SUMMARY OF CHANGES - PROTOCOL

For Protocol Amendment # to: Phase I Study of MLN0128 (TAK-228) (NSC# 768435) in Combination with Ziv-Aflibercept (NSC# 724770) in Patients with Advanced Cancers

NCI Protocol #: 9585
Local Protocol #: 2013-0665

NCI Version Date: October 8, 2019
Protocol Date: October 8, 2019

I. Protocol Changes by Rapid Amendment (Amendment 16/ Version 10; 07Oct2019)

#	Section	Page	Comments
1.	Throughout and Title Page	All	PI RESPONSE: The protocol version and version date was updated throughout the protocol on the title page and headers. Old Text: NCI Version Date: ~~June 11, 2019~~ Protocol Date: ~~June 11, 2019~~ Protocol Type / Version # / Version Date Original/ Local Version #00 / February 24, 2014 Amendment 01/ Local Version #01 / March 28, 2014 Amendment 02/ Local Version #02 / August 15, 2014 Amendment 03/ Local Version #03 / October 21, 2014 Amendment 04/ Local Version #03 / November 7, 2014 Amendment 05/ Local Version #03 / February 2, 2015 Amendment 06/ Local Version #03 / March 20, 2015 Amendment 07/ Local Version #04 / October 6, 2015 Amendment 08/ Local Version #05 / January 5, 2016 Amendment 09/ Local Version #06 / November 2, 2016 Amendment 10/ Local Version #07 / August 3, 2017 Amendment 11/ Local Version #07/ September 21, 2017 Amendment 12/ Local Version #07/ December 22, 2017 Amendment 13/ Local Version #07/ January 29, 2018 Amendment 14/ Local Version #08/ June 20, 2018 Amendment 15/ Local Version #09/ June 11, 2019 New Text: NCI Version Date**: October 8, 2019** Protocol Date: **October 8, 2019** Protocol Type / Version # / Version Date Original/ Local Version #00 / February 24, 2014

#	Section	Page	Comments
			Amendment 01/ Local Version #01 / March 28, 2014 Amendment 02/ Local Version #02 / August 15, 2014 Amendment 03/ Local Version #03 / October 21, 2014 Amendment 04/ Local Version #03 / November 7, 2014 Amendment 05/ Local Version #03 / February 2, 2015 Amendment 06/ Local Version #03 / March 20, 2015 Amendment 07/ Local Version #04 / October 6, 2015 Amendment 08/ Local Version #05 / January 5, 2016 Amendment 09/ Local Version #06 / November 2, 2016 Amendment 10/ Local Version #07 / August 3, 2017 Amendment 11/ Local Version #07/ September 21, 2017 Amendment 12/ Local Version #07/ December 22, 2017 Amendment 13/ Local Version #07/ January 29, 2018 Amendment 14/ Local Version #08/ June 20, 2018 Amendment 15/ Local Version #09/ June 11, 2019 **Amendment 16/ Local Version #10/ October 8, 2019**
2.	Collaborators	3	**PI RESPONSE: As part of a minor administrative change, the study personnel have been updated.** Old Text: Statistician: ~~Kenneth Hess, PhD~~ 1515 Holcombe Blvd., Unit ~~1411~~ Houston, Texas 77030 ~~713-794-4168~~ ~~khess@mdanderson.org~~ Study Coordinator: ~~Dipti Jain~~ 1515 Holcombe Blvd., Unit 455 Houston, Texas 77030 ~~713-792-1469~~ ~~713-606-3912~~ ~~djain@mdanderson.org~~ Responsible Research Nurse: ~~Valerie Marcott, RN~~ 1515 Holcombe Blvd., Unit 455 Houston, Texas 77030 ~~713-794-1014~~ ~~713-404-1550~~ ~~VDMarcot@mdanderson.org~~

#	Section	Page	Comments
			New Text: Statistician: **Suyu Liu, PhD** 1515 Holcombe Blvd., Unit **1409** Houston, Texas 77030 **713-563-4280** **syliu@mdanderson.org** Study Coordinator: **Fengying "Fiona" Ouyang** 1515 Holcombe Blvd., Unit 455 Houston, Texas 77030 **713-792-7192** **713-404-5990** **Fouyang@mdanderson.org** Responsible Research Nurse: **Jing Gong** 1515 Holcombe Blvd., Unit 455 Houston, Texas 77030 **713-563-8392** **713-606-3681** **jinggong@mdanderson.org**
3.	7.1 CAEPRs for CTEP IND Agent(s)	55	**PI RESPONSE: Per the Request a Rapid Amendment (RRA) for this NCI-CTEP trial, we have updated the protocol MLN0128 (TAK-228) risks based on the Comprehensive Adverse Events and Potential Risks (CAEPR) list (Version 2.3, July 28, 2019). This section now utilizes CTCAE 5.0 language, which is consistent with all other sections of the protocol.** • The SPEER grades have been updated. • The section below utilizes CTCAE 5.0 language unless otherwise noted. • Added New Risk: • Also Reported on MLN0128 Trials But With Insufficient Evidence for Attribution: Abdominal distension; Abdominal infection; Acidosis; Alkaline phosphatase increased; Alopecia; Ataxia; Blood and lymphatic system disorders - Other (hyperviscosity syndrome); Blood and lymphatic system disorders - Other (Raynaud's phenomenon); Blood

#	Section	Page	Comments

			lactate dehydrogenase increased; Bone pain; Chest wall pain; Colitis; Febrile neutropenia; Flu like symptoms; Fracture; Gastritis; Gastrointestinal disorders - Other (intestinal perforation); Gastrointestinal disorders - Other (salivary hypersecretion); General disorders and administration site conditions - Other (groin pain); GGT increased; Hemorrhoids; Hiccups; Hyperkalemia; Hypernatremia; Hypertension; Hyperuricemia; Ileus; Infections and infestations - Other (cystitis); Infections and infestations - Other (lower respiratory tract infection); Infections and infestations - Other (mucosal infection); Infections and infestations - Other (parotid gland); Injury, poisoning and procedural complications - Other (accidental overdose); Injury, poisoning and procedural complications - Other (postoperative fever); Injury, poisoning and procedural complications - Other (subdural hemorrhage); Lethargy; Lipase increased; Malaise; Metabolism and nutrition disorders - Other (severe chronic malnutrition); Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (non-hodgkin lymphoma); Nervous system disorders - Other (neuropathy peripheral); Papulopustular rash; Paresthesia; Pleuritic pain; Proteinuria; Radiculitis; Renal and urinary disorders - Other (strangury); Stroke; Vaginal hemorrhage; Ventricular arrhythmia
			• Increase in Risk Attribution:
			  • Changed to Likely from Less Likely: Constipation
			  • Changed to Less Likely from Also Reported on MLN0128 Trials But With Insufficient Evidence for Attribution: Acute kidney injury; Dyspepsia
			
			• Provided Further Clarification: (e.g., changed from 'arrhythmia' to 'ventricular fibrillation')
			  • Respiratory, thoracic and mediastinal disorders - Other (oropharyngeal pain) is now reported as Oropharyngeal pain.
			  • Eye disorders - Other (visual acuity reduced) is now reported as Vision decreased.
			  • Musculoskeletal and connective tissue disorder - Other (muscle spasms) is now reported as Muscle cramp.
			  • Personality change is now reported as Psychiatric disorders - Other (mental status changes).
			  • Urinary tract pain is now reported as Dysuria.
			
			PLEASE NOTE: The specific detailed changes listed here compare the new revised CAEPR Version 2.3, and associated

#	Section	Page	Comments

risk information for the ICD, to the most recent CAEPR Version 2.2. If your trial contains an older CAEPR version (i.e., does **NOT** currently contain CAEPR Version 2.2), you **MUST** include a description of any additional changes resulting from migration from the older CAEPR version.

