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A Phase I dose-escalation study of the BRAF inhibitor vemurafenib in combination with the MTOR inhibitor everolimus in subjects with advanced cancer

Javier Munoz

A PHASE I DOSE-ESCALATION STUDY OF THE BRAF INHIBITOR VEMURAFENIB IN COMBINATION WITH THE MTOR INHIBITOR EVEROLIMUS IN SUBJECTS WITH ADVANCED CANCER

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By

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DEDICATION

I dedicate this thesis to my family for their support.

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I acknowledge and appreciate the guidance received by Dr. Razelle Kurzrock, Dr. Filip Janku, Dr. Vivek Subbiah (current principal investigator for this phase I trial), and the members of my Advisory Committee (including Dr. Funda Meric-Bernstam, Dr. Karen Lu, Dr. Eduardo Vilar Sanchez, and Dr. Aung Naing) in order to finalizing this thesis and for their contribution to my career.

A PHASE I DOSE-ESCALATION STUDY OF THE BRAF INHIBITOR VEMURAFENIB IN COMBINATION WITH THE MTOR INHIBITOR EVEROLIMUS IN SUBJECTS WITH ADVANCED CANCER

(MDACC Protocol ID #: 2012-0153)

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ABSTRACT

Vemurafenib has been approved in the United States for the treatment of relapsed or refractory BRAF mutation positive malignant melanoma and is being investigated in various other malignancies. The RAS/RAF/MEK/ERK (MAPK) pathway is critical to cell proliferation in many human cancers. The mTOR inhibitors are well known to exert profound anticancer effects across malignancies through inhibition of the PTEN/PI3K/AKT/mTOR (mTOR) pathway. We hypothesize that the toxicity profile of the combination of vemurafenib and everolimus will be well tolerated. The primary objective is to find the maximum tolerated dose (MTD) and the toxicity of the combination of vemurafenib and everolimus following a standard 3 + 3 design. The most common diagnosis was melanoma in 5 out of 10 patients

(50%). Male patients in 7 out of 10 patients (70%). The average age was 63.5 years. Two out of 10 patients (20%) had partial responses and an additional 2 out of 10 patients (20%) had stable disease.

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ABBREVIATIONS:

Mitogen-associated protein kinase (MAPK)

Rapidly accelerated fibrosarcoma (RAF)

MAPK/ERK kinases (MEK)

Extracellular signal-regulated kinases (ERK)

Central nervous system (CNS)

Female (F)

Male (M)

Radiation (RT)

Hounsfield units (HU)

Guanine-nucleotide exchange factors (GEF)

Dose-limiting toxicity (DLT)

Maximum tolerated dose (MTD)

GTP-ase activating proteins (GAPs)

Rat sarcoma (RAS), Harvey (HRAS) and Kirsten (KRAS) rat sarcoma

BRAF (v-raf murine sarcoma viral oncogene homolog B1)

Guanosine triphosphate (GTP)

Son-of-sevenless (SOS1)

Common Terminology Criteria for Adverse Events (CTCAE)

Serious Adverse Experience (SAE)

Phosphatidylinositol 3-kinase (PI3K), Non-small cell lung cancer (NSCLC)

Lactate dehydrogenase (LDH)

Mammalian target of rapamycin (mTOR)

Fluorescence in-situ hybridization (FISH)

Genomic hybridization (CGH)

Polymerase chain reaction (PCR)

Anaplastic lymphoma kinase (ALK),

Food and Drug Administration (FDA)

Institutional Review Board (IRB)

Response Evaluation Criteria In Solid Tumors (RECIST)

SPECIFIC AIMS

(I) Background and significance: Vemurafenib has been approved in United States for treating relapsed/refractory BRAF mutation positive malignant melanoma and is being investigated in various other malignancies. The RAS-RAF-MEK-ERK (MAPK) pathway has been deemed critical to cell proliferation in several human cancer models. The frequency of such BRAF activating mutation and the resultant oncogene addiction makes mutated BRAF an extremely attractive target. Combining multiple agents with different mechanisms of action is now a paradigm in oncology phase I clinical trials. The BRAF inhibitor vemurafenib has been selected as the backbone of this trial. The mTOR inhibitors, such as everolimus and temsirolimus, are well known to exert profound anticancer effects across malignancies via inhibiting the PTEN-PI3K-AKT-mTOR (mTOR) molecular axis. It is possible that combining an inhibitor of BRAF plus an inhibitor of mTOR will be synergistic and might assist overcoming resistance to single agents targeted individually to the mTOR or MAPK signaling pathways. To our knowledge, there are no clinical trials currently evaluating such combination. Therefore, we have designed

a phase I trial evaluating such therapeutic combination in patients with advanced cancer.

(II) Hypothesis: We hypothesize that the toxicity of combined vemurafenib plus everolimus will be well tolerated.

(III) Specific aims:

Primary Objective: To determine the maximum-tolerated dose (MTD) of combined of vemurafenib and everolimus.

Secondary Objective: To describe preliminary antitumor activity (tumor response) of the combination of vemurafenib and everolimus. In the MTD expansion phase, correlate responses to treatment with mutations in the PTEN-PI3K-AKT-mTOR (mTOR) and/or RAS-RAF-MEK-ERK (MAPK) signaling axis and/or other signaling aberrations.

(IV) Brief methodology: The definition of dose-limiting toxicity (DLT) is any grade 3/4 that is non-hematologic; or any grade 4 hematologic side effect lasting two weeks or longer. Three patients will be treated per dose level with standard 3 + 3 design; three individuals will be dosed at dose level I and assessed for toxicity. If 0/3 individuals undergo DLT, the next 3-patient cohort will be dosed at the next higher level. If 1 of 3

individuals (1/3) dosed at a particular level undergoes DLT, then 3 more individuals will be dosed at the same dose. In summary, MTD is the highest level assessed with a DLT incidence lower than 33%. Patients will continue on the study until their disease has progressed, they elect to come off the study, they experience unacceptable toxicities.

INTRODUCTION:

The PTEN-PI3K-AKT-mTOR (mTOR) and RAS-RAF-MEK-ERK (MAPK) signaling axis (Figure 1) have been deemed key contributors to tumor growth and are among the most frequently activated in cancer. The RAS-RAF-MEK-ERK axis is one of the critical paths in neoplasia which may be constitutively activated via modification of particular proteins, as *BRAF*, that phosphorylates MEK on particular regulator residues as serine. Reports of mutated *BRAF* mutations have been mentioned at high frequency in multiple neoplastic disorders (e.g. 60% of melanoma [1], 30% to 50% in papillary thyroid cancer, 5% to 20% in colorectal cancer, and approximately 30% in ovarian cancer). Approximately 80% *BRAF* mutations that happen in human neoplastic disorders are characterized by mutated exon 15 resulting in the amino-acid V600E [1]. Such mutation imitates regulatory phosphorylation which intensifies *BRAF* activity ten-fold versus wild-type *BRAF* [1]. The frequency of this activating mutation and the resultant oncogene addiction makes mutated *BRAF* an extremely attractive target. Another putative RAF inhibitor, sorafenib, has achieved regulatory approval. However, it lacks specificity and potency against *BRAF* and its clinical activity is most likely due to inhibition of other targets. Other *BRAF* inhibitors have recently entered clinical trials, although vemurafenib was the first *BRAF* inhibitor that obtained FDA approval for *BRAF* mutated refractory malignant melanoma.

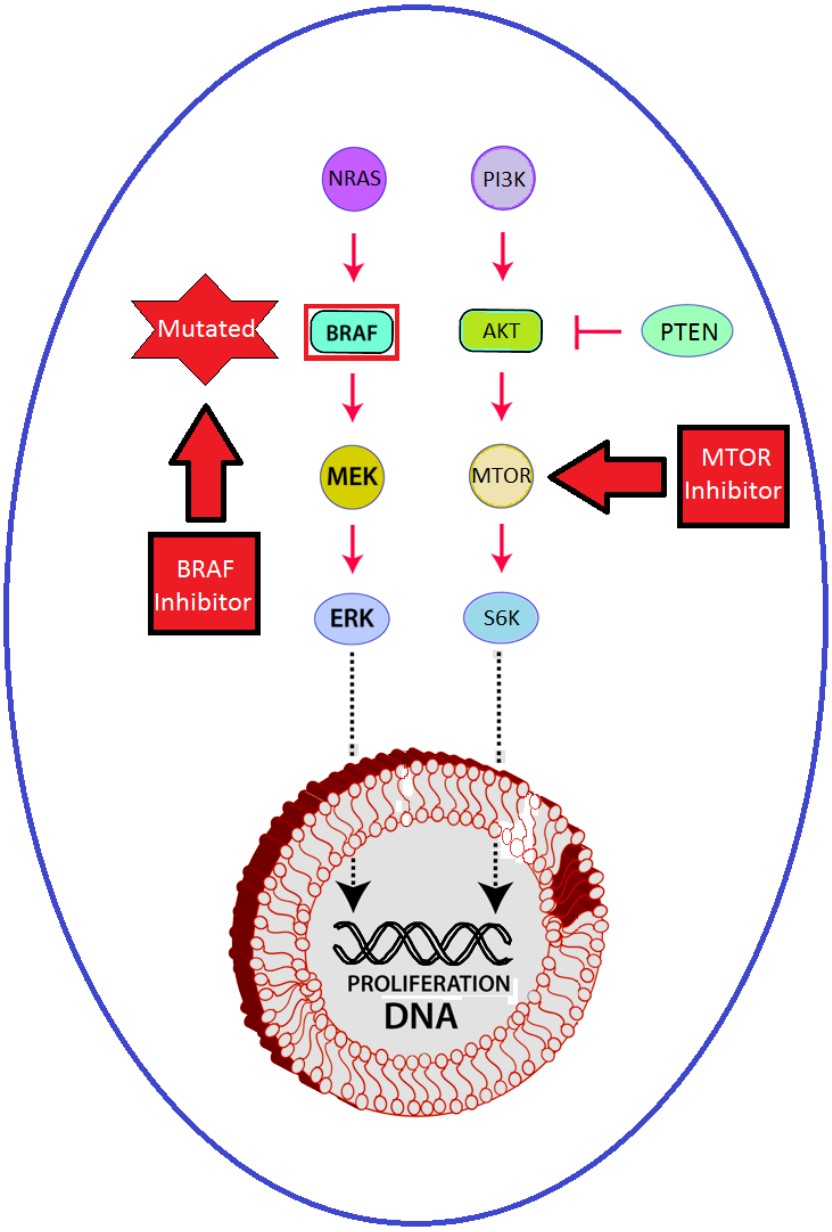

Figure 1. The PTEN-PI3K-AKT-mTOR (mTOR) and RAS-RAF-MEK-ERK (MAPK) signal axis. From Tsimberidou, Targeted Therapy in Translational Cancer Research, 1st edition. Copyright 2015 by John Wiley & Sons, Inc. Published with permission from Rightslink.

As a proof-of-concept, BRAF inhibition has been effective in other conditions as Hairy cell leukemia (Figure 2) [2] and Erdheim-Chester disease [3]. We attempted *BRAF* inhibition with vemurafenib in one patient from our retrospective review of our database of patients with Erdheim-Chester disease (Table 1) [3]; nevertheless therapy had to be halted due to toxicity. Haroche et al. has found *BRAF* mutations in 54% of cases of Erdheim-Chester disease and attempted *BRAF* inhibition in 2 patients with substantial and rapid clinical and biological improvement [4].

Activating mutations specific to the MAPK pathway affect the BRAF, GNAQ, and GNA11 genes (mutations frequently observed in melanomas and colorectal cancer), whereas mTOR pathway-specific alterations include PTEN and TSC1/2 loss, as well as activating mutations or amplification of PI3K (observed in various cancers). The mTOR and MAPK pathways are interconnected by homeostatic feedback loops resulting compensatory activation of one of the pathways in response to inhibition of the other. Cancer-driving receptor tyrosine kinases (EGFR, HER2, cMET, c-KIT) and the downstream effector RAS signal through both pathways simultaneously, and double mutations in the components of both pathways and/or concurrent up-regulation are frequently observed in associated tumors.

