroblastoma cells [82]. Activated ERK1/ERK2 levels also increased in these cells upon cisplatin treatment. MEK inhibitors blocked apoptotic cell death, which prevented the cisplatin-induced accumulation of p53 and Bax proteins [82].

It should be noted that the combination of MEK inhibitors and chemotherapeutic drugs may not always result in a positive interaction. In some cases, combination therapy results in an antagonistic response. For example, combining MEK inhibitors with betulinic acid, a drug toxic for melanoma cells, antagonized the normal enhancing effects of betulinic acid on apoptosis *in vitro* [83]. Furthermore, the precise timing of the addition of two agents is important as they may differentially affect cell-cycle progression; therefore, the order of administration may be important for a synergistic response to be obtained and perhaps to prevent an antagonistic response [83].

ENHANCING EFFECTIVENESS OF RAF/MEK AND PI3K/MTOR INHIBITORS WITH RADIOTHERAPY

Radiotherapy is a common therapeutic approach for treatment of many diverse cancers. A side effect of radiotherapy in some cells is induction of the Ras/Raf/MEK/ERK cascade [4]. Recently various signal transduction inhibitors have been evaluated as radiosensitizers. The effects of pre-treatment of lung, prostate, and pancreatic cancer cells with selumetinib were evaluated *in vitro* using human cell lines and *in vivo* employing xenografts [84]. The MEK inhibitor treatment radiosensitized the various cancer cell lines *in vitro* and *in vivo*. The MEK inhibitor treatment was correlated with decreased Chk1 phosphorylation 1-2 hrs after radiation. The authors noticed the effects of the MEK inhibitor on the G2 checkpoint activation after irradiation, as the MEK inhibitor suppressed G2 checkpoint activation. Since ERK1/ERK2 activity is necessary for carcinoma cells to arrest at the G2 checkpoint, suppression of phosphorylated Chk1 was speculated to lead to the abrogated G2 checkpoint, increased mitotic catastrophe and impaired activation of cell cycle checkpoints. Mitotic catastrophe was increased in cells receiving both the MEK inhibitor and radiation when compared to the solo-treated cells. It was also postulated in this study that the MEK inhibitor suppressed the autocrine cascade in DU145 prostate cancer cells that normally resulted from EGF secretion and EGFR activation. Suppression of this autocrine cascade by the MEK inhibitor may have served as a radiosensitizer to the radiation therapy. The other two cancer cell lines

examined in this study (A549 and MiaPaCa2) had *KRAS* mutations and both were radiosensitized by the MEK inhibitor. Although these studies document the ability of a MEK inhibitor to radiosensitize certain cells, clearly other cancer cell lines without activating mutations in the Ras/Raf/MEK/ERK pathway or autocrine growth stimulation should be examined for radiosensitization by the MEK inhibitor as the *KRAS* mutation may also activate the PI3K pathway which could lead to therapy resistance.

PI3K/Akt/mTOR inhibitors will sensitize the tumor vasculature to radiation both *in vitro* in cell lines and *in vivo* in xenogratfs [85, 86]. mTOR and radiation play critical roles in the regulation of autophagy [87, 88]. When mTOR is blocked by rapamycin there is an increase in autophagy. This is important as apoptotic cell death is a minor component to cell death in solid tumors. These studies document the potential beneficial use of combining mTOR inhibitors and radiation to improve the induction of autophagy in the treatment of solid tumors.

Just as new inhibitors are described, cells and tumors resistant to these inhibitors will also be discovered. Resistance to Gleevec (Imatinib) a BCR-ABL inhibitor has been well documented and novel inhibitors have been discovered to overcome this resistance [89]. Recently two distinct mechanisms for resistance to Raf inhibitors have been described [90, 91]. In one case, the *BRAF*-mutant melanoma cells that had been maintained in medium containing the B-Raf inhibitor AZ628 shifted their dependence from B-Raf to Raf-1 [91]. In another case, some B-Raf mutant melanoma cells may be intrinsically resistant to B-Raf inhibitors as a result of cyclin D amplification [91]. Some of these "additional" genetic mutations may be preexisting in the tumor cell population and upon culture of the cells or tumor in the presence of the Raf inhibitor; the "mutant-resistant" cells may take over the population.

KRAS AND PIK3CA MUTATIONS IN THE SAME CELL OR PATIENT CAN RESULT IN CONFERRING RESISTANCE TO RAPAMYCIN

Cancers containing PIK3CA mutations are often sensitive to the mTOR inhibitor rapamycin and the modified rapamycins (Rapalogs). However, PIK3CA-mutant cells that also have mutations at KRAS are resistant to Rapalogs [92, 93]. This maybe due to complicated feedback loops between the Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways wherein either mTORC1 inhibition leads to ERK1/2 activation by a p70S6K/PI3K/Ras dependent pathway or by the KRAS mutants activating $p90^{Rsk-1}$ which serves to activate eIF4B and rpS6 thereby bypassing mTOR-dependent activation.