New Text:

7.1.1.1 CAEPR for MLN0128 (TAK-228) (NSC# 768435)

Version 2.3, July 28, 2019[1]

Adverse Events with Possible Relationship to MLN0128 (TAK-228) (CTCAE 5.0 Term) [n= 390]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
CARDIAC DISORDERS			
		Cardiac arrest	
		Ventricular fibrillation	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
Constipation			***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 2)***
	Dry mouth		***Dry mouth (Gr 2)***
	Dyspepsia		
Mucositis oral			***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	General disorders and administration site conditions - Other (mucosal inflammation)		***General disorders and administration site conditions - Other (mucosal inflammation) (Gr 2)***
INFECTIONS AND INFESTATIONS			
	Urinary tract infection		***Urinary tract infection (Gr 2)***
INVESTIGATIONS			
	Creatinine increased		***Creatinine increased (Gr 2)***
		Electrocardiogram QT corrected interval prolonged	
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	Weight loss		***Weight loss (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 2)***
	Dehydration		***Dehydration (Gr 2)***

#	Section	Page	Comments

Hyperglycemia			***Hyperglycemia (Gr 3)***
	Hypokalemia		***Hypokalemia (Gr 2)***
	Hypomagnesemia		***Hypomagnesemia (Gr 2)***
	Hypophosphatemia		***Hypophosphatemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Back pain		***Back pain (Gr 2)***
	Pain in extremity		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Dysgeusia		***Dysgeusia (Gr 2)***
	Headache		***Headache (Gr 2)***
PSYCHIATRIC DISORDERS			
	Insomnia		
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 2)***
	Oropharyngeal pain		
		Pneumonitis	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Pruritus			***Pruritus (Gr 2)***
Rash maculo-papular			***Rash maculo-papular (Gr 2)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

Adverse events reported on MLN0128 (TAK-228) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that MLN0128 (TAK-228) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (hyperviscosity syndrome); Blood and lymphatic system disorders - Other (Raynaud's phenomenon); Febrile neutropenia
CARDIAC DISORDERS - Heart failure; Pericardial effusion; Sinus tachycardia; Ventricular arrhythmia
EYE DISORDERS - Blurred vision; Eye pain; Photophobia; Vision decreased
GASTROINTESTINAL DISORDERS - Abdominal distension; Colitis; Dysphagia; Esophagitis; Gastritis;

#	Section	Page	Comments
			Gastroesophageal reflux disease; Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (intestinal perforation); Gastrointestinal disorders - Other (salivary hypersecretion); Hemorrhoids; Ileus; Oral pain; Pancreatitis; Small intestinal obstruction; Small intestinal perforation; Toothache **GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (groin pain); Malaise; Non-cardiac chest pain; Pain** **HEPATOBILIARY DISORDERS - Gallbladder obstruction** **IMMUNE SYSTEM DISORDERS - Allergic reaction** **INFECTIONS AND INFESTATIONS - Abdominal infection; Infections and infestations - Other (cystitis); Infections and infestations - Other (lower respiratory tract infection); Infections and infestations - Other (mucosal infection); Infections and infestations - Other (parotid gland); Kidney infection; Lung infection; Papulopustular rash; Sepsis; Skin infection; Upper respiratory infection** **INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall; Fracture; Injury, poisoning and procedural complications - Other (accidental overdose); Injury, poisoning and procedural complications - Other (postoperative fever); Injury, poisoning and procedural complications - Other (subdural hemorrhage); Tracheal obstruction** **INVESTIGATIONS - Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Blood lactate dehydrogenase increased; Cholesterol high; GGT increased; Lipase increased; Lymphocyte count decreased; Neutrophil count decreased; White blood cell decreased** **METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperkalemia; Hypernatremia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia; Metabolism and nutrition disorders - Other (severe chronic malnutrition); Metabolism and nutrition disorders - Other (vitamin D deficiency)** **MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Chest wall pain; Flank pain; Generalized muscle weakness; Muscle cramp; Myalgia** **NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (non-hodgkin lymphoma); Treatment related**

#	Section	Page	Comments
			secondary malignancy **NERVOUS SYSTEM DISORDERS - Ataxia; Intracranial hemorrhage; Lethargy; Nervous system disorders - Other (carotid artery occlusion); Nervous system disorders - Other (neuropathy peripheral); Paresthesia; Radiculitis; Stroke; Tremor** **PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Psychiatric disorders - Other (mental status changes)** **RENAL AND URINARY DISORDERS - Dysuria; Hematuria; Proteinuria; Renal and urinary disorders - Other (strangury)** **REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Vaginal hemorrhage** **RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchopulmonary hemorrhage; Epistaxis; Hiccups; Hypoxia; Nasal congestion; Pleural effusion; Pleuritic pain; Pneumothorax; Postnasal drip; Productive cough** **SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Rash acneiform; Urticaria** **VASCULAR DISORDERS - Flushing; Hypertension; Hypotension; Thromboembolic event** **Note: MLN0128 (TAK-228) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.**
4.	13.1 Study Design/ Endpoints	89	PI RESPONSE: The statistical collaborator was updated. Old Text: Dr. ~~Kenneth Hess~~ (~~Professor of~~ Biostatistics at UT MD Anderson Cancer Center) will perform all statistical analyses. New Text: Dr. **Suyu Liu** (Biostatistics at UT MD Anderson Cancer Center) will perform all statistical analyses.

NCI Protocol #: 9585

Local Protocol #: 2013-0665

TITLE: PHASE I STUDY OF MLN0128 (TAK-228) (NSC# 768435) IN COMBINATION WITH ZIV-AFLIBERCEPT (NSC# 724770) IN PATIENTS WITH ADVANCED CANCERS

Coordinating Center: The University of Texas, MD Anderson Cancer Center

***Principal Investigator:** Aung Naing
1515 Holcombe Blvd, Unit 455
Houston, Texas 77030
Phone: 713-563-0803
Fax: 713-563-0566
Email: anaing@mdanderson.org

Co-Investigators:

Funda Meric-Bernstam, MD
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
Phone: 713-745-4453
Fax: 713-404-3176
Email: fmeric@mdanderson.org

Siqing Fu, MD, PHD
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
Phone: 713-792-7960
Email: siqingfu@mdanderson.org

David S. Hong, MD
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
Phone: 713-563-5844
Email: dshong@mdanderson.org

Filip Janku, MD
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
Phone: 713-563-2632
Email: FJanku@mdanderson.org

Daniel Karp, MD
1515 Holcombe Blvd., Unit 455

Houston, TX 77030
Phone: 713-745-7398
Email: dkarp@mdanderson.org

Sarina Piha-Paul, MD
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
Phone: 713-794-4225
Email: spihapau@mdanderson.org

Vivek Subbiah, MD
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
Phone: 713-563-0393
Email: vsubbiah@mdanderson.org

Apostolia Tsimberidou, MD, PHD
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
Phone: 713-792-4259
Email: atsimber@mdanderson.org

Shubham Pant, MD
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
Phone: 713-745-6601
Email: spant@mdanderson.org

Jordi Rodon Ahnert, MD, PHD
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
Phone: 713-792-5603
Email: jrodon@mdanderson.org

Timothy A. Yap, MD, PHD
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
Phone: 713-563-1784
Email: tyap@mdanderson.org

Collaborators

Shalini Dalal, MD*
Palliative Care
1515 Holcombe Blvd., Unit 1414
Houston, TX 77030
Phone: 713-792-6085
Email: sdalal@mdanderson.org

Jean-Bernard Durand, MD*
Cardiology
1515 Holcombe Blvd., Unit 1451
Houston, TX 77030
Phone: 713-792-6239
Email: jdurand@mdanderson.org

Mouhammed A. Habra, MD*
Endocrine Neoplasia and HD
1515 Holcombe Blvd., Unit 1461
Houston, TX 77030
Phone: 713-792-2841
Email: mahabra@mdanderson.org