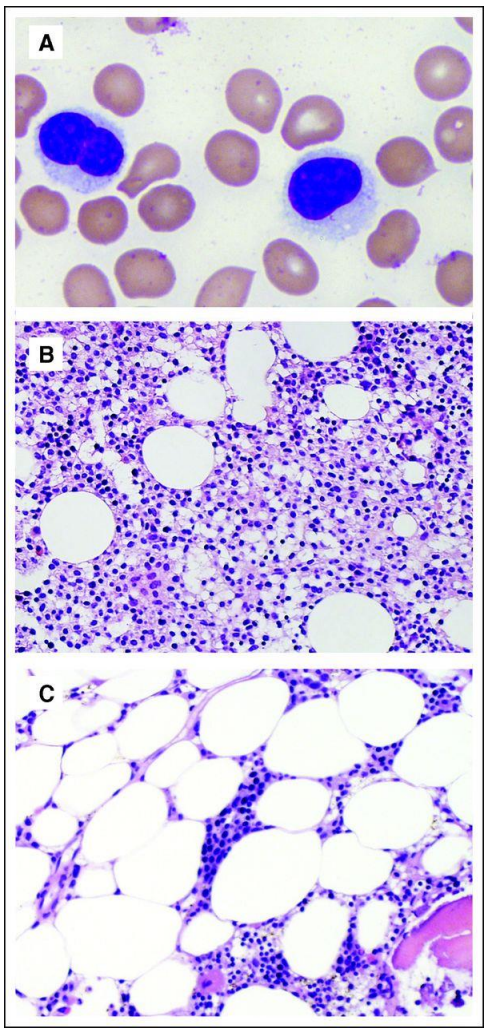

Figure 2. Pathology slides showing Hairy cell leukemia status post vemurafenib. Bone marrow (panel A) displays hairy cells. Four months later, bone marrow biopsy displays decreasing hairy cells from 68% (panel B) to 10-20% (panel C). Originally published by the American Society of Clinical Oncology. Munoz J, Schlette E, Kurzrock R. J Clin Oncol. 31 (20), 2013 Jul 10:e351-2. Published with permission from Rightslink.

Pathology	Sex	Age at diagnosis (y)	Initial presentation	Involvement	Treatment	Comments (best response)
ECD	M	55	Retro-orbital pain	Retro-orbital, bone, retroperitoneum, CNS	IFN-α	IFN April 2000 to October 2010 then lost to follow-up
ECD	M	48	New-onset atrial fibrillation	Cardiac (valve and pericardiac), retroperitoneum, omental, bone, diabetes insipidus	Steroids, imatinib, IFN-α, anakinra	Imatinib (follow-up unclear) IFN January 2011 until January 2014 (ongoing) Anakinra February 2012 until January 2014 (ongoing)
ECD	M	36	Ascites	Perihepatic, pericardiac, renal, bone, pleural, bone marrow	IFN-α	IFN May 2008 until at least December 2009 (then lost to follow-up)
ECD	M	44	Slurred speech	CNS, retroperitoneum, hydronephrosis, bone	Cladribine, imatinib	Cladribine for 5 cycles from 2008 to 2009 (no response). Imatinib from 2009 until January 2014 (ongoing)
ECD/ LCH	F	31	Ataxia	CNS, bone, lungs, diabetes insipidus, panhypopituitarism	RT to brain, IFN-α, imatinib	IFN January 2005 to March 2009. Imatinib April 2009 to at least until September 2011 (then lost to follow-up)
ECD	F	64	Shortness of breath	Lung, subcutaneous, skin, cecal, adrenal, hilar, bone	Imatinib	Imatinib July 2009 to December 2009 (then lost to follow-up)
ECD	F	51	Bone pain	Bone, lung, diabetes insipidus, panhypopituitarism	Steroids, RT, IFN-α, cladribine, imatinib	IFN April 2002 until April 2007 (at which time there was progression) Cladribine for 5 d in 2008 Imatinib from July 2009 until November 2009 Patient now deceased
ECD	F	46	Mental status changes	CNS, bone, retro-orbital, lungs	IFN-α, steroids, RT, imatinib	IFN 2007 to 2010 (at which time there was progression) Imatinib from July 2010 to August 2010 Patient now deceased
ECD	F	60	Subcutaneous nodules	Bone, subcutaneous	IFN-α, imatinib, IFN-α, anakinra, cladribine	IFN June 2010 to August 2010 (stopped because of toxicity) Imatinib August 2010 to October 2010 (then progression) IFN November 2010 until 2012 Anakinra February 2012 until 2013 Cladribine April 2013 to November 2013 (then progression)
ECD/ LCH	F	76	Bone pain	Bone, carotid artery	Vemurafenib, IFN-α	Vemurafenib July 2012 to September 2012 (stopped because of toxicity) IFN October 2012 to March 2013 (stopped because of toxicity)
ECD	F	44	Polydipsia	Bone, CNS, diabetes insipidus, hydronephrosis	IFN-α	IFN December 2008 until at least February 2012 (then lost to follow-up)
ECD	M	68	Abdominal pain	Bone, cardiac, retro-orbital, perirenal	IFN-α	IFN September 2010 until unknown (then lost to follow-up)
ECD	F	43	Polydipsia	Bone, diabetes insipidus	Anakinra	Anakinra May 2012 until January 2014 (ongoing)
ECD	M	27	Skin lesions	Skin	Imatinib, IFN-α, anakinra, PUVA	Imatinib May 2012 to June 2012 (then progression) IFN October 2012 until September 2013 (then progression) Anakinra added to IFN from March 2013 to September 2013 (then progression) PUVA for skin lesions from September 2013 to January 2014 (ongoing)

Table #1 - Fourteen individuals with Erdheim-Chester disease (ECD) or Langerhan cell hystiocytosis (LCH). Only patients with adequate follow-up were included. Abbreviations: Central nervous system (CNS); female (F); male (M); radiation (RT). Reprinted from Mayo Clin Proc, 89(7), Munoz J, Janku F, Cohen PR, Kurzrock R. Erdheim-Chester disease characteristics and management, 985-96, 2014, with permission from Elsevier. Published with permission from Rightslink.

Therefore, as supported by the concept of synthetic lethality, simultaneous inhibition of the MAPK and mTOR signaling cascades may lead to significantly enhanced antitumor activity compared to inhibition of either cascade alone. Indeed, preclinical data suggests simultaneous blockade of MEK and mTOR substantially enhances antineoplastic action in different tumor xenografts as prostate, colorectal, thyroid, pancreatic, and liver cancer. In vitro, cells carrying dual mutated PI3K/KRAS show increased sensitivity to combined MEK plus mTOR targeted agents.

These findings support the hypothesis that such combination therapy may demonstrate activity for various indications, particularly tumor types characterized by frequently occurring mutations in the respective pathways. The feasibility of concomitant inhibition of MAPK and mTOR pathways is being actively explored (http://clinicaltrials.gov), and includes trials of MEK inhibitors GSK1120212 and AZD6244 combined with the mTOR inhibitors, everolimus and temsirolimus, respectively. Furthermore, mutated PI3K and mutated members of MAPK have been frequently found in neoplastic disorders.

For example, mutated PIK3CA coincide with mutated RAS, as KRAS-NRAS, and mutated *BRAF*. Janku et al. [5] showed that PIK3CA mutations occurred in 54 (11%) of 504 patients tested; whereas mutated *BRAF* had been reported in 31 (9%) of 361 individuals. *BRAF* mutations were seen in 44% (23/52) of patients with melanoma. Regardless of histology, mutated RAS (KRAS, NRAS)

or BRAF had been reported in 47% of individuals with mutated PIK3CA versus 24% wild type PIK3CA. PIK3CA mutations were observed in 20% of individuals with mutated RAS or *BRAF* versus 8% with wild type BRAF or wild type RAS [5].

Everolimus has the same mechanism of action as an immunosuppressant and an antitumor agent. Everolimus works via inhibition of mTOR (mammalian target of rapamycin) which is a protein kinase implicated in cell cycle control, specifically cellular progression from the G1 to S phase. Furthermore, mTOR is located downstream from PI3K and AKT. Then eIF4E-binding protein or 4E-BP1 and p70-S6-kinase (S6K) are located downstream from mTORand subsequently translate and regulate mRNAs encoding proteins. The currently FDA approved mTOR inhibitors are temsirolimus, which is intravenous, and everolimus, which is oral.

The proposed trial seeks to establish the MTD of combined vemurafenib plus everolimus in individuals with advanced resistant solid malignancies characterized by the prevalence of MAPK and mTOR pathway alterations. In addition to clinical safety evaluations, this study provide data regarding pharmacokinetics regarding such combination. Pharmacodynamic activity and preliminary evaluation of antitumor activity of the combination will be evaluated to select indications for further development of the combination in phase II trials.

The rest of this chapter named introduction is based upon: Munoz J, Janku F, RAS-RAF-MEK pathway: Aberrations and therapeutic possibilities (Submitted for publication, Book chapter, Targeted Therapy in Cancer, Wiley). The mitogen-activated protein kinases (MAPK) belong to a group of threonine- serine kinases that form a cascade of molecular signals, eventually leading to proliferation, survival, differentiation and cell fate determination [6].

The MAPK network is organized hierarchically (Figure 3) beginning with cell membrane receptors subject to external stimuli (such as hormones, cytokines and growth factors). These successively initiate proliferation from the cell membrane to the nucleus as MAPK's become phosphorylated by MAPK's kinases (MAPKK's), which subsequently are phosphorylated by MAPKK's kinases (MAPKKK's) that further become active via other kinases located the nearby cell membrane [6]. The primary MAPK network is RAS:RAF:MEK:ERK [7] axis, composed by Rat Sarcoma (or RAS), rapidly accelerated fibrosarcoma (or RAF), MAPK/ERK kinase (MEK); plus extracellular signal-regulated kinases (ERK). Once up-regulated, carcinogenesis is initiated. An inherited deregulated MAPK pathway, usually due to heterozygous mutations [8], causes several phenotypic conditions marked by cognitive defects, facial dysmorphism, cardiac defects, and an increased risk of malignancies, known as the neuro-cardio-facial-cutaneous syndrome family [8].

Other components of this intricate network (Figure 3) include BRAF (or vraf murine sarcoma viral oncogene homolog-B1) [9], whose designation stems from

the original identification of RAF during an exploration of retroviral oncogenes. Initially RAF-1 was discovered (now called CRAF) in 1985, then ARAF in 1986, and subsequently BRAF in 1988 [10].

Hierarchically, the apex of the cascade is composed of HRAS, KRAS, and NRAS [10]. The next layer is formed by the MAPKKK, including ARAF, BRAF, and CRAF. These can homodimerize or heterodimerize [10]. MEK1 and MEK2 compose MAPKK, which completes the network with ERK1 and ERK2, and MAPK [10]. Although the MAPK network is generally shown as a linear path in cartoons (Figure 1), in reality it branches out and interacts with molecular members of other pathways including mTOR [11].

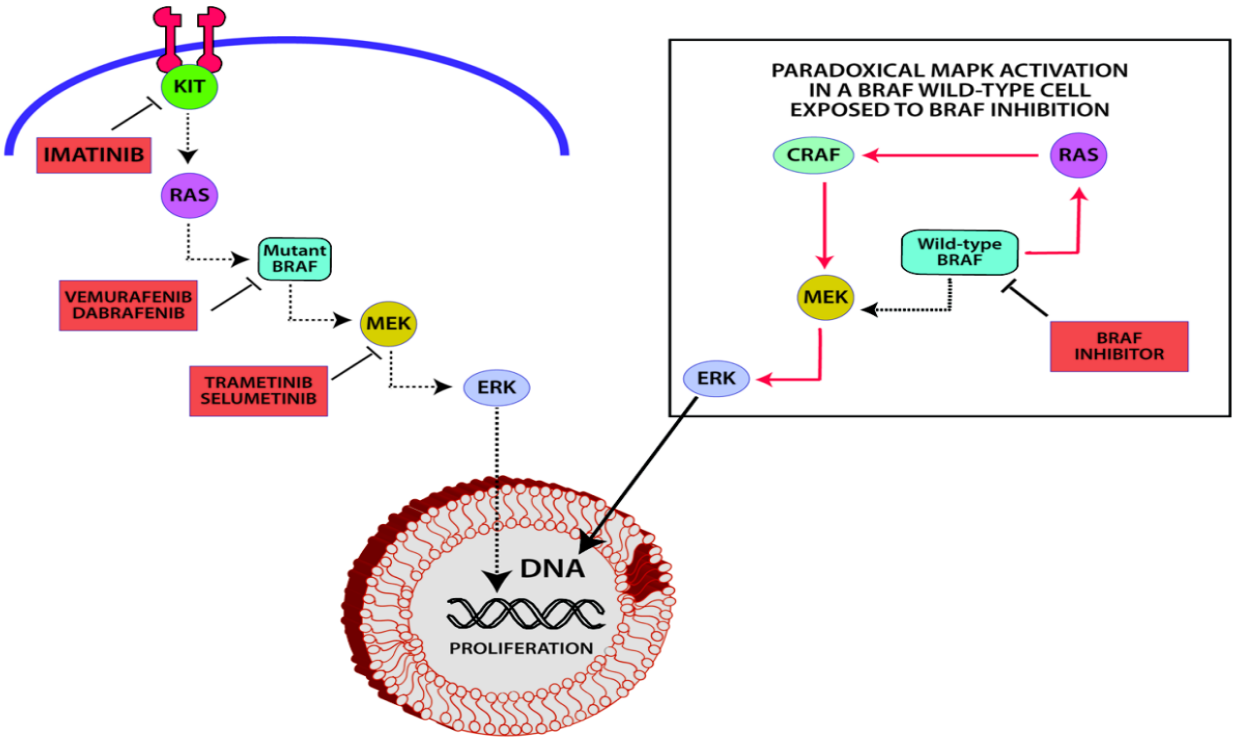

Figure 3. Simplified diagram of the MAPK signaling pathway. Following stimulation of a cell-surface receptor (e.g., KIT), From Tsimberidou, Targeted Therapy in Translational Cancer Research, 1st edition. Copyright 2015 by John Wiley & Sons, Inc. Published with permission from Rightslink.