IDENTIFICATION OF NOVEL SITES IN THE PIK3CA GENE WHICH CONFER RESISTANCE TO PI3K INHIBITORS

A group of highly-gifted graduate students and their colleagues developed an innovative approach to identify residues in *PIK3CA* that will result in resistance or increased sensitivity to PI3K inhibitors [94]. Frequently mutations in kinases which confer resistance to inhibitors occur in the gatekeeper residues that block drug binding. In an insightful study performed by Zunder and colleagues, they took advantage of the fact that yeast do not contain or express *PIK3CA* and that the product of *PIK3CA* (PI3K) is normally toxic to yeast [94]. Therefore introduction of membrane-localized *PIK3CA* into yeast resulted in yeast toxicity, however, when they treated the transfected yeast with a PI3K inhibitor, the yeast survived. They found that certain mutations in *PIK3CA* would confer resistance to the PI3K inhibitors, preventing growth, in transfected yeast at drug concentrations which would allow normal membrane-localized *PIK3CA*-transfected yeast to grow. Unlike with BCR-ABL inhibitor resistant mutations, these *PIK3CA* mutations did not reside in the classic gatekeeper residues. As a biological bonus, they also identified some mutations in *PIK3CA* (L814C) that conferred enhanced sensitivity to PI3K inhibitors. These mutations allowed the growth of the mutant *PIK3CA*-transfected yeast at inhibitor concentrations that would normally suppress the growth of yeast bearing the WT membrane-localized *PIK3CA*. Furthermore, such information is valuable for the design of novel PI3K inhibitors that will be effective in the treatment of cancer patients which become resistant to the first generation of PI3K inhibitors.

SUMMARY OF RAF/MEK/ERK AND PI3K/PTEN/AKT/MTOR PATHWAYS INHIBITORS EVALUATED IN CANCER THERAPY AND IN CLINICAL TRIALS

In Table 1, a detailed summary of many of the various Raf, MEK, PI3K, Akt and mTOR inhibitors which have been evaluated in preclinical and cancer clinical trials is presented [89, 95-178]. Clearly targeting these activities involved in normal and cancerous growth has become an intensely investigate field. Perhaps some of the most recent success has arisen in targeting mTOR. The regulation of mTOR and its subsequent effects on protein translation is critically implicated in many cancers [171-181] and is also involved in cell differentiation [182-185], cancer initiating cells [187-198] and other important cellular processes as will be discussed below.

NOVEL USES OF RAF/MEK AND PI3K/AKT/MTOR INHIBITORS: TARGETING CANCER INITIATING CELLS (CICS)

An overview of the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in some of novel aspects of their usage is presented in Figure 4. Targeting these pathways may be an approach to overcome chemotherapeutic drug resistance. An area of intense research interest in experimental therapeutics is the cancer stem cell, more appropriately referred to as the cancer initiating cell (CIC) [89, 187-243]. CICs often share some properties with drug resistant cells as they both are often resistant to chemotherapeutic and hormonal based therapies. The abilities of the various Raf, MEK and mTOR inhibitors as well as the natural product resveratrol to target and suppress the proliferation of CICs are beginning to be examined [185, 191-195, 212-220]. It is not clear whether Raf or MEK inhibitors will specifically target CICs [193, 194]. CICs have unique properties from the majority of the particular cancer (often called bulk cancer) as they can be both quiescent and also resistant to chemotherapeutic and hormonal based drugs, often due to their increased expression of proteins involved in drug transport as well as PI3K/PTEN/Akt/mTOR pathway [89, 193, 194, 197-200, 224-226]. However, under certain conditions, they resume proliferation and hence should be potentially susceptible to: Raf, MEK, PI3K, Akt, mTOR and other inhibitors Targeting the Raf/MEK/ERK and PI3K/PTEN/mTOR pathways could be very important in terms of CIC elimination.

The "tumor microenvironment" most likely plays critical roles in CIC survival and also reemergence and subsequent metastasis [206-211]. Combinations of cytotoxic chemotherapeutic drugs and inhibitors which target the Raf/MEK/ERK, PI3K/PTEN/mTOR and upstream kinases may be an eventual approach to target the tumor microenviroment, however, specificity of targeting may be a significant problem. The ability to target the tumor microenvironment is a challenging issue.