Ravi Murthy, MD*
Interventional Radiology
1515 Holcombe Blvd., Unit 1471
Houston, TX 77030
Phone: 713-745-0856
Email: rmurthy@mdanderson.org

Chaan Ng, MD*
Diagnostic Radiology
1515 Holcombe Blvd., Unit 1473
Houston, TX 77030
Phone: 713-792-6759
Email: cng@mdanderson.org

Sinchita Roy Chowdhuri, MD, PHD*
Pathology
1515 Holcombe Blvd., Unit 0085
Houston, TX 77030
Phone: 713-563-9344
Email: sroy2@mdanderson.org

Ana M. Ciurea, MD*
Dermatology
1515 Holcombe Blvd., Unit 0434
Houston, TX 77030
Phone: 713-563-6645
Email: amciurea@mdanderson.org

Nizar M. Tannir, MD
Genitourinary Medical Oncology
1515 Holcombe Blvd., Unit 1374
Houston, TX 77030
Phone: 713-563-7265
Email: ntannir@mdanderson.org

Peter Kim, MD*
Cardiology
1515 Holcombe Blvd., Unit 1451
Houston, TX 77030
Phone: 713-792-6246
Email: pkim@mdanderson.org

Ala Abudayyeh, MD*
Nephrology
1515 Holcombe Blvd., Unit 1468
Houston, TX 77030
Phone: 713-404-9366
Email: aabudayyeh@mdanderson.org

* Collaborators who will not be consenting or treating patients on trial.

Statistician:

Suyu Liu, PhD
1515 Holcombe Blvd., Unit 1409
Houston, Texas 77030
713-563-4280
syliu@mdanderson.org

Study Coordinator:

Fengying "Fiona" Ouyang
1515 Holcombe Blvd., Unit 455
Houston, Texas 77030
713-792-7192
713-404-5990
fouyang@mdanderson.org

Responsible Research Nurse:
Jing Gong
1515 Holcombe Blvd., Unit 455
Houston, Texas 77030
713-563-8392
713-606-3681
jinggong@mdanderson.org

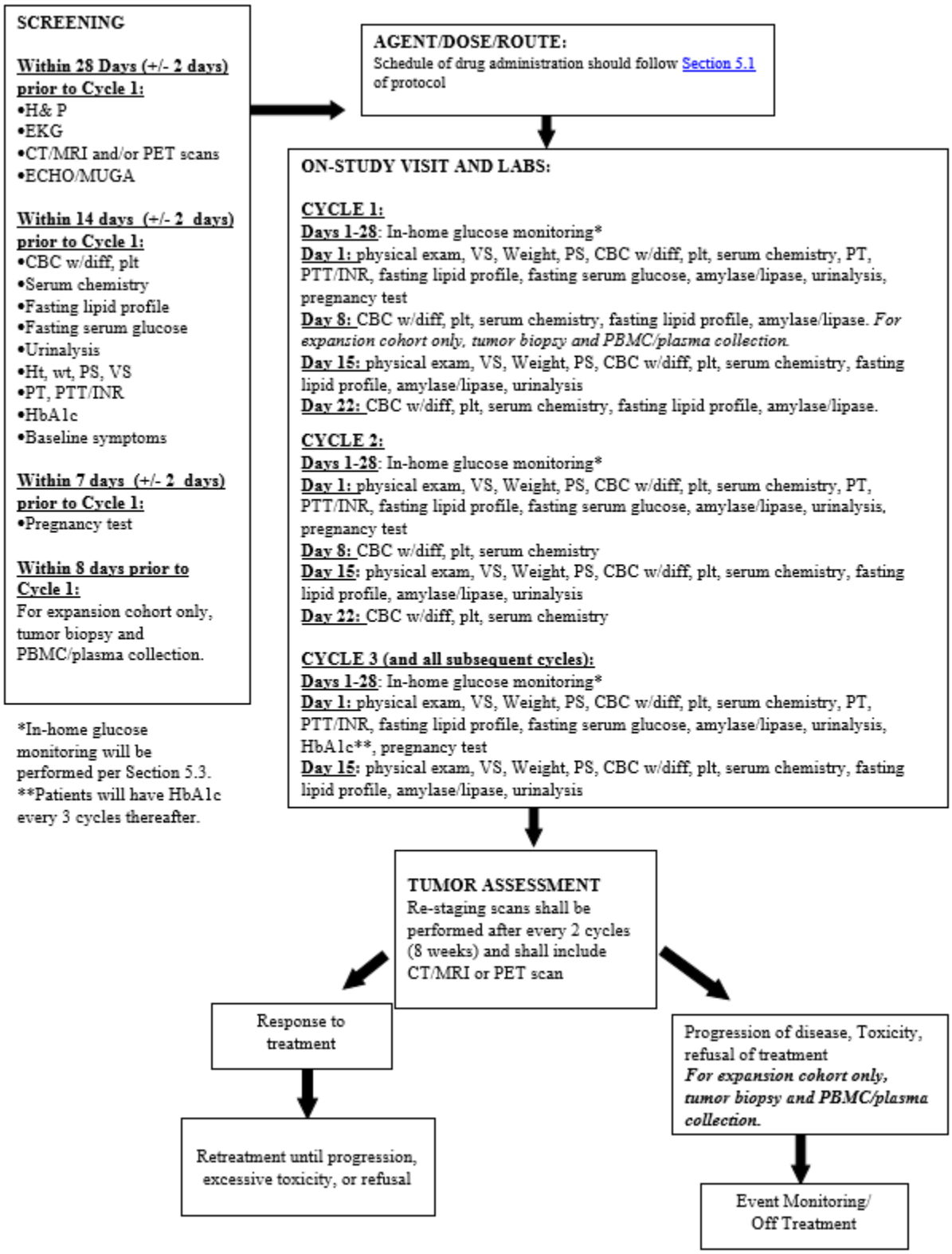
Responsible Data Manager:
Senait Fessahaye
1515 Holcombe Blvd., Unit 455
Houston, Texas 77030
713-563-9897
713-792-6402
snfessah@mdanderson.org

NCI-Supplied Agent(s): MLN0128 (TAK-228) (NSC# 768435), Millennium Pharmaceuticals, Inc. IND #: **121931**
IND Sponsor: DCTD, NCI

Additional NCI-Supplied Agent(s): Ziv-Aflibercept (NSC# 724770), Sanofi Aventis Corporation
IND #: 100137
IND Sponsor: DCTD, NCI