Germline mutations in the MAPK pathway are associated with developmental abnormalities [12]. Somatic mutations and acquired aberrations in the MAPK pathway, particularly *RAS* and *BRAF* mutations, are associated with malignancies [13]. For example, the MAPK pathway is activated in most melanomas [14]. Furthermore, targeted therapy selectively or non-selectively inhibiting those aberrations with small molecules has shown benefit [13]. Here, the

currently known MAPK pathway mutations and therapeutic possibilities suggested by these biomarkers are explicated.

Given the complexity of crosstalk among downstream signals, a working hypothesis underlying this phase I study would be that BRAF plus mTOR blockers will likely become synergistic. Additionally, combination therapy with drugs that target different key signal transduction pathways may help overcome both intrinsic and acquired resistance in individuals with prior exposure to RAS/RAF/MEK and/or mTOR inhibitors. Furthermore, vemurafenib has been chosen as a BRAF inhibitor because of its potency and there is ample evidence in the literature in BRAF mutation positive metastatic melanoma to support its efficacy [15]. Additionally, its strategic location at the top of the RAS:RAF:MEK:ERK (MAPK) axis is expected to benefit and currently no RAS inhibitors are available. Of interest, pre-clinical plus clinical trials show that combined BRAF plus mTOR inhibitors is efficacious, especially in tumors with co-existing BRAF and PI3K/AKT/mTOR aberrations [16-19].

Combining multiple agents with different mechanisms of action is now a paradigm in oncology phase I clinical trials. Vemurafenib has received FDA approval in the United States for treating refractory BRAF mutation positive malignant melanoma and is being investigated in various other malignancies. The RAS:RAF:MEK:ERK axis is very important when it comes to cell proliferation in many human cancers. The frequency of this activating mutation and the resultant

oncogene addiction makes mutated BRAF an extremely attractive target. The BRAF inhibitor vemurafenib has been selected as the backbone of this trial. The mTOR inhibitors, such as everolimus and temsirolimus, are well known to exert profound anticancer effects across malignancies via inhibiting the PTEN:PI3K:AKT:mTOR axis [20]. We hypothesize that combining a BRAF plus an mTOR inhibitor may be synergistic and assist in overcoming resistance to single agents targeted to the PTEN:PI3K:AKT:mTOR (mTOR) and/or RAS:RAF:MEK:ERK (MAPK) signal axis. To our knowledge, there are no clinical trials currently evaluating such combination. Therefore, we have designed a phase one trial evaluating such combination in cancer patients.

BACKGROUND:

The RAS family: HRAS, KRAS and NRAS

The rest of this chapter named background is based upon: Munoz J, Janku F, RAS-RAF-MEK pathway: Aberrations and therapeutic possibilities (Submitted for publication, Book chapter, Targeted Therapy in Cancer, Wiley). The RAS (rat sarcoma) genes were named because of the similarity of their sequences to the Harvey rat sarcoma virus (or HRAS) and Kirsten rat sarcoma virus (or KRAS) [21]. Bos et al. [22] in 1989 reported *RAS* mutations in pancreatic adenocarcinoma

(90%), colon cancer (50%), lung cancer (30%), and thyroid tumors (50%). *KRAS* mutations occur most frequently (approximately 85%), then *NRAS* (approximately 15%), and *HRAS* (less than 1%). KRAS, NRAS and HRAS have a high degree of homology and are expressed in many tissues. On average, somatic mutated *RAS* ensue in as many as 30% of malignancies [22], although deregulated RAS activation can occur without *RAS* mutation in the setting of up-regulated upstream stimuli signal transducers or down-regulated downstream negative feedback. These pivotal RAS molecules are small G proteins, or guanosine triphosphate (GTP) / GTP-ases, frontline master regulators activating an intracellular network of signals that ultimately lead to gene expression and proliferation. Small G proteins also include RRAS, MRAS, Rap-2A, among others [23]. Guanine nucleotide-exchange factors (GEF) remove guanosine diphosphate (GDP) from inactive GDP-bound RAS. Consequently, RAS has a greater proclivity to bind to the more prevalent GTP that then converts into its active form, GTP-bound RAS. In summary, RAS proteins are governed via connecting to GTP and/or GDP, that subsequently produces active or inactive proteins [24]. RAS proteins are tightly regulated, due to a fined tuned balance between GDP/GTP switching, activators such as GEF and natural inhibitors such as GTPase activating proteins (GAP).

RAS (HRAS:KRAS:NRAS) can carry aberrations that impair the alteration or balance from GTP-active versus GDP-inactive form of RAS. From those mutations, Gly12Val is the most frequent *HRAS* aberration in malignancies, accounting for approximately 45% of total somatic *HRAS* gene mutations. The

frequency of particular mutations depends on whether aberrations of a particular gene are germline or acquired mutations. Interestingly, germline *KRAS* mutations are rare in human malignancies; where acquired somatic *KRAS* mutations occur far more frequently.

The congenital RAS-opathies: Germline mutations of *RAS*

A phenotypic spectrum is linked to a disturbed MAPK pathway causing genotype-phenotype associations such as *RAS* aberrations [25] and neuro/cardio/facial/cutaneous disorders. These are known as RAS-opathies and include Noonan syndrome [26] (predisposed to juvenile myelomonocytic leukemia), LEOPARD [27], Neurofibromatosis type 1 [28] (predisposing individuals to myeloid malignancies such as juvenile myelomonocytic leukemia), Costello syndrome [29] (which can result in solid tumors such as rhabdomyosarcoma), cardiofaciocutaneous syndrome [30, 31] (associated with acute lymphoblastic leukemia), and other Noonan-like syndromes.

Each gene in the MAPK pathway, located on different chromosomes, encodes a different protein so it is not surprising that different mutations manifest clinically as different diseases [25]. The clinical presentation of these diseases is not, however, exclusively associated with a particular mutation in these RAS-

opathies. An example is the relatively common Noonan syndrome, which has been associated with multiple MAPK aberrations (*KRAS:NRAS:BRAF:MEK1)*. The son of sevenless (SOS1) gene is a type of GEF, which as explained above, alters the GDP/GTP balance involving RAS. Clinical overlap among these hyperactive RAS syndromes [32] is likely due to an interplay among multiple members of MAPK. Cardiofaciocutaneous syndrome were linked to a varied array of mutated *KRAS:BRAF:MEK1:MEK2* genes in as many as 90% of patients. One exception is a germline missense mutated *HRAS* proto-oncogene causing confirmed Costello syndrome in almost a 100% of affected patients. By the same token, neurofibromatosis type 1 (NF1) is secondary to heterozygous *NF1* gene loss-of-function, which regulates expression of neurofibromin, a RAS GTPase, a large ubiquitous protein highly expressed in neurons, Schwann cells, and leukocytes accounting for the clinical stigmata of neurofibromas. *NF1* is a tumor suppressor gene and patients are thus prone to second-hit malignancies as neurofibromin is a protein with GAP activity (a negative controller of MAPK axis). Patients with type-1 neurofibromatosis have more benign tumor development called neurofibromas and malignancies such as peripheral nerve sheath tumors [33], sarcoma, GIST, and other types of neoplasia [34], and juvenile myelomonocytic leukemia [28]. Costello syndrome can present with sarcomas, neuroblastoma, and other types of neoplasia [29].

Even though the genotype-phenotype relationship is not completely clear [12], mutations in *KRAS* affect the skin and may develop leukemia. Mutations in

HRAS (such as Costello syndrome) manifest via skin abnormalities and tumor growths. For example, patients with Noonan syndrome have a small increased likelihood of developing malignancies [35]. LEOPARD syndrome has been associated with leukemia, neuroblastoma, and melanoma [35, 36].

The acquired RAS-opathies: Melanoma and NRAS

Once a receptor is stimulated by cytokines or growth factors, the receptor gets attached to Src homology 2 (SH2) domain that recruits SOS, subsequently disrupting the homeostatic GDP/GTP balance. Cell receptor stimulation causes RAS to dissociate from GDP and RAS binding to GTP, activating MAPK pathway downstream components including RAF and MEK [37]. RAS activation is restricted by GTP-ase activity or GAP's that balances active-GTP-attached RAS versus inactive-GDP-attached RAS. Mutations in *RAS* proteins change the amino-acids (as G12:G13:Q61), modifying hydrolysis from the binding of RAS to GTP, thus activating the MAPK pathway. *BRAF* mutations have been seen in 50 to 70% of patients with melanoma [38, 39], whereas somatic *NRAS* mutations are found in 15-30% of cases, producing a constitutively active NRAS protein, which stimulates the MAPK pathway. It has been suggested that an interaction exists between NRAS and c-Met, epidermal growth factor receptor (EGFR), and KIT. Most patients with melanoma have a hyperactive MAPK pathway; thus, it is not surprising that a

MEK inhibitor such as MEK162 is associated with positive outcomes in *NRAS* mutant melanoma.

RAF family: ARAF, BRAF, and CRAF

The ARAF:BRAF:CRAF proteins belong to the serine/threonine group of kinases downstream from RAS, and upstream from MEK1/2. Even though ARAF:BRAF:CRAF are siblings from a family, they have distinct characteristics; BRAF would the powerful stimulator of MEK after comparison versus its relatives, ARAF and CRAF. For example, BRAF and CRAF have essential differences in binding to RAS [40] and are governed by distinct autoregulatory mechanisms [41]. Of the 3RAF isoforms, BRAF is most frequently involved in cancer (approximately 7% in general and 70% melanoma) [42]. Most mutated *BRAF* arise within the kinase domain, leading to V600E substitute that stimulates MAPK [1]. Somatic mutated *BRAF* were commonly documented in multiple malignancies; nevertheless aberrations in ARAF and CRAF are rarely seen. Despite the fact that multiple mutated germline *BRAF* were documented, germline *BRAF* mutations rarely promote tumorigenesis as they do not have the malignant potential of the Val600Glu *BRAF* mutation. Interestingly, germline and somatic amino acid shifts may up-regulate or down-regulate the mutant kinase.

BRAF inhibitors

Regarding RAF in tumorigenesis, although BRAF is the main RAF subtype overall such neoplasia characterize only a portion of malignancies. To further complicate this picture, CRAF and BRAF can act in concert through heterodimerization (Figure 3). Sorafenib, a RAF inhibitor that also blocks other tyrosine kinases along with vascular endothelial growth factor, was not effective treating patients with melanoma *BRAF* V600E mutations and phase three studies did not endorse beneficial effects from adding sorafenib to standard of care [43, 44] despite initial promising results [45]. It may well be that other activated pathways such as PI3K will need to be abrogated to produce a more beneficial response [46]. Vemurafenib spearheads the list of approved BRAF inhibitors and prolonged overall survival (6-month survival rates of 84% versus 64%) compared to dacarbazine in *BRAF* V600-mutant melanoma randomized into the BRIM-3 study [15], results subsequently confirmed by an extended follow up [47]. Another BRAF inhibitor, dabrafenib received FDA approval in U.S. (05/29/2013) to treat *BRAF* V600E mutant melanoma [48]. The approval of dabrafenib was established on improved progression-free survival (median 5.1 versus 2.7 months) compared to dacarbazine in an international, open-label phase three study in 250 individuals with *BRAF* V600E mutant melanoma [49].

Management of melanoma in the era of B-RAF inhibitors

The management of early-stage high-risk cutaneous melanoma with local resection plus adjuvant interferon alfa has been described elsewhere [50]. Managing metastatic melanoma is however more complicated. Despite the high toxicity and low cure rate of high-dose interleukin-2, only recently have newer agents revolutionized the management of metastatic melanoma such as the immunotherapy ipilimumab, the monoclonal antibody directed against CTLA 4, or a targeted therapy as vemurafenib (FDA approved if there is a V600 driver mutant in the *BRAF* gene, which is present in approximately 50% of patients) [51, 52]. This approval was based on clinical trials demonstrating prolongation of overall survival in this population. The replacement of glutamic-acid by valine at amino-acid 600 or V600E mutation was found in approximately 80% of cases [52]; whereas the substitution of lysine for valine (V600K mutation) was shown in 20% of cases.