Recently miRNAs have been shown to regulate many genes involved in drug resistance and likely CIC regulation [200, 223]. miRNAs specific of the 3'UTR of PTEN have been shown to be upregulated in certain ovarian cancer cells and can cause resistance to cisplatin [223]. One can also hypothesize that there may be altered expression of similar or additional miRNAs in CICs which will alter their sensitivities to mTOR and other inhibitors. The p53 pathway and genome stability/instability play key roles in regulating many aspects of cell growth including CICs [225-243]. We know very little about the changes in p53 and genome stability/instability that may occur in the initial CIC to more "malignant" CICs which may be present at later stages of tumor progression. As we learn more regard the effects of p53 and DNA damage responses on CIC and they development, we may be able to more effectively target these biochemical events from happening and inhibit tumor progression.

TARGETING THE RAF/MEK/ERK AND PI3K/PTEN/AKT/MTOR PATHWAYS TO SUPPRESS CELLULAR SENESCENCE/QUIESENCE

The Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways also play critical roles in the regulation of cellular senescence and quiescence [227-242]. Escape from drug-induced senescence has also been associated with drug resistance and CICs [227]. Often an additional key molecule implicated in: DNA damage responses, cellular senescence and drug resistance is p53, whose activity can be regulated by both the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways. These pathways exert their effects on p53 itself (post-translation modification by ERK and many other kinases as well as on the p53 inhibitor MDM-2 [231-236, 239-242]. mTOR can modulate the ability of p53 to promote senescence or quiescence [239, 240]. Genomic instability and epigenomic regulation can also have key effects on CIC generation and cellular senescence/quiescence [233-240] and can be regulated in part by mTOR [239-242].

TARGETING THE RAF/MEK/ERK AND PI3K/PTEN/AKT/MTOR PATHWAYS TO SUPPRESS CELLULAR AND ORGANISMAL AGING

While we have discussed the roles of these pathways in cancer in significant detail, an additional important aspect of targeting the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways is to halt cellular aging, which in the end, kills all those who either do not have or have been fortunate to survive cancer and other diseases. There is an emerging scientific field which documents that slowing the growth process and stimulating metabolism will slow aging and perhaps dementia as well [243-273]. Indeed, caloric restriction may be critically important in suppression of aging as well as cancer [263-273]. Many studies have indicated that inhibiting the PI3K/PTEN/Akt/mTOR and Raf/MEK/ERK pathways will influence aging [251, 254, 255, 256]. These experiments have led to innovative hypothesis that cellular senescence results from the hyper-activation of proliferative pathways and that drugs (*e.g.,* Metformin) and signal transduction inhibitors (*e.g.,* Raf, MEK, PI3K, mTOR inhibitors) can inhibit cellular proliferation and cellular aging [251, 254, 255, 256, 271]. Similar effects on the prevention of cellular senescence were observed with Resveratrol, the active component contained in the skins of red grapes which was shown to also inhibit mTOR and p70S6K cellular senescence [193, 194, 252, 256, 257, 259]. Additional

studies have shown that the commonly-prescribed diabetes drug Metformin will also inhibit mTOR and prevent cellular aging [246, 247, 266, 270, 271]. Since both the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways interact to regulate the activity of mTOR and downstream components of this pathway are critical for both mRNA stability and protein translation of genes involved in critical growth and survival, it is believed that by inhibiting some of these key pathways, it may be possible to prevent cellular aging.

CONCLUSIONS

Various pharmaceutical companies have developed inhibitors to the Ras/Raf/MEK/ERK pathway. Initially MEK inhibitors were shown to have the most specificity. However, these inhibitors may have limited effectiveness in treating human cancers, unless the particular cancer proliferates directly in response to the Raf/MEK/ERK pathway. Moreover, MEK inhibitors are often cytostatic as opposed to cytotoxic, thus their ability to function as effective anti-cancer agents in a monotherapeutic setting is limited, and they may be more effective when combined with chemo or radiotherapy. Raf inhibitors have also been developed and some are being used to treat various cancer patients (*e.g.,* Sorafenib). This particular Raf inhibitor also inhibits other receptors and kinases which may be required for the growth of the particular cancer. This promiscuous nature of Sorafenib has contributed to the effectiveness of this particular Raf inhibitor for certain cancers. Mutant specific Raf and PI3K inhibitors are also being developed. This is perhaps the most exciting area in terms of inhibitor development as it may result in the effective targeting of the mutant gene promoting the proliferation of the particular tumor. However, problems have been identified with certain B-Raf mutant allele inhibitors as they will also result in Raf-1 activation if Ras is mutated. Combination therapy with either a traditional drug/physical treatment or another inhibitor that targets a specific molecule in a different signal transduction pathway is also a key approach for improving the effectiveness and usefulness of MEK and Raf inhibitors.