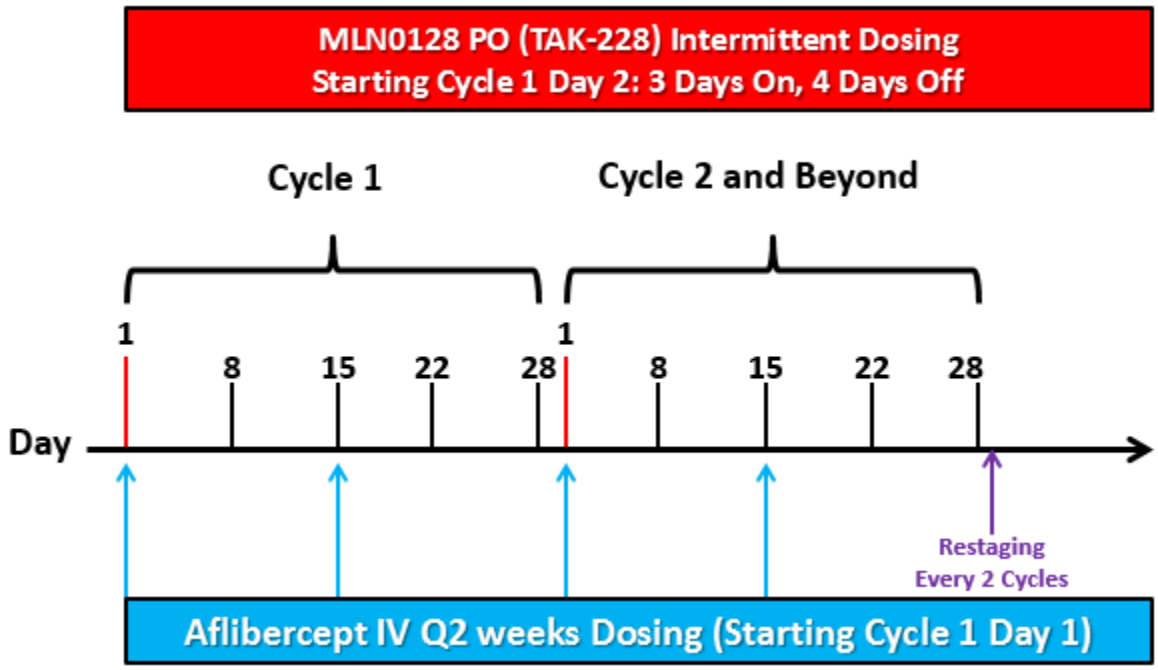
Protocol Type / Version # / Version Date
Original/ Local Version #00 / February 24, 2014
Amendment 01/ Local Version #01 / March 28, 2014
Amendment 02/ Local Version #02 / August 15, 2014
Amendment 03/ Local Version #03 / October 21, 2014
Amendment 04/ Local Version #03 / November 7, 2014
Amendment 05/ Local Version #03 / February 2, 2015
Amendment 06/ Local Version #03 / March 20, 2015
Amendment 07/ Local Version #04 / October 6, 2015
Amendment 08/ Local Version #05/ January 5, 2016
Amendment 09/ Local Version #06/ November 2, 2016
Amendment 10/ Local Version #07/ August 3, 2017
Amendment 11/ Local Version #07/ September 21, 2017
Amendment 12/ Local Version #07/ December 22, 2017
Amendment 13/ Local Version #07/ January 29, 2018
Amendment 14/ Local Version #08/ June 20, 2018
Amendment 15/ Local Version #09/ June 11, 2019
Amendment 16/ Local Version #10/ October 8, 2019

SCHEMA


SCREENING
Within 28 Days (+/- 2 days) prior to Cycle 1:
•H& P
•EKG
•CT/MRI and/or PET scans
•ECHO/MUGA
Within 14 days (+/- 2 days) prior to Cycle 1:
•CBC w/diff, plt
•Serum chemistry
•Fasting lipid profile
•Fasting serum glucose
•Urinalysis
•Ht, wt, PS, VS
•PT, PTT/INR
•HbA1c
•Baseline symptoms
Within 7 days (+/- 2 days) prior to Cycle 1:
•Pregnancy test
Within 8 days prior to Cycle 1:
For expansion cohort only, tumor biopsy and PBMC/plasma collection.
*In-home glucose monitoring will be performed per Section 5.3.
**Patients will have HbA1c every 3 cycles thereafter.
AGENT/DOSE/ROUTE:
Schedule of drug administration should follow Section 5.1 of protocol
ON-STUDY VISIT AND LABS:
CYCLE 1:
Days 1-28: In-home glucose monitoring*
Day 1: physical exam, VS, Weight, PS, CBC w/diff, plt, serum chemistry, PT, PTT/INR, fasting lipid profile, fasting serum glucose, amylase/lipase, urinalysis, pregnancy test
Day 8: CBC w/diff, plt, serum chemistry, fasting lipid profile, amylase/lipase. For expansion cohort only, tumor biopsy and PBMC/plasma collection.
Day 15: physical exam, VS, Weight, PS, CBC w/diff, plt, serum chemistry, fasting lipid profile, amylase/lipase, urinalysis
Day 22: CBC w/diff, plt, serum chemistry, fasting lipid profile, amylase/lipase.
CYCLE 2:
Days 1-28: In-home glucose monitoring*
Day 1: physical exam, VS, Weight, PS, CBC w/diff, plt, serum chemistry, PT, PTT/INR, fasting lipid profile, fasting serum glucose, amylase/lipase, urinalysis, pregnancy test
Day 8: CBC w/diff, plt, serum chemistry
Day 15: physical exam, VS, Weight, PS, CBC w/diff, plt, serum chemistry, fasting lipid profile, amylase/lipase, urinalysis
Day 22: CBC w/diff, plt, serum chemistry
CYCLE 3 (and all subsequent cycles):
Days 1-28: In-home glucose monitoring*
Day 1: physical exam, VS, Weight, PS, CBC w/diff, plt, serum chemistry, PT, PTT/INR, fasting lipid profile, fasting serum glucose, amylase/lipase, urinalysis, HbA1c**, pregnancy test
Day 15: physical exam, VS, Weight, PS, CBC w/diff, plt, serum chemistry, fasting lipid profile, amylase/lipase, urinalysis
TUMOR ASSESSMENT
Re-staging scans shall be performed after every 2 cycles (8 weeks) and shall include CT/MRI or PET scan
Response to treatment
Retreatment until progression, excessive toxicity, or refusal
Progression of disease, Toxicity, refusal of treatment
For expansion cohort only, tumor biopsy and PBMC/plasma collection.
Event Monitoring/ Off Treatment

Dose Escalation Schedule		
Dose Level	**Dose***	
	MLN0128 (TAK-228) (mg PO for 3 days on and 4 days off) Starting on Cycle 1 Day 2	**Ziv-Aflibercept (mg/kg IV Q 2 weeks) Starting on Cycle 1 Day 1**
Level -1	3	2
Level 1	4	2
Level 2	4	3
**Doses are stated as exact dose in units (*e.g., *mg/m^2, mcg/kg, etc.) rather than as a percentage.* *As of Amendment 13/ Version 07, all newly enrolled patients will be given the milled formulation of MLN0128 (TAK-228), and a new dose escalation is being explored. Starting with Amendment 14/Version 08, the dose escalation will be re-started at Dose Level 1 with the updated dose level escalation.* *Both study drugs will have a +/- 2 day window. If the ziv-aflibercept dosing is rescheduled, then the MLN0128 (TAK-228) dosing will be adjusted accordingly.*		

Dose Escalation Treatment Schema



In cases where a subject misses dosing at his/her dosing time, the subject may still take the dose within 12 hours of the regular dosing time (subjects should not take 2 consecutive daily doses within 12 hours of each other). Subjects who vomit after receiving MLN0128 (TAK-228) will not receive a replacement dose within 24 hours. If confirmed that the study drug has been vomited, the dose should be noted as having been missed.