To date, no randomized comparison has been undertaken between immunotherapy with ipilimumab, high-dose interleukin-2 and BRAF inhibitors or the appropriate sequencing of such agents. Nevertheless, it is indicated that all patients be assessed, minimally for *BRAF* mutation, or to test for a more comprehensive panel of mutations. If a comprehensive mutational evaluation is not available, in the absence of *BRAF* V600E mutation, screening for non-V600E *BRAF* mutations, other MAPK aberrations (e.g., *NRAS*) and KIT should be done. Just as distinct malignancies based on their organ of origin have different

frequencies of a particular aberration (Figure 4) [1]; there are particular phenotypic characteristics that correlate with the genotype of patients with melanoma [53]. As an example, an acral melanoma may carry a *KIT* mutation (approximately 15-20%) instead of a *BRAF* mutation. Initial phase two trials of imatinib for unselected metastatic melanoma showed limited activity [54-56]; nevertheless a subsequent phase two trial with selected individuals harboring a *KIT* mutation or amplification showed 23.3% response [57]. The MAPK pathway and microphthalmia-associated transcription factor (MITF) were associated in melanocyte differentiation/survival [58, 59]. MITF is phosphorylated by the MAPK pathway [60] and MITF mutation has been associated with familial and sporadic melanoma [61]. In addition to MITF, specific aberrations have been correlated with particular subtypes of melanoma such as BRAF/NRAS in conjunctival melanomas (*BRAF* mutations in 29% and *NRAS* mutations in 18%) [62], *KIT* mutations or amplifications in acral (36%) and mucosal (39%) melanomas [63], and *GNAQ*/*GNA11* in uveal melanomas (*GNAQ* in 45% and GNA11 in 32%) [64] (Figure 4). Furthermore, *BRAF* mutations are common in vertical growth phase melanoma and metastatic melanoma (62-72%) [65], whereas *BRAF* mutations are rare in radial growth phase melanomas (10%) [65] or in in situ melanoma (5.6%) [66]. Finally, mutated *BRAF* has been reported in non-malignant growths (82% in nevi) [9, 66] suggest that *BRAF* mutations are involved in collaboration with other molecular aberrations in carcinogenesis rather than being solo founder mutations. As an example, mutant *BRAF* had been reported in 29% of invasive melanoma versus 5.6% of in situ melanomas. Mutant *NRAS* had been described in 5.2% of primary melanomas and in no in situ

melanomas [66]. These *NRAS* & *BRAF* mutations seem to occur prematurely during melanoma-genesis while remaining present during worsening of disease [14].

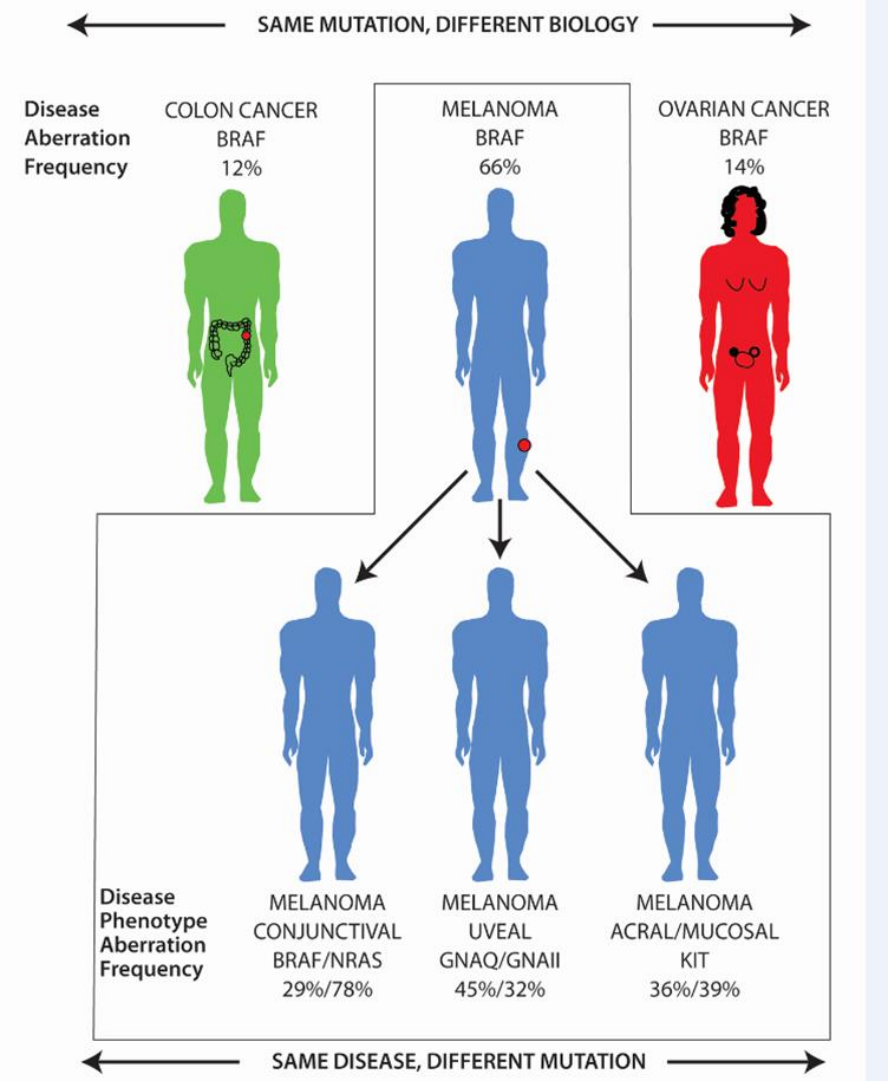

Figure 4. Genotypes and phenotypes of malignancies expressing BRAF mutations. From Tsimberidou, Targeted Therapy in Translational Cancer Research, 1st edition. Copyright 2015 by John Wiley & Sons, Inc. Published with permission from Rightslink.

Comorbidities, performance status, drug toxicities, pace of disease progression, and presence of brain metastases are factors to be considered in choosing the appropriate course of therapy. For example, unfit patients with fast-paced bulky disease and central nervous disease involvement are unlikely to benefit from high-dose interleukin-2, although BRAF inhibition can salvage patients in that scenario. Ipilimumab, an antibody-based immunotherapy directed against the CTLA-4 checkpoint, may need a prolonged period of time to show activity, and would not be appropriate in the setting aggressive disease progression. In contrast, BRAF inhibitors are a very attractive targeted therapy in melanoma. Both vemurafenib [67, 68] and dabrafenib [69, 70] have reported activity with melanoma that invaded the central nervous system.

CRAF story

Downstream RAS, such next line of activated molecules includes BRAF and CRAF. No reports of activating mutations of CRAF have been documented so far, whereas BRAF kinase domain mutations are as common as 50% in melanoma. As a result, it has been suggested that there is single-step activation between RAS and BRAF, but that multiple-steps might be involved between RAS and CRAF [71]. BRAF inhibitor drugs abrogate MAPK in mutated *BRAF* cell lines, whereas BRAF inhibitors may paradoxically stimulate the MAPK pathway within wild-type BRAF cells [72-74]. Despite being relatively safe, dermatologic toxicity [75] was seen with

these inhibitors. These include cutaneous squamous cell carcinoma in 12% [76], sometimes developing within weeks of starting a BRAF inhibitor suggesting preexisting *RAS* mutations in other skin areas due to paradoxical activation of the MAPK pathway (*HRAS* mutations in 41% of 29 samples with cutaneous squamous cell carcinoma or keratoacanthomas) [77]. It has been suggested that combining a MEK inhibitor and a BRAF inhibitor may decrease toxicity caused by paradoxical stimulation of MAPK axis. As a more ominous complication, a patient exposed to vemurafenib developed fast worsening of chronic myelomonocytic leukemia related to mutant *RAS* [78].

Primary and secondary resistance to BRAF inhibitors

Despite high initial responses as high as 48% [15], primary and secondary resistance to vemurafenib has been reported and most melanoma patients exposed to vemurafenib eventually develop resistance (Figure 5). Thus, combinatorial trials using BRAF inhibitors as a backbone or small molecules targeting other areas of the MAPK pathway are suggested to overcome resistance. Tissue samples obtained during the phase two BRIM2 study showed an association between decreased ERK phosphorylation and objective responses, whereas increased ERK phosphorylation and the development of secondary *NRAS* (Q61) or *MEK1* (Q56P) or *MEK1* (E203K) mutations were associated with acquired resistance [79].

Thus, re-stimulation of MAPK seems to develop resistance to drugs that abrogate BRAF. Interestingly, resistance hasn't been linked with developing a second mutation that impairs drug binding to BRAF, a mechanism observed in other malignancies. Other possible mechanisms that can cause resistance include MAPK pathway reactivation via alternative means like insulin growth factor receptor-1 (or IGF-1R)/PI3K axis activation [80, 81], PD-L1 expression [82], increased cyclin D1 expression [83], elevated CRAF protein levels [84], production of shortened forms of BRAF proteins due to aberrant RNA splicing [85], *NRAS* (Q61) mutations [79], *MEK1* (Q56P, E203K, C121S, or F129L) mutations [79, 86, 87], and ERK activation through bypassing mechanisms including COT activation and receptor tyrosine kinase as PDGFRβ upregulation [88, 89]. Conversely, clinical response associated with BRAF inhibition lead to decreased phosphorylated ERK levels [90].

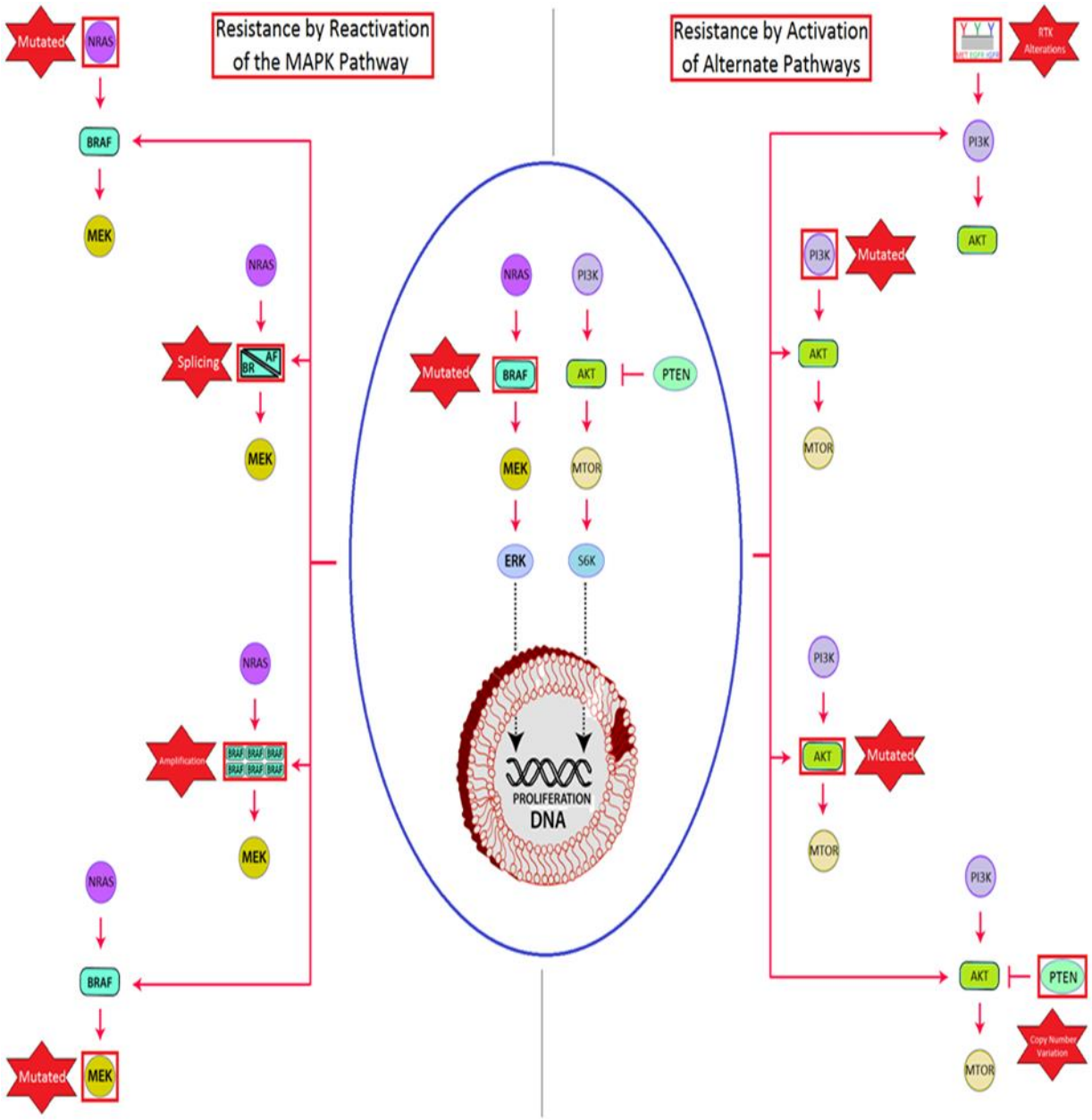

Figure five. Mechanistic diagrams regarding resistant malignant cells to BRAF inhibition. From Tsimberidou, Targeted Therapy in Translational Cancer Research, 1st edition. Copyright 2015 by John Wiley & Sons, Inc. Published with permission from Rightslink.