Modified rapamycins, Rapalogs are being used to treat various cancer patients, (*e.g.,* patients with RCC and HCC). While Rapalogs are effective and their toxicity profiles are well know, one inherent property is that they are not very cytotoxic when it comes to killing tumor cells. This inherent property of rapamycins, may also contribute to their low toxicity in humans.

Mutations at many of the upstream receptor genes or Ras can result in abnormal Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathway activation. Hence targeting these cascade components with small-molecule inhibitors may inhibit cell growth.. The usefulness of these inhibitors may depend on the mechanism of transformation of the particular cancer. If the tumor exhibits a dependency on the Ras/Raf/MEK/ERK pathway, then it may be sensitive to Raf and MEK inhibitors. In contrast, tumors that do not display enhanced expression of the Ras/Raf/MEK/ERK pathway may not be sensitive to either Raf or MEK inhibitors but if the Ras/PI3K/Akt/mTOR pathway is activated, it may be sensitive to specific inhibitors that target this pathway. Some promising recent observations indicate that certain CICs are sensitive to mTOR inhibitors, documenting their potential use in the elimination of the cells responsible for cancer re-emergence [185, 191]. Some CICs may be sensitive to Resveratrol. Finally, it is likely that many of the inhibitors that we have discussed in this review will be more effective in inhibiting tumor growth in combination with cytotoxic chemotherapeutic drugs or radiation.

Some scientists and clinicians have considered that the simultaneous targeting of Raf and MEK by individual inhibitors may be more effective in cancer therapy than just targeting Raf or MEK by themselves. This is based in part on the fact that there are intricate feed-back loops from ERK which can inhibit Raf and MEK. For example when MEK1 is targeted, ERK1,2 is inhibited and the negative feed-back loop on MEK is broken and activated MEK accumulates. However, if Raf is also inhibited, it may be possible to completely shut down the pathway. This is a rationale for treatment with both MEK and Raf inhibitors. Likewise targeting both PI3K and mTOR may be more effective than targeting either PI3K or mTOR by themselves. If it is a single inhibitor which targets both molecules, such as the new PI3K and mTOR dual inhibitors this becomes a realistic therapeutic option. Finally, an emerging concept is the dual targeting of two different signal transduction pathways, Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR for example. This has been explored in some preclinical models as discussed in the text. The rationale for the targeting of both pathways may be dependent on the presence of mutations in either/or both pathways or in upstream Ras in the particular cancer which can activate both pathways. However, it is not clear, at this point in time, that the targeting of two different kinases in the same pathway or two different kinases in two different pathways with two different inhibitors will be performed clinically in the near future. While it may be scientifically interesting and effective it may be clinically impractical. It might make more clinical sense to target one kinase and also use a chemotherapeutic drug which will kill the cells.

It is not always clear why a particular combination of a signal transduction inhibitor and chemotherapeutic drug works in one tumor type but not at all in a different tumor type. This has also been experience with the development of individual chemotherapeutic drugs, some work in some cells but not others. This may result from many different complex interacting events. Some of these events could include: percentage of cells in different phases of the cell cycle, persistence of CICs and many other factors. Finally,

chemotherapeutic drug therapy and other types of therapy (radiotherapy, antibody therapy) may induce certain signalling pathways (*e.g.*, the reactive oxygen species generated by chemotherapy and radiotherapy induce the Ras/Raf/MEK/ERK pathway). The induction of these signalling pathways may counteract some of the effects of the signal transduction inhibitors.

Scientists and clinicians often have an intentionally narrow view of a particular topic. For example, cancer researchers predominantly feel that Raf, MEK, PI3K, Akt and mTOR inhibitors will suppress the growth of malignant cancer cells. Yet MEK and mTOR and other inhibitors may also be useful in the treatment of autoimmune and allergic disorder where there is abnormal cellular proliferation. Recently it has been observed that the suppression of the Ras/Raf/MEK/ERK and Ras/PI3K/Akt/mTOR pathways may prevent the induction of cellular senescence and aging. Clearly, these later two clinical topics, immune disorders and aging, greatly enhance the potential clinical uses of these targeted therapeutic drugs.

ACKNOWLEDGMENTS.

This work was supported in part by grants from: Fondazione del Monte di Bologna e Ravenna, MinSan 2008 "Molecular therapy in pediatric sarcomas and leukemias against IGF-1 receptor system", PRIN 2008 and FIRB 2010 (RBAP10447J) to AMM.

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