Dose Expansion Treatment and Correlative Study Schema

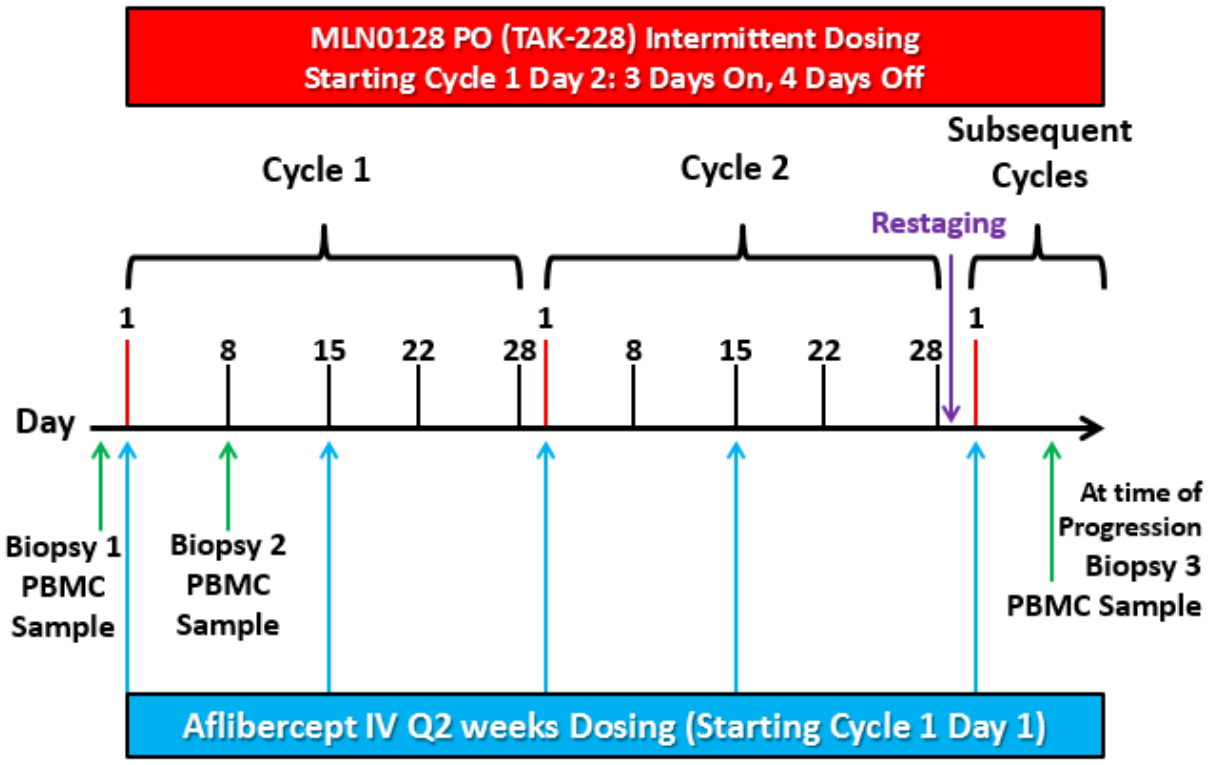

MLN0128 PO (TAK-228) Intermittent Dosing
Starting Cycle 1 Day 2: 3 Days On, 4 Days Off
Cycle 1
Cycle 2
Subsequent Cycles
Restaging
Day
1
8
15
22
28
1
8
15
22
28
1
Biopsy 1 PBMC Sample
Biopsy 2 PBMC Sample
At time of Progression Biopsy 3 PBMC Sample
Aflibercept IV Q2 weeks Dosing (Starting Cycle 1 Day 1)

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To evaluate safety and tolerability, determine maximum tolerated dose (MTD) and recommend a phase II dose of the combination of MLN0128 (TAK-228) with ziv-aflibercept in patients with advanced cancers refractory to standard therapy.

1.2 Secondary Objectives

1.2.1 To give early indication of efficacy by evaluation of tumor size.

1.2.2 To evaluate Akt/mTOR signaling and adaptive responses; testing phosphorylation levels of biomarkers such as, but not limited to, VEGF1 and 2, AKT and 4E-BP1 following treatment with MLN0128 (TAK-228) and ziv-aflibercept in PBMCs and biopsy samples during expansion cohort.

2. BACKGROUND

2.1 Study Disease(s)

PI3K/AKT/mTOR Pathway and Cancer

The PI3K/AKT/mTOR pathway is one of the most frequently deregulated pathways in human tumors, although mTOR mutations have not been reported. Pathway activation occurs either by mutation or upregulation of upstream proteins (such as PI3K or AKT) or through loss of PTEN (mutation or epigenetic silencing). Enhanced growth factor receptor signaling via amplification, mutation or autocrine stimulation, can also lead to pathway activation. Finally, amplification of BCL2, constitutive activation of the Ras/RAF/MEK pathway, c-Abl kinase and cyclin D1 overexpression have been shown to activate the PI3K/AKT/mTOR pathway and are present in variable amounts in solid tumors and hematological malignancies. Around 70% of late-stage cancer patients have specific activation patterns in at least one of these pathways and blocking these effects at the point of convergence is a rational approach in cancer therapy (Meric-Bernstam and Mills, 2004). Deregulation of the pathway is characterized by high mTORC1 and mTORC2 activity, associated with high pS6K and pAKT levels. This is particularly prominent in solid tumors from lung, breast, head and neck, ovary and colon (Dancey, 2004; Georgakis and Younes, 2006; Fasolo and Sessa, 2008).

2.2 MLN0128 (TAK-228) (INK128)

MLN0128 (TAK-228) is an orally bioavailable, potent, highly selective, adenosine 5' triphosphate (ATP)-competitive inhibitor of the kinase activity of mammalian target of

rapamycin (mTOR) (Chiang *et al*., 2007). mTOR is a serine/threonine kinase belonging to the phosphatidylinositol 3 kinase (PI3K)-related kinase superfamily. It functions as a sensor of mitogen activity, cellular energy and nutrient levels, and it is a central controller of cell growth.

Mechanism of Action

The mTOR is a kinase that regulates cell growth, translational control, angiogenesis, and cell survival by integrating nutrient and hormonal signals. mTOR kinase plays a key role in several pathways that are frequently dysregulated in human cancer (Chiang *et al*., 2007). mTOR exists in two complexes, one with raptor, which is rapamycin-sensitive, and the other with rictor, which is rapamycin insensitive. mTOR complex 1 (mTORC1) phosphorylates 4EPB1 and p70S6 kinase and results in translation of proteins involved in cell cycle progression. The mTOR complex 2, rapamycin-insensitive (mTORC2), has been shown to directly phosphorylate and activate the upstream kinase AKT at serine 473. Inhibition of mTORC1 inhibits the negative feedback loop between S6 kinase and insulin receptor substrate 1 (Figure 1) (Naing, 2013). This results in an increase in PI3K and AKT activity and may explain, at least in part, the limited activity of rapamycin analogs in a number of tumor types.

Inhibiting mTOR may inhibit abnormal cell proliferation, tumor angiogenesis, and abnormal cellular metabolism, thus providing the rationale for mTOR inhibitors as potential agents as either monotherapy or in combination with other chemotherapeutic agents in the treatment of solid tumor and hematological malignancies. MLN0128 (TAK-228) inhibits both mTORC1 and mTORC2 complexes by inhibiting mTOR kinase. It, therefore, controls both S6K and AKT; and is expected to have a broader spectrum of activity than rapamycin and its analogs in man.

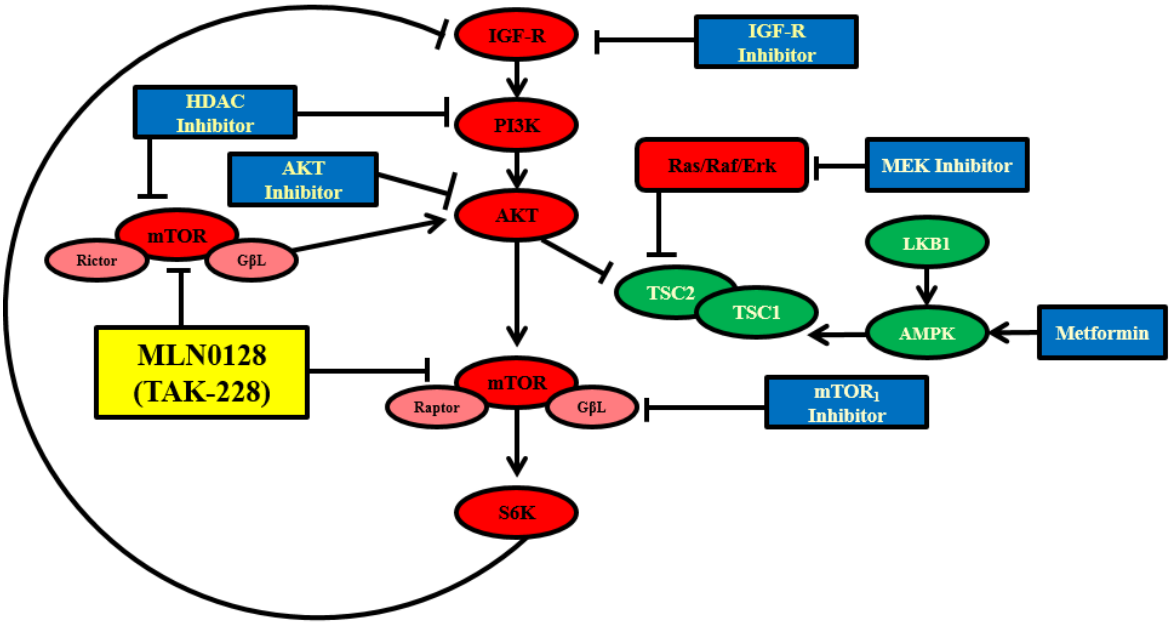


Figure 1: Inhibition of mTORC1 and mTORC2 by dual kinase inhibitor (Modified figure from Naing, 2013)

To address the incomplete inhibition of mTOR by rapalogs, MLN0128 (TAK-228) was developed. MLN0128 (TAK-228) is a potent, selective, orally bioavailable, ATP-competitive inhibitor of mTOR that is currently in phase 1 clinical trials. MLN0128 (TAK-228) targets both

mTORC1 and TORC2, and is designed to overcome the shortcomings of the current rapalogs to achieve greater clinical benefit (Investigator's Brochure, 2017).