MEK inhibitors - The MEK family: MEK1 and MEK2

RAS activation is followed by activation of RAF (ARAF:BRAF:CRAF), subsequently MEK (MEK1A1/MEK1A2), then finally ERK (ERK1:ERK2). ERK is the final step of the pathway and acts upon multiple proteins. MEK1:MEK2 genes encode kinases that activate ERK proteins, their only known substrate. MEK kinase activity has been documented as inducing proliferation, although no *MEK* mutations have been associated with triggering development of cancer or primary resistance to vemurafenib. Interestingly, a *MEK1* C121S mutation was recently seen in a melanoma case that became vemurafenib-resistant. The mutant had not been present before vemurafenib therapy, supporting the role of molecular evolution in therapeutic resistance. MEK aberrations have however been linked to some neurocardiofacialcutaneous syndromes. In melanoma, the *BRAF* V600E mutation correlates with response to MEK inhibitors in preclinical models and clinical studies. Trametinib abrogates MEK1/MEK2 in patients with prior anti-BRAF therapy for mutant *BRAF* V600E/V600K [91]. Trametinib (2 mg/day orally) was FDA approved based on improved PFS (median 4.8 versus 1.5 months) and overall survival (6-month survival rate of 81% versus 67%) compared to chemotherapy (dacarbazine or paclitaxel) in the phase 3 METRIC study in 322 individuals that have *BRAF*V600E–positive advanced melanoma [92]. Individuals status post chemotherapy or immunotherapy were included, whereas prior BRAF inhibitors were not allowed and no responses to trametinib were observed [93].

Trametinib received FDA approval [91] when combined with dabrafenib for initial treatment for mutant *BRAF* V600E/V600K melanoma. Overall response with dabrafenib (150 mg) combined with trametinib (1 or 2 mg) had been reported as 76% versus 54% with single agent dabrafenib (P=0.03) [94]. Squamous cell skin cancer developed not as frequently in the dual drug group compared to monotherapy (7% versus 19%), whereas pyrexia developed more often in the dual drug combination versus monotherapy (71% versus 26%) [94].

Other MEK inhibitors under development are selumetinib, MEK162 and others [95]. The combination of selumetinib plus dacarbazine was compared to single-agent dacarbazine in a phase 2 study with randomization that accrued 91 *BRAF* mutant individuals, showing improved progression-free survival (5.6 versus 3.0 months) but not an improvement in survival [96]. Furthermore, a phase II trial assessed MEK162 in 71 individuals with melanoma carrying V600 *BRAF* (41 cases) or *NRAS* mutations (30 individuals) with partial response of 20% in both groups (8/41 cases in *BRAF* mutations plus 6/30 patients in *NRAS* mutations) [97].

ERK inhibitors

A novel selective ERK1/2 inhibitor SCH772984 that may work in cases resistant to BRAF or MEK inhibitors while producing improvement in xenografts [98]. Further data regarding ERK inhibition is eagerly awaited.

Implications of aberrations in the MAPK pathway in the management of lung carcinoma

Personalization of genotype-driven treatment for metastatic lung cancer is promising with multiple driver mutations have been identified such as *EGFR*, *ALK*, *ROS1*, *BRAF*, *NRAS*, *KRAS, among others*. *BRAF* mutants have been reported in 1-3% of patients with NSCLC [99-101]. The trial testing dabrafenib in *BRAF* V600E metastatic NSCLC is ongoing (NCT01336634). *NRAS* mutations were seen in less than 1% (1 of 195) of NSCLC [102]. *KRAS* mutants seem to be more common in smokers [103]. *KRAS* mutations were seen in 22% of smokers with lung adenocarcinomas, whereas transition *KRAS* mutations were seen in 15% of non-smoker patients with lung adenocarcinoma [104]. The effect of mutated *KRAS* on 300 of 1,543 individuals with early NSCLC status post adjuvant chemotherapy following resection didn't show a statistically significant differences in survival versus wild-type *KRAS* in pooled-analysis of four clinical studies [105]. In the metastatic setting, mutated *KRAS* conferred a worse prognosis compared to mutated *EGFR* [106], whereas *KRAS* mutation was prognostic for reduced PFS in the ones that received erlotinib-maintenance although didn't show statistically significant difference in survival compared to wild-type *KRAS* [107]. KRAS mutations herald patients with colon malignancies that are resistant to cetuximab. Nevertheless, responses to cetuximab were maintained in phase 3 studies of NSCLC [72, 108]. In the absence of current KRAS targeted drugs, the therapeutic

emphasis for *KRAS*-mutant lung carcinoma is to target molecules located downstream from activated KRAS, which is supported in pre-clinical models [18]. Objective responses had been documented in 16/43 individuals (37%) status post docetaxel plus selumetinib compared with none of 40 KRAS-mutated individuals with advanced NSCLC receiving docetaxel plus placebo [109]. Clinical trials evaluating MEK inhibitors combined with chemotherapy in *KRAS*-mutated NSCLC are underway (NCT01192165, NCT01362296).

Acquired mutations in the MAPK pathway in other malignancies

Broadly stated, *RAS* mutations are present in as many as 30% of all malignancies [10], whereas *BRAF* mutations are found in as many as 60% (melanomas 60%; thyroid neoplasia 50%; colon malignancies 20%) [10]. Activating *RAS* oncogenic mutations (*NRAS*, *HRAS* and *KRAS* in decreasing frequency) are more frequently seen in follicular-subtype thyroid neoplasia (80%) than papillary-subtype thyroid cancer (20%) [110]. *RAS* mutations have been linked to worse outcome in thyroid neoplasia [111]. On the other hand, 43.8% of 500 patients with papillary thyroid cancers were found to have *BRAF* mutant state, which were linked to higher invasiveness [112]. The *BRAF* V600E mutation has been linked with high-risk clinicopathological factors [113] and increased cancer-related mortality in individuals with papillary-subtype thyroid cancer [114]. BRAF

inhibitors explored in preclinical mice models of thyroid carcinoma decreased levels of phosphorylated MEK and ERK [115].

Erdheim-Chester disease (ECD) has been felt to be characterize as non-Langerhans cell histiocytosis. Because it could co-exist in the setting of Langerhans histiocytosis [116, 117], it is believed that these conditions may overlap pathologically and therapeutically [118]. *BRAF* mutations were found 54% (13/24) of patients with Erdheim-Chester disease and [4] in 38% (11/29) to 57% (35/61) of patients with Langerhans cell histiocytosis [119, 120]. Subsequently, three patients with relapsed mutated *BRAF* V600E Erdheim-Chester disease displayed positive outcomes status post BRAF inhibitor vemurafenib [121]. Individuals with classic hairy cell leukemia almost always carry the V600E BRAF mutation [122], whereas approximately 50% of variant hairy cell leukemia carry *MAP2K1* gene (encoding *MEK1*) mutations [123] instead of *BRAF* mutations [124]. Some have suggested that patients with exon 15 *BRAF*V600E-negative hairy cell leukemia should be screened for exon 11 (F468C and D449E) mutations [72]. Case reports of clinical improvement after exposure to the BRAF inhibitor vemurafenib have been described [72, 125] and clinical studies are in progress to determine the protagonist part of BRAF inhibition for hairy cell leukemia (NCT01711632). *BRAF* kinase mutants have been documented in 4% of multiple myeloma [126]. The case report of an individual with mutated *BRAF*V600E multiple

myeloma documented response to low-dose BRAF inhibition with vemurafenib [127].

Big results for small molecules

Although aberrations in the MAPK pathway have been known to contribute to deregulated growth, both in inherited developmental disorders and acquired mutations, rendering patients prone to malignancies, only until recently have inhibitors been developed that match their respective targets. Initial investigations on the MAPK pathway were based on pre-clinical models of acute growth factor exposure in the lab, which do not correlate with a normal physiological state in vivo, hence, the utility of MAPK pathway inhibitors is being tested in the clinic and the challenges of developing a state of BRAF inhibitor resistant disease need to be studied (Figure 4). Three agents have been approved by the FDA for use in *BRAF*-mutant metastatic melanoma, the BRAF blockers (vemurafenib & dabrafenib) and the MEK blocker (trametinib). Further exploration of MAPK inhibition in other malignancies is eagerly awaited. Molecular stratification, and targeted therapy of the MAPK network poses us for success while offering the opportunity to launch a decisive attack against cancer.

PATIENTS AND METHODS:

Patient Eligibility:

Inclusion Criteria:

- Confirmed BRAF mutated status.
- Measurable or non-measurable disease.
- Individuals with advanced cancer.
- Refractory to standard of care.
- Three weeks post prior treatment.
- ECOG better than two.
- Adequate organ and marrow as per standard (e.g. ANC>1.0).
- Contraception if needed during and thirty days post study.
- Able to understanding/signing/consenting our study.

Exclusion Criteria:

- Poorly controlled additional conditions.
- Poor organ function (e.g. creatinine worse than 2.0).
- Pregnancy.
- Status pot stem cell transplantation.

- Allergy to vemurafenib or everolimus.
- Recent surgical procedure (within a month).
- Patients with a baseline QTc > 500 ms.

Treatment Plan:

This is a phase one, single hospital, open-label, dose-escalation trial of vemurafenib plus everolimus, dosed in combination to individuals with metastatic or advanced solid malignancies. Dose escalation for such study will examine eligible patients with various tumor types. The study will be piloted at the MD Anderson Cancer Center in Texas. Other premedications may be substituted or not used at all based on physician discretion. Patients will continue treatment until their disease worsens, their side effects become too severe, the patient's physician feels we shouldn't continue, or election to withdraw from study. A patient may also be discontinued due to a concurrent illness that prevents further administration of treatment. Premedication, precautions, route, and schedule for each medication are described in the tables below. Each study medication in this protocol has been approved by the FDA and is commercially available. Other investigational drugs beyond vemurafenib and everolimus are not allowed.

Concomitant medications:

Vemurafenib plus everolimus will be the only chemotherapy drugs (or

agents used with anti-neoplastic intent) given in this study. No other chemotherapeutic or anticancer agents may be administered. Individuals will not enroll in other clinical protocol that administers a treatment or uses a device as treatment while enrolled in this study except for supportive care trials. Irradiation is not allowed during the study, except for palliation purpose at the discretion of the Investigator. Administration of other chemotherapy, immunotherapy or antitumor hormonal therapy during the study is not allowed. Supportive care, including, but not limited to, antinausea drugs can be administered if approved by the treating physician. Because all the agents are commercially available and are FDA approved drugs, all institution standard guidelines for these drugs may be used as per treating physician. Concurrent treatment with bisphosphonates is allowed for patients who received stable doses prior to study entry. All concomitant treatments, including blood and blood products, must be documented and recorded. Erythropoietin may be administered as per treating physician consistent with local guidelines. Granulocyte stimulating factors should be administered according to institutional guidelines. As the proposed agents in this trial have extensive metabolism through the CYP450 3A4 substrates, patients should have a 5 half-life washout period or 4-week washout period, whichever is shorter, prior to receiving the investigational agents.

Overlapping toxicities for vemurafenib plus everolimus: Headache (18-37%). Peripheral edema (15-40%). Rash (20-50%). Diarrhea (20-45%). Fatigue (10-30%). Abnormal liver function tests (20-60%).

Study schema:

Starting Dose	Dose Level 1

Dose-escalation schedule for vemurafenib plus everolimus treatment arm A dose and schedule (28-day cycle)

Dose Level	Vemurafenib* PO BID (mg)	Everolimus PO daily (mg)
Level -1	480 mg	2.5 mg
Level 0	720 mg	2.5 mg
Level 1	720 mg	5.0 mg
Level 2	720 mg	10 mg
Level 3	960 mg	10 mg

Table 2. Dose-escalation schedule for vemurafenib plus everolimus (28-day cycle). The starting dose level is Level 1. If all patients tolerate dose Level 1, then we will dose escalate to dose Level 2. However, if dose Level 1 is intolerable, then we will de-escalate to dose Level -0. If dose Level 0 is intolerable, then we will de-escalate to dose Level -1.

Pretreatment Evaluation:

- Complete history and physical examination within 2 weeks of C1D1.
- 12-lead ECG within 4 weeks of C1D1. Vemurafenib can cause Qtc prolongation and monitoring is recommended as per package insert.
- Laboratory studies: CBC with differential, BUN, creatinine, potassium, magnesium, total bilirubin, SGPT [ALT], glucose, triglyceride, cholesterol, and urine pregnancy test. Dyslipidemia is a known side effect of everolimus and

needs to be monitored as per package insert.

- Radiologic evaluation of measurable disease and pertinent tumor markers within four weeks before starting treatment. If the patient does not have radiologically measurable disease but has cutaneous measurable disease, this must be documented at the pretreatment evaluation physical examination.

Evaluation during study:

- Physical examinations at least once per cycle (28 days). This includes skin examination every 2 cycles.
- Labs should be performed at least once per cycle including CBC with differential, BUN, creatinine, potassium, glucose, triglycerides, cholesterol, total bilirubin, and SGPT [ALT].
- Radiologic evaluations and pertinent tumor markers will be repeated after two cycles of treatment.
- ECGs monthly for three months then q3 months.

Evaluation of Toxicity:

- The MTD will be defined by DLTs that occur in the first cycle (induction phase). All enrolled participants will be considered in the DLT analysis. Neupogen/neulasta are allowed in this trial. Correctable electrolyte imbalances and alopecia are not considered DLTs.

- Three individuals will be dosed per level. If there are no side effects, the next cohort will be treated with a 100% increment. If there is Grade 1 toxicity, a 50% increment will occur. If there is Grade 2 toxicity, a 25% increment will occur. If there is Grade 3 or 4 toxicity due to study drug, there will be an expansion to six patients. If no other individuals have Grade 3 or 4 side effects, then next cohort will be treated with a 25% dose increase. If a second patient has Grade 3 toxicity, then the MTD is exceeded. The next lower dose level will be expanded to six patients. The MTD is the highest level with less than 1/6 individuals with Grade 3 or higher toxicity.
- Patients will continue on the study until their disease has progressed, they elect to come off the study, they experience toxicities that warrant coming off trial.
- No maximum number of cycles if benefiting clinically.
- If a response has been observed in a particular tumor type with the study drug or drug combination, then the study may be expanded to include a total of 14 participants with that tumor type. All enrolled participants will be considered in the DLT analysis.
- Up to 3 additional patients may be added to a cohort for evaluation of correlative studies.

Response Criteria:

While primary objectives of this study include the evaluation of dose-ranging

experience and the toxicity observed, an attempt to evaluate efficacy will require the following criteria for response. Patients with lymphoma will be measured per the CHESON criteria and all others will be evaluated with the RECIST criteria. For details of the CHESON criteria and RECIST criteria, please see Appendix A.

Criteria for Removal from the Study:

- If a Progression of disease per WHO or RECIST criteria as described previously. (Exception: If the patient is deriving clinical benefit).
- The development of unacceptable toxicity.
- Physician recommendation for patient removal.
- Patient elects to discontinue further treatment on the study medications.

Reporting Requirements:

Evaluation of Toxicity/Adverse Events: Evaluation of toxicity during the conduct of the study will be done following the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE). Known grade I and II toxicities and all clinically insignificant toxicities will not be tabulated for FDA approved drugs. The study uses FDA approved agents with known toxicity profiles. Therefore, Grade 1 and 2 toxicities (related or unrelated) will not be collected or documented as these are not considered clinically significant in this patient population and/or they are expected for these study agents. Grade 3 and 4

toxicities that are felt to be treatment related and unexpected (per package insert) will be documented. Unless otherwise documented in the electronic medical record as clinically significant and study drug related, all lab abnormalities will be assumed to be related to the patient's other co-morbid conditions, prior therapies, other concomitant therapies/medications, or underlying cancer. Serious Adverse Events will be reported per standard IRB reporting requirements. Serious Unexpected problems will be reported per standard IRB reporting requirements. Assessment of Intensity: Maximum intensity should be assigned to an adverse experience. Intensity will be assigned a Grade of 1-5. Final arbitration of intensity in cases of differing assessments by different practitioners will be the attending physician. Day to day fluctuations of intensity may not be recorded but rather the worst grade over the longest time period.

Statistical Considerations:

- This is a descriptive study with no formal statistical hypothesis to test.
- This protocol will utilize a standard 3 + 3 design.
- If a response has been observed in a particular tumor type with the study drug or drug combination, then the study may be expanded to include a total of 14 participants with that tumor type. All enrolled participants will be considered in the DLT analysis. If at any time more than or equal to one-third of the participants at a level develop DLT, that dose is above MTD.

- Patients can be added at the highest dose level seemed safe to date. All patients are considered in the DLT analysis. Up to 3 additional patients may be added to a cohort for evaluation of correlative studies. These patients will be considered in the DLT analysis.
- There will be no intra-patient dose escalation.
- Expected sample size. Approximately up to 35-45 patients will be treated in this study including patients treated in dose expansion cohort at MTD. The estimated accrual rate is 1-3 patients per month.

Dose Delays and Modifications:

- If Grade 3 toxicity occurs (DLT), dose reduction by 50% is allowed after patient recovers. The drug that will be reduced is the one that the physician feels has most likely caused the toxicity. If the drug that caused the toxicity is not known, the patient will be dose reduced to the previous dose level.
- If Grade 3 o/4 toxicity that is known to be related to one drug in the regimen, then that drug may be de-escalated to the prior dose level after the patient recovers to </= Grade 1 toxicity.
- If Grade 3/4 toxicity for which it is unclear which drug is the cause of the toxicity, then the drug which was dose escalated at the current dose level may be de-escalated to the prior dose level after the patient recovers to </= Grade 1 toxicity.
- If Grade 3/4 toxicity at level one, and if the toxicity is known to be related

to one drug in the regimen, then a dose reduction of 50% of that drug is permitted after the patient recovers to </= Grade 1 toxicity.

- If Grade 3/4 toxicity at level one, and if it is unclear which drug is the cause of the toxicity, then a dose reduction of 50% of all drugs in the regimen is permitted after the patient recovers to </= Grade 1 toxicity.

Correlative Studies (optional):

This phase I study will utilize novel technologies to analyze key downstream pathways efficiently so as to provide high impact data that may further expand our understanding of the biology of BRAF activation. Two major pathways appear to be involved in downstream signaling. The PI3K [128] and the MAPK axis are involved in proliferation and cell cycle progression. Aberrations in the PI3K/AKT/PTEN/RAF/RAS/GNAQ and related pathways may be assessed in tumor tissue or circulating blood.

Calendar:

Baseline assessment led within 14 days before C1D1. Baseline screening imaging can take place up to 4 weeks before the protocol starts. If screening procedures were performed within 7 days prior to Cycle 1 Day 1 it may be counted as baseline visit and Cycle 1 Day 1 labs and exams may not have to be repeated. Routine lab studies, physical exams, vital signs, weight, performance

status and scans will have a flexibility window of +/- 7 days.

	Screening	Cycle 1				Cycle 2 and beyond				Off Study[b]
		Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4	
Informed consent	X									
Medical history	X									
Physical Exam	X				X				X	X
Height	X									
Weight	X				X				X	X
Vital signs	X				X				X	X
Performance status	X				X				X	X
CBC w/diff, plts	X				X				X	X
Serum chemistry	X				X				X	X
PT/INR	X									
Urinalysis	X									X
EKG	X				X				X	
lipid	X				X				X	
Tumor assessment	X									
Adverse event evaluation	X	continuous								
Pregnancy test	X									
PD sampling	X									

Table 3. Study calendar.

- Physical examinations at least once per cycle. ECG at least once per cycle per vemurafenib's package insert.
- Labs should be performed at least once per cycle including CBC with differential, BUN, creatinine, potassium, glucose, triglycerides, cholesterol, total bilirubin, and SGPT[ALT].
- Radiologic evaluations and pertinent tumor markers will be repeated after two cycles of treatment.
- Testing and drug administration will take place as per protocol schedule unless patient/logistical/medical reasons intervene.
- Note: Evaluation that can occur once per cycle can be done at any point in

the cycle.

PRELIMINARY RESULTS:

This study has so far enrolled 10 patients as of April 2014 (Table 4). The most common diagnosis was melanoma in 5 out of 10 patients (50%). Male patients in 7 out of 10 patients (70%). The average age was 63.5 years. The average number of cycles on study was 3.2 and the average duration on study was 102.8 days. All patients had *BRAF* mutations (Table 5), particularly *BRAF* V600E mutation in 8 out of 10 patients (80%). Two out of the 10 patients (20%) had partial responses and additional 2 out of the 10 individuals (20%) displayed stability on imaging studies. Five patients out of 10 (50%) had received vemurafenib previously (Table 6). The two patients that displayed partial responses did not have melanoma (they had papillary thyroid cancer and NSCLC, respectively) with none of them had not received vemurafenib previously. The 77-year-old male with papillary thyroid carcinoma was chemotherapy and *BRAF* inhibitor naïve prior to receiving vemurafenib plus everolimus displaying a 36% response as per RECIST 1.1 (Figure 6). The 55-year-old female with NSCLC status post multiple lines of chemotherapeutic agents and also the *BRAF* inhibitor dabrafenib prior to starting vemurafenib plus everolimus displaying a 39% response as per RECIST 1.1 (Figure 7).

Case	Diagnosis	Age	Gender	Dose (mg)	Cycles	Duration (days)	Response
1	Melanoma	65	M	720/5	8.00	224.00	SD
2	Melanoma	68	M	720/5	3.00	96.00	PD
3	Melanoma	45	F	720/5	2.00	57.00	PD
4	Colorectal	63	M	720/5	3.00	99.00	PD
5	Melanoma	77	F	720/5	1.00	45.00	PD
6	Appendix	78	M	720/5	5.00	154.00	SD
7	NSCLC	55	F	720/10	4.00	132.00	-39%
8	Melanoma	66	M	720/10	1.00	30.00	PD
9	Esophagus	41	M	720/10	1.00	49.00	PD
10	Thyroid	77	M	720/10	4.00	142.00	-36%

Table 4. Characteristics of patients enrolled. M: Male. F: female. SD: Stable disease. PD: Progressive disease. Cycles: Number of cycles. Response: Best response. Appendix: Appendicular carcinoma. Thyroid: Papillary thyroid carcinoma. NSLCL: non-small cell lung cancer.

Case	Response	Mutation analysis
1	SD	**BRAF V600E**
2	PD	**BRAF V600K**, MET T1010I
3	PD	**BRAF V600E**
4	PD	**BRAF V600E**, SMAD4 P356R, TP53 R213*, KIT M541L
5	PD	**BRAF V600E**, CDKN2A R58*, PIK3CA E545K, MET N375S
6	SD	**BRAF V600E**, TP53 R110L
7	-39%	**BRAF V600E**, IDH1 R132C, PPP2R1A R183
8	PD	**BRAF V600E**, KIT M541L
9	PD	**BRAF Q609***, KRAS A146P, FBXW7 S478F, KIT G498S, KIT M541L, STK11 D23E
10	-36%	**BRAF V600E**, PIK3CA H1047R, RET Q626K

Table 5. Relationship between responders and mutational status. Response: Best response. SD: Stable disease. PD: Progressive disease.

Case	Response	Previous therapies
1	SD	Cisplatin, vinblastine, and dacarbazine; **vemurafenib**
2	PD	Dacarbazine, vinblastine, cisplatin, IL-2
3	PD	Cisplatin, bendamustine, dacarbazine; ipilimumab; IL2; carboplatin, paclitaxel; **vemurafenib**
4	PD	FOLFOX, bevacizumab; FOLFIRI, cetuximab; mitomycin-C; FOLFIRI, cetuximab; **vemurafenib**
5	PD	**Vemurafenib**; trametinib and **dabrafenib**
6	SD	FOLFOX; FOLFIRI
7	-39%	Carboplatin, paclitaxel; erlotinib; pemetrexed, rituximab; vinorelbine; gemcitabine; **dabrafenib**; MDX 1105 (anti-PD-L)
8	PD	Temozolomide, ipilimumab; GSK1120212 (MEK inhibitor); **vemurafenib**; **vemurafenib** and sorafenib; carboplatin, paclitaxel
9	PD	Docetaxel, fluoracil, oxaliplatin; irinotecan, cisplatin
10	-36%	Radiation

Table 6. Relationship between responders and previous therapies. Response: Best response. SD: Stable disease. PD: Progressive disease. IL-2: Interleukin 2.

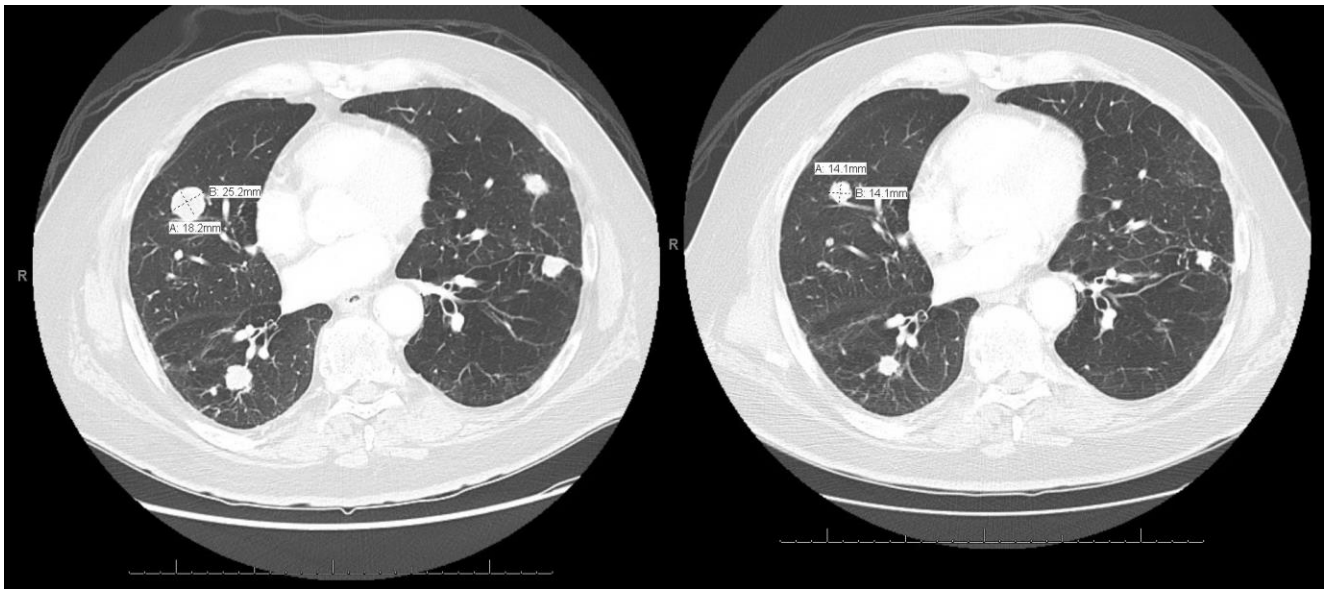

Figure 6. The 77-year-old male with papillary thyroid carcinoma was chemotherapy and *BRAF* inhibitor naïve prior to receiving vemurafenib plus everolimus displaying a 36% response as per RECIST 1.1.