Nonclinical Studies with MLN0128 (TAK-228) in Human Tumor Models

MLN0128 (TAK-228) selectively and potently inhibits mTOR kinase (IC50 = 1.1 nM), inhibits mTORC1/2 signaling, and prevents cellular proliferation. The *in vitro* cellular potency of MLN0128 (TAK-228) was not diminished in the presence of human whole blood components.

MLN0128 (TAK-228) displayed cellular inhibition of TORC1 and TORC2 pathways with IC50 less than 10 nM. MLN0128 (TAK-228) is believed to have the potential of achieving greater clinical benefit than the currently available rapalogs. *In vitro* studies have demonstrated that MLN0128 (TAK-228) MLN0128 selectively and potently inhabits the mTOR kinases, but relative to mTOR inhibition MLN0128 (TAK-228) has >100-fold less potency as an inhibitor of Class I (PI3 kinase isoforms α, β, γ, δ), class II (PI3KC2α and PI3K2C β), and class III (VPS34) PI3K family members, as well as PI3Kα and PI3Kβ. (Investigator's Brochure, 2017). MLN0128 (TAK-228) was also found to inhibit (>80%) of the biochemical activity of only five kinases (mTOR, DNA-PK, PDGFR α, Flt3, and CK1 epsilon kinases) out of a panel of 222 protein kinases. MLN0128 (TAK-228) inhibited ligand binding of 10 receptor and intracellular protein kinases including (ACVR1, BMPR1B, CSF1R, CSNK1D, CSNK1E, DDR1, MEK1, MEK2, PDGFR α, and RIPK2) out of a panel of 402 distinct kinases. In PTEN deficient cells, MLN0128 (TAK-228) had pharmacodynamic properties comparable to dual inhibition of TORC1 and PI3K utilizing a combination of rapamycin and PI-103.

The pharmacodynamics and antitumor activity of MLN0128 (TAK-228) was studied *in vivo* in murine xenograft models of human glioblastoma, NSCLC, breast cancer, renal cell cancer, endometrial adenocarcinoma, and castration-resistant prostate cancer (CPRC). Consistent with the mode of action, MLN0128 (TAK-228) inhibited phosphorylation of downstream modulators of mTORC1 (namely 4EBP1 and S6) and mTORC2 (namely AKT [S473]) in human U87 glioblastoma tumor xenograft models in mice at doses as low as 0.1 mg/kg. Additionally, MLN0128 (TAK-228) showed strong tumor growth inhibition (TGI) in all 8 xenograft models at tolerable oral (PO) doses from 0.15 mg/kg (daily [QD]; tested in MDA-MB-361 breast carcinoma) to 3.0 mg/kg (every other day [Q2D] or once weekly [QW]; tested in all models) (Investigator's Brochure, 2017).

In addition to single-agent activity in these xenograft models, MLN0128 (TAK-228) was combined with the standard of care (SOC) agent paclitaxel in the breast and endometrial models. The combination of MLN0128 (TAK-228) with paclitaxel resulted in enhanced antitumor activity and reduced tumor burden. When tumors were pretreated with paclitaxel, there was an added benefit in tumor reduction compared to the effects of simultaneous administration of both agents, consistent with the mechanism of action of paclitaxel. Findings from these nonclinical pharmacology studies suggest that MLN0128 (TAK-228), alone and in combination with paclitaxel, has therapeutic potential as an orally administered mTORC1/2 inhibitor for the treatment of cancers associated with dysregulated activation of the PI3K/AKT/mTOR pathway, such as renal cell, endometrial, breast, lung, and prostate cancers(Investigator's Brochure, 2017).

In preliminary work, we evaluated the antitumor efficacy of MLN0128 (TAK-228) in intrinsically rapamycin-resistant and rapamycin-sensitive cancer cell lines (Figure 2). We demonstrated that both rapamycin-sensitive and resistant cancer cell lines were responsive to clinically achievable levels of MLN0128 (TAK-228) (Figure 2A). Evaluation of the effect of MLN0128 (TAK-228) on downstream signaling in multiple cell lines have shown more robust inhibition of mTOR targets S6K and 4E-BP1, as well as inhibition of phospho-Akt Ser473. There was, however, only transient inhibition of phospho-Akt Thr 308, with subsequent upregulation (Figure 2B). Further, in some *in vivo* models MLN0128 (TAK-228) had a significantly greater antitumor efficacy compared to rapamycin (unpublished data).

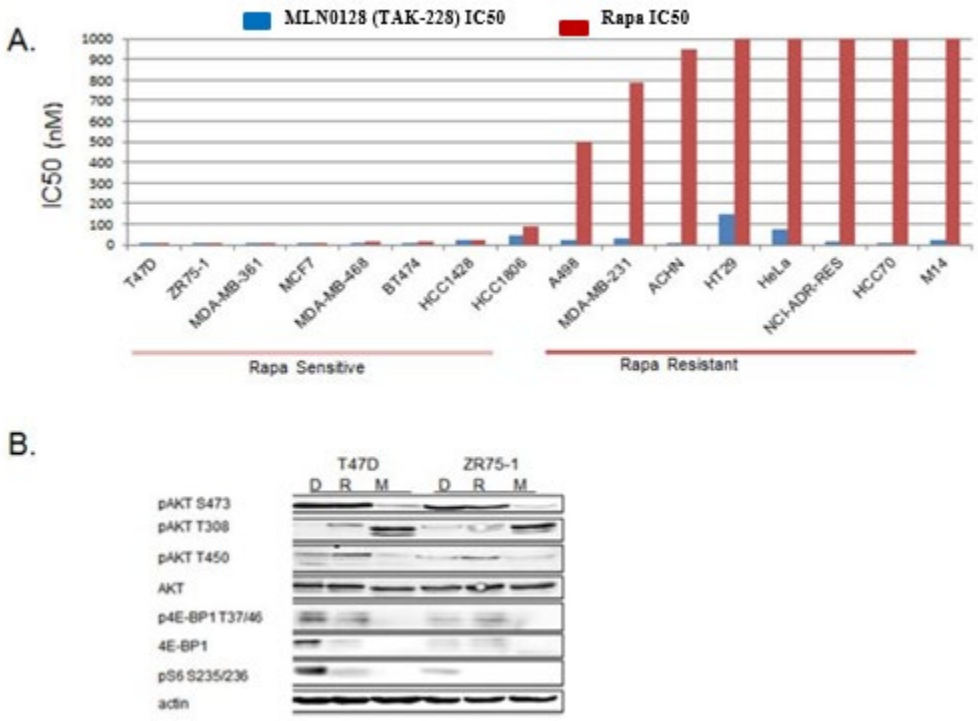


Figure 2. *In vitro* efficacy of MLN0128 (TAK-228). A. A panel of cancer cell lines with differential sensitivity to rapamycin was treated with increasing doses of MLN 0128 and rapamycin; Half maximal inhibitory concentrations (IC50s) were determined by Sulforhodamine B colorimetric (SRB) assay. **B.** Cell lines were treated with vehicle, rapamycin or MLN0128 (TAK-228) for 24 hours and cell signaling was assessed by Western blotting. Results from 2 of 13 cell lines evaluated are shown**.**