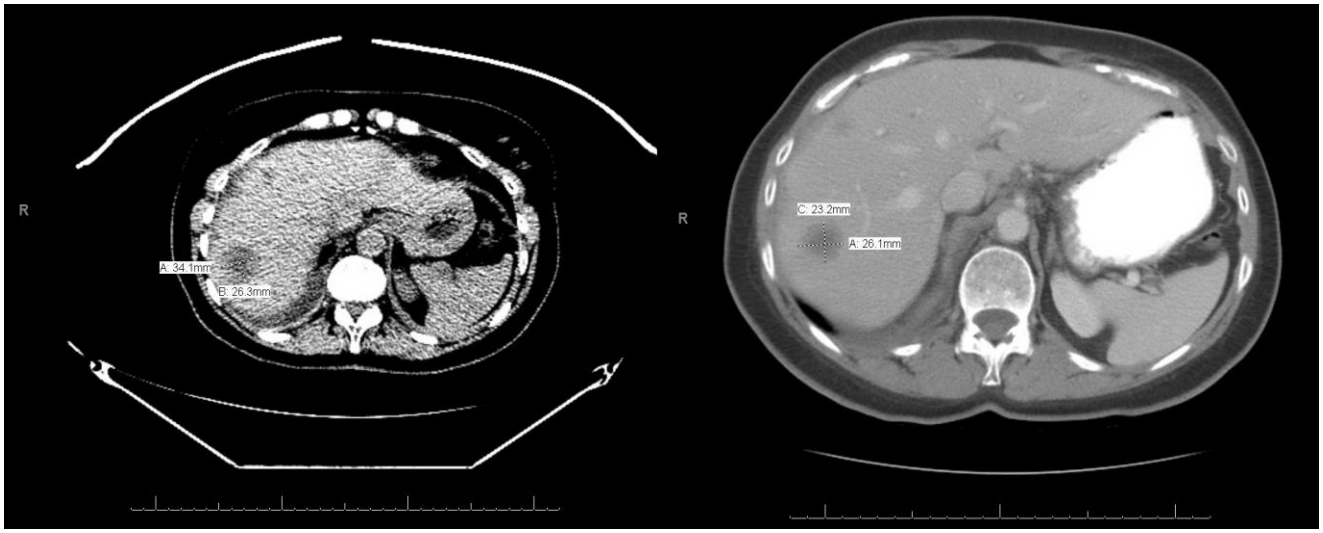

Figure 7. The 55-year-old female with NSCLC status post multiple lines of chemotherapeutic agents and also the *BRAF* inhibitor dabrafenib prior to starting vemurafenib plus everolimus displaying a 39% response as per RECIST 1.1.

DISCUSSION:

Searching under the street light:

We have been looking for diagnostic and prognostic cues “where the light is” for the longest time in many fields in medicine including oncology. Old age and elevated lactate dehydrogenase helped us to understand some conditions although most patients were still treated blindly with non-targeted cytotoxic chemotherapy in the absence of better stratification models. Cytogenetics, able to assess large chromosomal structure deviations, heralded a step forward. At the end of the day, a very small group of patients respond dramatically to non-targeted cytotoxic chemotherapy in most malignancies when we are shooting in the dark; whereas a relatively larger group of patients respond to matched targeted treatments while avoiding therapeutic misses or near-misses under the bright shining light of molecular profiling. Time and scientific progress brought upon us multiple novel molecular profiling platforms that have facilitated the process of finding the right treatments for the right subgroups of patients. As a disclaimer, some profound responses seen in the targeted era are short-lived and perhaps combinatorial trials will overcome therapeutic resistance in the years to come.

Cancer is not only one disease. For example, ALK altered neoplasia ("ALK-oma") is approximately four percent of lung carcinoma. Matching drugs to

targets showed impressive results, albeit brief, hence it has been suggested that combination therapy will be needed to overcome resistance to single agents [129].

Targeting the MAPK & mTOR: MAPK is a group of serine/threonine kinases that form a cascade of molecular signals that eventually lead to proliferation, survival, differentiation and cell fate determination. The MAPK network is organized hierarchically starting at the level of the cell membrane receptors with external stimuli (as hormones, cytokines and growth factors) successively communicating a message of proliferation all the way to the nucleus as MAPK's, MAPKK's, and MAPKKK's. The main identified MAPK network is the pathway conformed by RAS-RAF-MEK-ERK, and, if up-regulated, it leads to carcinogenesis. Inherited deregulated MAPK pathway, usually due to heterozygous mutations, cause several phenotypic conditions [130] with cognitive defects, facial dysmorphism, cardiac defects, and increased risk of malignancies; coined as neuro-cardio-facial-cutaneous syndrome family. Other players in this intricate network include BRAF with a designation which stems from its original identification of RAF during retroviral oncogenes. Initially RAF-1 was discovered (now called CRAF) in 1985, then ARAF in 1986, and subsequently BRAF in 1988. Hierarchically, the top of the cascade is aligned by HRAS, KRAS, and NRAS. The next layer is formed by the MAPKKK including ARAF, BRAF, and CRAF that may homodimerize or heterodimerize. MEK1 and MEK2 line up as MAPKK to culminate the network into ERK1 and ERK2, the

MAPK. Despite the fact that we illustrate the MAPK network as a linear path, it actually branches out and interacts with molecular members of other pathways including mTOR.

Novel molecular testing - Coming of age:

Morphologically, many tumors look alike (i.e. some aggressive lymphomas, Ewing sarcomas or desmoplastic small round cell tumors) and, let alone, light microscopy would have major difficulties settling this diagnostic matter. Diagnostic tools to detect molecular aberrations may detect specific mutations already known to be common in a particular malignancy or find previously unidentified mutations. Molecular diagnosis include new-comers as transcriptome sequencing and proteomics that may also permit the identification of novel biomarkers for targeted treatments.

Prognostication - Is LDH still valuable in the molecular era?

Definitively yes (at least at present). Molecular tools are not meant to replace clinical and laboratorial variables but rather supplement and enrich the information available at reach in each particular case. The ubiquitous elevated lactate dehydrogenase (LDH) is a poor's men prognosticator in both benign (i.e. pancreatitis) and malignant (i.e. lymphoma) conditions. The value of age or LDH assessing risk and prognosis has been well established over the years in

different prognostic scales (international prognostic index, follicular lymphoma international prognostic index) [131]. Immunohistochemistry alone will misclassify an abundant group of patients; immunohistochemistry plus molecular profiling and clinical variables as age or race will be a robust way of understanding a patient's tumor. Mechanistically, whether or not these laboratorial variables (LDH, beta-2 microglobulin, albumin, etc.) are superficial surrogates of a much deeper biology of the tumor and/or the host remains to be seen. That said, a feeling of unfairness settles in when comparing the humble LDH to the power unlocked by the prowess in generating data from the human genome project as it is identifying candidate genes for genetic cancer predisposition, loss-of-function for tumor suppressing genes versus gain-of-function oncogenes as possible targets. Furthermore, this loss-of-function for tumor suppressing genes may occur secondary to hypermethylating the promoter for that particular gene, abrogation of one genetic copy (allele) or loss of heterozygosity, and abrogation of both gene copies (parental alleles) or homozygous deletion. Molecular studies are meant to compliment, not replace, the breadth of knowledge regarding prognostic and diagnostic variables that we already have in hand.

The Vogelstein model - Sequential versus catastrophic aberrations:

The Vogelstein model speaks about a step-by-step malignant evolution [132]. The Knudson's "two-hit" theory speaks about an initial mutation followed by a second mutation can develop neoplasia [133]. A similar pattern of a

subsequent molecular cascade promoting proliferation has been suggested to occur with retinoblastoma, helicobacter pylori, and other infectious agents as Epstein Barr virus and human papilloma virus; although it has been difficult to confirm. Multiple genetic and epigenetic aberrations following the Vogelstein model in colorectal cancer have been tried to apply to other malignancies; nevertheless these mutations do not necessarily follow a sequential pattern of acquisition and accumulation of molecular abnormalities. The adenoma/carcinoma stepwise approach (initiation, promotion, progression) of the Vogelstein theory has been challenged as some aberrations are present at early stages and no longer found in advanced disease, and the dynamic malignant process of de-differentiation/re-differentiation does not seem to be a linear process of rather irreversible molecular aberrations. Such bidirectional differentiation dynamism is exemplified by beta-catenin as part of the Wnt signaling pathway, which under normal conditions regulates embryonic morphogenesis depending on a temporal coordination of events; but also when hyperactive, perhaps due to molecular perturbations produced by Helycobacter pylori, can stimulate proliferation and tumor invasion.

Solid tumors - Much to learn from their hematologic counterparts:

Neoplastic cells convey advantages over the soon-to-be outnumbered normal cells resulting in a clonal evolution and expansion of the fittest. *BRAF* mutations are common in some solid malignancies but rare in liquid neoplasms

with the exception of Hairy cell leukemia. Trisomy 8 is common in some liquid malignancies but rare in solid neoplasms with the exception of desmoid tumors.

Passed discrepancies, the anti-apoptotic family of the B cell leukemia/lymphoma 2 or Bcl-2, with their members, Bcl-xL and Bcl-2, has been identified in both liquid and solid tumors as head and neck malignancies. Beyond AML, multiple molecular markers have appeared in recent times in other hematological malignancies as myelodysplastic syndromes (TET2, DNMT3A, ASXL1, EXH2, U2AF1, etc.), chronic lymphocytic leukemia (NOTCH1, XPO1, MYD88, KLH6, etc.), acute lymphoblastic leukemia (ETV6, RUNX1, rearrangements of the cytokine receptor gene CRLF2, alterations of the lymphoid transcription factor gene IKZF1 or IKAROS, etc.), and multiple myeloma (FGFR3, MMSET, MAFB, etc.). Hematological malignancies are ahead of their solid counterparts in part due to the ease of genetic evaluation (e.g. peripheral blood assessment). It remains to be seen whether most of these aberrations are “driver” mutations creating oncogene-addiction, or mere “passenger” mutations admiring the landscape while our patients cruise through the path of molecular evolution. Complex as it seems, these multiple aberrations will soon stratify patients in different small subsets of mutations and once found to be druggable targets, extrapolations may be made across malignancies in clinical trials (i.e. BRAF inhibition with vemurafenib in BRAF-mutated Hairy cell leukemia).

From myeloma to melanoma and back - More than phonetics:

Most melanomas carry aberrations in MAPK which is a cascade of activating phosphorylation including KRAS, BRAF (and/or a parallel path through CRAF that requires additional steps for activation), MEK, and finally ERK. The microphthalmia-associated transcription factor (MITF), critical in the process of melanin production, has been found to be mutated in some instances as well. Clinically, melanoma has been divided into cutaneous, uveal and acral/mucosal. Molecularly, these clinical subgroups have their own characteristic aberrations as BRAF/NRAS mutations, GNAQ/GNA11/BAP1 or BRCA1-associated protein 1, and KIT, respectively. Up close and personalized, melanoma could be tackled by targeted inhibition of BRAF, MEK, NRAS, and KIT. MEK inhibitors as trametinib and MEK162 have shown responses in patients with melanomas harboring BRAFV600 & NRAS mutations. BRAF inhibitors as vemurafenib and dabrafenib (GSK2118436) displayed dramatic improvements in BRAF-mutated melanoma. Imatinib may have activity in KIT-mutant melanoma although initial trials have been disappointing. Interestingly, BRAF inhibition may cause worsening of pre-existent RAS mutated conditions and newly diagnosed squamous cell carcinoma, keratoacanthoma, second primary melanomas and worsening of RAS-mutant chronic myelomonocytic leukemia have been described. Not surprisingly, the pairing of BRAF plus MEK inhibitors in clinical trials appears to produce less dermatologic toxicity perhaps due to further downstream blockade of possible escape pathways. As angiogenesis seems to be hyperactive in melanoma,

bevacizumab has been evaluated with carboplatin plus paclitaxel. Despite the fact that bevacizumab targets the vascular endothelial growth factor or VEGF some may argue that we do not really have a molecular target at this point that would help us to select patients. Desperate to find a subgroup that will be setup for therapeutic success, investigators looked back at the subset of patients with elevated LDH only to find overall survival benefit after bevacizumab challenge which proves our point that even the most extensive molecular evaluations might not completely replace basic laboratorial data. Multiple myeloma has many things to learn from melanoma as the search for oncogenic drivers, targeted therapy and molecular stratification of patients. Melanoma has many things to learn from multiple myeloma as the investigator's drive to design combinatorial trials to overcome resistance as quadruplets (i.e. VCRD or bortezomib, cyclophosphamide, lenalidomide, dexamethasone) and then some (i.e. VDT-PACE or bortezomib-dexamethasone-thalidomide, and cisplatin-doxorubicin-cyclophosphamide-etoposide). Myeloma trials do not only vehemently group drugs together, but they also bring up new agents aggressively to the frontline in newly diagnosed patients, instead of waiting for heavily pretreated individuals that are less likely to respond, which has allowed them to obtain responses near 100% and word in the street is that a cure is near for this plasma cell dyscrasia. Interestingly, a very small subset of patients (4%) with multiple myeloma will carry BRAF mutations [126]. The value of targeting BRAF-mutant myeloma patients with BRAF inhibitors is currently unknown. NRAS (24%) and KRAS (27%) mutations have been found in myeloma as well. If we erase the arbitrary

limits between malignant hematology and oncology, myeloma and melanoma are not so far apart beyond linguistic connotations; and a successful therapeutic formula may be extrapolated from one disease to another irrespective of its chapter in medicine textbooks.