MLN0128 (TAK-228) Nonclinical Toxicology and PK studies

PK Studies

MLN0128 (TAK-228) was rapidly absorbed after oral administration to mice, rats, dogs, and monkeys, with high oral bioavailability. MLN0128 (TAK-228) did not inhibit P-glycoprotein (P-gp). A study of the tissue distribution of [14C] MLN0128 (TAK-228) showed that [14C] MLN0128 (TAK-228) was rapidly and widely distributed throughout the body in Long-Evans rats; radioactivity was eliminated from most tissues at 48 hours post dose, and from all but the

adrenal cortex, adrenal gland, adrenal medulla, eye, liver, and uveal tract at 168 hours. MLN0128 (TAK-228) displayed dose proportional plasma exposures and a moderate propensity to cross the blood-brain barrier. MLN0128 (TAK-228) was modestly bound to human plasma proteins (approximately 70%). MLN0128 (TAK-228) inhibited breast cancer resistance protein (BCRP), organic cation transporter (OCT)1, and OCT2 (Investigator's Brochure, 2017).

M1, the single metabolite (monohydroxylation product) observed in human microsomal incubations, was also observed in rats and monkeys, the species used for the Good Laboratory Practice (GLP) toxicology studies. Recently completed in vitro metabolism experiments in human hepatocytes using ^{14}C-labeled TAK-228 suggest that TAK-228 is metabolized primarily via CYP1A2 (approximately 31%-40%), with a minor contribution from CYP3A4 (approximately 11%-22%). These data suggest that TAK-228 is also metabolized by direct glucuronidation (approximately 22%) and an unidentified non-uridine diphosphate glucuronosyl transferase pathway (approximately 18%). The new data differ from the previous in vitro CYP phenotyping data obtained using recombinant CYP enzymes, which suggested the involvement of CYP2C9 (approximately 35%), CYP2C19 (approximately 28%), and CYP3A4 (approximately 28%) in TAK-228 metabolism. In addition, physiologically based PK modeling and simulation using the new metabolism data for TAK-228 suggest that the risk for a metabolism-based drug-drug interaction with TAK-228 appears to be low.

Toxicology

The MLN0128 (TAK-228) toxicology program consisted of single- and repeat-dose studies in rats and monkeys, single-dose studies in dogs, and an Ames genotoxicity study. The toxicologic profiles obtained in the non-GLP-compliant and GLP-compliant studies were generally consistent. The observed toxicities were consistent between rats and monkeys, with no apparent sex differences (Investigator's Brochure, 2017).

The toxicity profile of MLN0128 (TAK-228) in rats and monkeys, as established in GLP-compliant repeat-dose studies, is consistent with pharmacologic inhibition of mTORC1/2 activity. The dose limiting toxicities (DLTs) of MLN0128 (TAK-228) in rats and monkeys were secondary to an exaggerated pharmacologic response and consisted of body weight loss and associated clinical observations that included gastrointestinal (GI) distress and decreased activity, appetite, and body temperature. Adverse effects in rats included body weight loss, decreased activity, increased glucose and insulin levels, alterations in white blood cells (WBCs), bone marrow and lymphoid depletion, thymic necrosis, oligospermia, testes degeneration/ atrophy, nonglandular stomach epithelial degeneration/ ulceration/ hyperplasia, and alveolar histiocytosis. The microscopic findings observed in the testes, epididymides, and nonglandular stomach were not resolved after a 14-day recovery period, while partial to complete resolution was seen in the lungs, thymus, and bone marrow. The adverse effects in monkeys included decreased activity, appetite, and body weight; increased glucose and insulin; lymphoid and bone marrow depletion; adrenal hypertrophy/hyperplasia; pancreatic and salivary gland acinar cell secretory depletion; GI tract erosion and ulceration; and skin ulceration/epidermal hyperplasia. The findings in the pancreas, adrenal glands, and salivary glands may have been related to a stress response or reduced food consumption. The findings were generally reversible after a 14-day recovery period (Investigator's Brochure, 2017).

The findings in rat and monkey repeat-dose toxicology studies with MLN0128 (TAK-228), including bone marrow and lymphoid depletion, GI and skin effects, and effects on glucose and insulin levels, can be monitored in clinical trials. The toxicities seen in the repeat-dose toxicology studies, such as GI effects and glucose and insulin increases, are consistent with the treatment-emergent adverse events (TEAEs), including mucositis and hyperglycemia, observed to date in patients receiving MLN0128 (TAK-228). MLN0128 (TAK-228) was negative for mutagenicity in the Ames assay, and shows low potential for phototoxicity (Investigator's Brochure, 2017). MLN0128 (TAK-228) has a low potential to affect the hERG potassium ion channel, and did not affect cardiovascular parameters in vivo in telemeterized monkeys (Investigator's Brochure, 2017).

Summary of MLN0128 (TAK-228) Clinical Studies

Single-agent MLN0128 (TAK-228) had initial clinical development in two phase 1 studies in subjects with advanced solid malignancies and hematologic malignancies (multiple myeloma [MM] and Waldenstrom macroglobulinemia [WM]), and in a third study in combination with paclitaxel with or without trastuzumab in subjects with advanced solid tumors (Investigator's Brochure, 2017).

Study INK128-001

Study INK128-001 evaluated safety and anti-tumor activity of MLN0128 (TAK-228) in subjects with advanced solid malignancies. As of 13 September 2016, 198 subjects have been treated in Study INK128-001. As of the cutoff date, treatment-emergent SAEs had been reported for 84 patients (42%) in this study. The most commonly reported (≥4 patients, overall) preferred terms were stomatitis in 7 patients (4%), pneumonia in 6 patients (3%), abdominal pain, acute kidney injury, or anemia in 5 each (3%), and asthenia or vomiting in 4 each (2%).

All 116 patients in the dose escalation phase reported at least 1 treatment-emergent adverse event (TEAE). Across all dosing schedules in this phase, the most frequently occurring TEAEs were hyperglycemia, nausea, and vomiting. There were no meaningful trends across dosing schedules or dose levels in the incidence of these TEAEs.

The most commonly reported TEAEs among patients receiving QD dosing (n=31) were hyperglycemia (84%) and nausea (52%). The incidence of hyperglycemia increased in a dose-dependent manner. A total of 19 QD patients (61%) overall reported at least 1 TEAE of Grade 3 or greater severity. The most commonly reported TEAEs of Grade 3 or greater severity were hyperglycemia (6 patients [19%] overall) and lymphopenia (3 patients [10%] overall).

Among patients receiving QW dosing (n=30), the most commonly reported TEAEs were nausea (73%), vomiting (67%), and hyperglycemia (60%). Clear dose relationships were not evident among the most commonly reported TEAEs, but the most common TEAEs were generally reported more frequently by patients in the 30 and 40 mg than patients in the 7 and 10 mg QW dose groups. A total of 18 patients (60%) overall reported at least 1 TEAE of Grade 3 or greater severity. The most common TEAE of Grade 3 or greater

severity was fatigue (3 patients [10%] overall). TEAEs of Grade 3 or greater severity were reported more frequently by patients in the 40 mg than patients in the other QW dose groups.