Lung cancer - Divide and conquer:

Multiple drugs have increased the armamentarium against NSCLC including erlotinib for EGFR and crizotinib for anaplastic lymphoma kinase (ALK) and ROS1 translocation. KRAS mutations are being challenged by selumetinib which is an oral MEK inhibitor downstream from KRAS (non-impressive results from farnesyl protein transferase inhibitors in NSCLC population so far), PIK3CA mutations with ridaforolimus which is an mTOR inhibitor, BRAF mutations with vemurafenib which is a BRAF inhibitor, RET translocations with vandetanib (multi-tyrosine kinase drug), DDR2 mutation with dasatinib, HER2 expression with afatinib which is an EGFR/HER2 tyrosine kinase inhibitor (non-impressive results of trastuzumab in the HER2 amplified NSCLC population so far), and MET expression with tivantinib which is a MET tyrosine kinase inhibitor. Interestingly, crizotinib was originally developed as a MET inhibitor and it seems to be active in patients with NSCLC that express MET. Needless to say, crizotinib as an ALK inhibitor is also under evaluation in hematologic malignancies as anaplastic lymphoma which gave birth to the name of such tyrosine kinase (ALK or anaplastic lymphoma kinase).

Prognostic versus predictive - The nail and the hammer:

Just as when you choose your specialist in medicine, you choose your disease (i.e. an orthopedic surgeon recommending an arthroscopic procedure for osteoarthritis). When you have a hammer, everything looks like a nail; thus it is not surprising that oncologists have been treating patients blindly with cytotoxic chemotherapy for the longest time. Following the above example, immunohistochemistry revealing HER-2 overexpressed breast cancer, reminds us of the abundant benefits brought upon patients after finding a nail (i.e. the abnormal HER-2 pathway) and hammering it at frontline, relapse and even during continuation despite progression (trastuzumab, lapatinib, T-DM1). Interestingly, a tumor being identified as HER2-enriched subtype (high HER2 expression and low basal/luminal expression) does not automatically mean that it is a clinically HER2 overexpressed breast cancer. On the other hand, some HER2-enriched subtype tumors are clinically HER2-negative breast cancers and the possible use of HER2 as a therapeutic target in such population is under evaluation. At the end of the day, it makes sense that the evaluation of multiple genes involved in the production of a protein (i.e. HER2 hormonal receptor) might be a more thorough assessment than the evaluation of an abnormality by a single immunochemistry stain (HER2 positivity 2+). HER2-overexpression has been a well-known factor heralding poor prognosis (i.e. increased brain metastasis, shorter survival, etc.), although it is until the development of HER2-

targeted therapy that a negative factor became a positive predictive one. Similarly, AML has new poor prognostic aberrations (i.e. FLT3) and perhaps it will be under the development of novel drugs (i.e. novel FLT3 inhibitors as quizartinib) that a negative factor will become a positive one. From a therapeutic point of view, discovering targeted therapy directed against the ubiquitous p53 and KRAS mutations may become the “holy grail” for multiple tumors, including gastrointestinal malignancies, nevertheless currently they remain in the dark. With the arrival of more refined molecular diagnostic tests, a multitude of nails have been brought to our working table; nevertheless at the end of the day having endless nails are worthless gadgets without having a hammer to use them. It will take time and heavy investment on research to pair druggable aberrations to matched targeted therapies.

CML - A full circle:

CML is used across the board as the paradigm of personalized medicine and physician-scientists are in a quest to find the next “imatinib-like” agent; nevertheless its impressive results, imatinib is not magic and a percentage of patients are resistant and ultimately progress. Molecular evaluation was critical to understand why this occurred (i.e. T315I mutation) and the development of second and third generation tyrosine kinase inhibitor to overcome such resistance. Interestingly, the advent of the powerful pan-ABL1 kinase inhibitor ponatinib [134] with response rates near 100% in patients with or without the

current known resistant mutations makes unnecessary testing for these aberrations.

A word of caution:

Targeted single-agent therapy is no panacea. Ovarian high grade serous carcinomas also express high levels of estrogen receptors albeit response rates to hormonal manipulation have been low (approximately 10%) [135]. Targeting c-kit in GIST only showed a 38% response [136], and acute myeloid leukemia, no clinical responses as single agent maneuver [137], do not yield the same responses hence generalizations should be made cautiously. Furthermore, sporadic medullary thyroid cancer may show responses to RET inhibitors even without RET mutations. Technical limitations may include the fact that gene profiles obtained from formalin-fixed samples might not be the same as assessing banked frozen tissue. To further complicate the situation, different particular platforms have different specific requirements (frozen unfixed sample placed, within a brief lapse of time following surgery, in a particular container to be sent to a particular molecular profiling company). Profiling different areas of a same tumor, or different metastatic sites stemming from a single tumor, might provide discordant results due to tumor heterogeneity. Timing (early versus late) and location (local versus distant) might also factor in as these variables might be a surrogate of the existence of a different disease that evolves over time; thus it is critical to apply molecular profiling data to the exact setting that was initially

studied. Beyond offering potential targets for treatment selection that can augment the chances of observing responses; high-risk versus low-risk discordance between molecular and clinical predictors will be expected in a subgroup of patients and treatment decisions might be difficult until large prospective matters settle this matter.

Personalized cancer drugs with their potent, albeit transient, responses may never “cure” patients because cancer genomes are not the only culprit for malignant development or poor prognosis. The molecular makeup of the host and the tumor microenvironment probably play a role as well. A limited tumor-only molecular evaluation of genes could potentially misclassify a patient as low risk of mortality (i.e. chronic lymphocytic leukemia), when such patient actually carried high risk variables for heart disease or thromboembolic events, which sadly would ultimately cause the patient’s demise in a swift fashion. The new molecular technologies will be able to locate minuscule numbers of abnormal genes despite being admixed among countless normal genes which will be used to our advantage when it comes to screening; although a diagnostic conundrum may arise after discovering a new mutation associated with particular genotype-phenotype malignant presentations in an asymptomatic patient (prophylactic surgical removal versus watch-and-worry with aggressive interval screening).

Future directions - On the non-labeling of patients and combinatorial clinical trials:

Light microscopy can only illuminate so far when it comes to poorly differentiated malignancies. In the case of carcinomas of unknown primary, an aberration-specific approach may guide our therapeutic decisions in lack of an organ-specific approach. Certain gene expression profiles obtained from tumors may guide towards the normal tissue of origin in a site-specific approach. As such, a treatment paradigm has risen that knows no boundaries when it comes to differentiating malignant hematology versus oncology (i.e. vemurafenib use in BRAF-mutant Hairy cell leukemia) or site of origin (i.e. HER2 overexpressed gastric cancer as “druggable” aberrations previously believed to be exclusive of breast cancer). In the past, familial neoplasms shed light upon the presence of inherited genetic germline mutations as trials studied the affected kindred. In the future, each individual patient may be a trial of its own as molecular profiling might reveal acquired somatic genetic mutations amenable to targeted therapy. As a cautionary note, finding a target in a pathway is not a guarantee of response. Heavily pretreated PIK3CA-mutated colon neoplasia that received trials incorporating PI3K/AKT/mTOR inhibitors showed minimal activity [138].

Finding an aberrant pathway (i.e. PDGFRA) may unify both common and rare tumors and standardize a therapy (i.e. imatinib mesylate for KIT-negative GIST, Erdheim-Chester disease, desmoid tumors, and dermatofibrosarcoma

protuberans). It has been a decade since the Human Genome Project; nevertheless translating such massive collection of information to the clinic has remained an elusive goal due to technical and economic issues. In this world of instant gratification, molecular profiling and genome sequencing during routine checkups will soon no longer be utopia (Illumina Inc., Life Technologies, Oxford Nanopore Technologies, Noblegen Biosciences, etc.). As seen through deep gene-sequencing, cancer is not only one disease; hence the true challenge will be to match a tumor to a drug. Beyond wide genome profiling, high-throughput technology will also evaluate proteins (proteomics), or cellular metabolism (metabolomics) that can be exploited towards prognostic stratification and therapeutic decision-making. At the end of the day, you do not want to treat your patients with cancer based on the molecular analysis performed at diagnosis five years ago; you want real-time high-throughput molecular data now.

Phase I clinical trial design looking for the maximum tolerated dose comes from the original chemotherapy trials; perhaps trials looking for the minimum effective dose are more applicable to the era of targeted agents. With limited resources, the mathematical number of possible combinations of targeted agents is not feasible to pursue unless we have a strong pre-clinical rationale in drug development.

Conclusion:

Protocol 2012-0153 is currently IRB approved and already enrolling patients to determine MTD plus preliminary antitumor activity (tumor response) of the combination of vemurafenib and everolimus. Suggested completion timeframe for protocol 2012-0153 is within three years of accrual start date. Preliminary data from initial ten patients enrolled show encouraging results.

APPENDIX: DRUG INFORMATION

Everolimus:

Everolimus (Afinitor®), side effects: Angioedema, marrow suppression, generalized/localized edema, azoospermia/oligospermia, infection, malignancy, mucositis/stomatitis, nephrotoxicity, noninfectious pneumonitis (which might require dosage modification or corticosteroid therapy) and wound healing complication. Side effects occurring in more than 10% of cases as follows: Cardiovascular: Peripheral edema (4% to 45%), hypertension (4% to 30%). Central nervous system: Fatigue (7% to 45%), fever (19% to 32%), headache (18% to 30%), seizure (SEGA: 29%), personality change (SEGA: 18%), insomnia (9% to 17%), dizziness (7% to 14%). Dermatologic: Rash (18% to 59%), acneiform dermatitis (SEGA: 25%; RCC: 3%), cellulitis (SEGA: 21%), nail disorders (5% to 22%), pruritus (14% to 21%), dry skin (13% to 18%), contact dermatitis (14%), excoriation (14%), acne (11%). Endocrine & metabolic: Hypercholesterolemia (17% to 77%), hyperglycemia (12% to 75%; Grades 3/4: <1% to 17%), hypertriglyceridemia (≤73%), bicarbonate decreased (≤56%), hypophosphatemia (13% to 40%), hypocalcemia (17% to 37%), hypoglycemia (≤32%), hypokalemia (12% to 23%), hyperlipidemia (renal transplant: 21%), hyperkalemia (renal transplant: 18%), dyslipidemia (renal transplant: 15%), hypomagnesemia (renal transplant: 14%), hyponatremia (≤16%), albumin decreased (≤13%).

Gastrointestinal: Stomatitis (oncology uses: 44% to 86%; Grade 3: 4% to 7%; Grade 4: <1%; renal transplant: 8%), diarrhea (19% to 50%; Grade 3: ≤5%; Grade 4: <1%), constipation (11% to 38%), abdominal pain (3% to 36%), nausea (26% to 32%: Grade 3: 1% to 2%), anorexia (1% to 30%), vomiting (15% to 29%; Grade 3: 1% to 2%), weight loss (9% to 28%), taste alteration (10% to 19%), gastroenteritis (1% to 18%), xerostomia (8% to 11%). Genitourinary: Urinary tract infection (renal transplant: 16% to 22%; RCC 5%), dysuria (renal transplant: 11%). Hematologic: Anemia (26% to 92%; Grades 3/4; 13% to 15%), leukopenia (oncology uses: 26% to 54%; renal transplant 3%), and others. Neuromuscular & skeletal: Weakness (19% to 33%), arthralgia (≤15%), back pain (11% to 15%), limb pain (10% to 14%). Otic: Otitis (SEGA: 14% to 36%). Renal: Creatinine increased (11% to 50%), hematuria (renal transplant: 12%). Respiratory: Upper respiratory infection (16% to 82%), sinusitis (3% to 39%), cough (7% to 30%), dyspnea (20% to 24%; Grade 3: 2% to 6%; Grade 4: ≤1%), epistaxis (≤22%), pneumonitis (14% to 17%; Grade 3: 3% to 4%), nasal congestion (14%), rhinitis (14%), pharyngitis (4% to 11%). Miscellaneous: Infection (RCC: All infections: 37%; Grade 3: 7%; Grade 4: 3%; renal transplant: 62%).

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