Among patients receiving QD×3d QW dosing (n=33), the most commonly reported preferred terms were nausea (73%), stomatitis (70%), hyperglycemia (67%), decreased appetite and vomiting (61% each), and diarrhea (52%). Clear dose relationships were not evident among the most commonly reported TEAEs. A total of 25 patients (76%) overall reported at least 1 TEAE of Grade 3 or greater severity. The most common (≥10% of patients) TEAEs of Grade 3 or greater severity were hyperglycemia (7 patients [21%] overall) and asthenia, hypophosphatemia, and stomatitis (each reported by 5 patients [15%] overall). TEAEs of Grade 3 or greater severity were reported less frequently by patients in the 6 and 9 mg QD×3d QW than patients in the other dose groups.

The most commonly reported TEAEs among patients receiving QD×5d QW dosing (n=22) were nausea and stomatitis (59% each) and hyperglycemia and vomiting (55% each). Events of stomatitis, vomiting, and decreased appetite increased in a dose-dependent manner across the dose levels in the QD×5d QW schedule. A total of 14 patients (64%) overall reported at least 1 TEAE of Grade 3 or greater severity. The most common (≥10% of patients) TEAEs of Grade 3 or greater severity were asthenia and stomatitis (each reported by 3 patients [14%] overall). TEAEs of Grade 3 or greater severity were reported more frequently by patients in the 13 mg than patients in the 7 and 10 mg QD×5d QW dose groups.

All 82 patients in the expansion phase reported at least 1 TEAE. Across dosing schedules in this phase, the most frequently occurring TEAEs were fatigue, nausea, and hyperglycemia. There were no meaningful differences between dosing schedules in the overall incidence of these TEAEs or Grade 3 or greater TEAEs, and the pattern of these events was generally similar in both schedules.

Among patients receiving 5 mg QD dosing during the Expansion Phase (n=39), the most commonly reported (>50% of patients) preferred terms were fatigue (64%), nausea (54%), and decreased appetite and diarrhea (51% each). A total of 30 patients (77%) in the 5 mg QD dose group reported at least 1 TEAE of Grade 3 or greater severity. The most common (≥10% of patients) TEAEs of Grade 3 or greater severity were hyperglycemia and fatigue (each reported by 5 patients [13%]) and hypophosphatemia (4 patients [10%]).

The most commonly reported TEAEs among patients receiving 30 mg QW dosing (n=17) were nausea (76%), hyperglycemia (71%), fatigue (65%), vomiting (59%), and stomatitis (53%). A total of 9 patients (53%) in the 30 mg QW dose group reported at least 1 TEAE of Grade 3 or greater severity. The most common (≥10% of patients) TEAEs of Grade 3 or greater severity were hyperglycemia, fatigue, hypophosphatemia, nausea, and pain in extremity (each reported by 2 patients [12%]).

Among patients receiving 40 mg QW dosing (n=26), the most commonly reported (>50%

of patients) preferred terms were fatigue (92%), nausea, hyperglycemia, and vomiting (77% each), and decreased appetite (54%). A total of 19 patients (73%) in the 40 mg QW dose group reported at least 1 TEAE of Grade 3 or greater severity. The most common (≥10% of patients) TEAEs of Grade 3 or greater severity were hyperglycemia (6 patients [23%]), fatigue (4 patients [15%]), and anemia and dehydration (each reported by 3 patients [12%]).

In Study INK128-001, as of 13 September 2016, the maximum tolerated doses (MTD) for all 4 schedules had been determined: for the QD dosing the MTD is 6 mg QD, for the QDx3dQW dosing, the MTD is 16 mg; for the QDx5dQW dosing, the MTD is 10 mg; and for the QW dosing schedule, the MTD is 40 mg. The MTDs for each of the 4 schedules was determined by evaluation of cohorts of 6 evaluable patients. At each MTD, up to 6 additional patients were enrolled to further evaluate safety and tolerability. A significant proportion of patients treated at the MTDs required dose modifications due to drug-related AEs beyond 1 or 2 cycles, and therefore were not representative of a recommended phase 2 dose. The study is currently further evaluating doses at less than the MTD for QDx3dQW and QDx5dQW, to determine a dose(s) and schedule(s) to be studied further in the expansion phase of the study, as well as in future phase 2 studies. The dose escalation portion of the study has evaluated dose regimens ranging from 2 to 7 mg QD, 7 to 40 mg QW, 6 to 20 mg QDx3dQW, and 7 to 13 mg QDx5dQW (Investigator's Brochure, 2017).

Study INK128-002
Study INK128-002 is evaluating safety and anti-tumor activity of MLN0128 (TAK-228) in subjects with hematologic malignancies (MM and WM). As of 09 December 2012, 39 subjects have been treated in Study INK128-002. The most common AEs (≥ 20%), regardless of causality were nausea (56%), fatigue (49%), hyperglycemia (38%), thrombocytopenia (36%), diarrhea (28 %), decreased appetite and vomiting (23% each), and stomatitis/anemia (21% each). Most commonly reported (at least 2 subjects) Grade ≥ 3 AEs, regardless of causality included thrombocytopenia (12%) and hypocalcemia, hypokalemia, and pneumonia (8 % each). In Study INK128-002, dose escalation is completed, with 4 mg determined as the MTD for the QD schedule, and 9 mg determined as the MTD for the QDx3dQW schedule (Investigator's Brochure, 2017).

Study INK128-003
Study INK128-003 is evaluating safety and anti-tumor activity of MLN0128 (TAK-228) in subjects with advanced solid tumors in combination with paclitaxel (and trastuzumab for HER2+ subjects). As of 09 December 2016, 67 subjects have been treated in Study INK128-003. The most common AEs (≥ 20%), regardless of causality were fatigue (67%); nausea (60%); diarrhea (52%); vomiting (46%), decreased appetite and hyperglycemia (43% each), stomatitis (40%), anemia and dehydration (37% each), asthenia (33%), hypokalemia (28%), neutropenia and urinary tract infection (27% each), decreased weight (22%), and dizziness and hypophosphatemia (21% each). Most commonly reported (at least 2 subjects) Grade ≥ 3 AEs, regardless of causality, include neutropenia (21%); hypophosphatemia (15%); diarrhea and hyperglycemia (12% each), fatigue, hypokalemia, and vomiting (10% each) (Investigator's Brochure, 2017).

In Study INK128-003, dose escalation is completed, with 8 mg of MLN0128 (TAK-228) QDx3dQW being selected for the dose expansion phase of the study. The QDx5dQW and QW schedules were abandoned before MTDs were declared, as these schedules were viewed as less convenient relative to the QDx3dQW schedule, from the perspective of administering the paclitaxel and trastuzumab combination (Investigator's Brochure, 2017).

Study MLN0128-1004
Study MLN0128-1004 was a phase 1 open-label study to evaluate the safety, tolerability, and PK of TAK-228 as a single agent and in combination with paclitaxel in adult patients with advanced nonhematologic malignancies. As of the data cutoff date for the primary CSR (30 June 2016), 5 patients were ongoing in the study. The study had 3 arms that enrolled in parallel. The MTD for QD dosing of milled TAK-228 was 3 mg TAK-228. The highest doses of milled TAK-228 that were tested and deemed safe in the QW and the QD×3d QW+paclitaxel cohorts of this study were 30 mg TAK-228 and 6 mg TAK-228, respectively.

A total of 61 patients were enrolled in the study and received at least 1 dose of study drug: 19 patients in the Single-Agent QD Arm, 22 patients in the Combination Arm, and 20 patients in the Single-Agent QW Arm.

Fifteen patients (78.9%) in the PK run-in period and all patients (100.0%) in the study treatment period of the Single-Agent QD Arm reported at least 1 TEAE. The most commonly reported (≥25%) preferred terms were vomiting (26.3%) during the PK run- in period and fatigue (58.8%); pruritus (47.1%); decreased appetite, diarrhea, and nausea (35.3% each); and insomnia (29.4%) during the study treatment period. Clear dose relationships were not evident among the most commonly reported TEAEs.

Twenty-one patients (95.5%) in the Combination Arm reported at least 1 TEAE. The most commonly reported (≥25%) preferred terms were diarrhea (63.6%), nausea (54.4%), decreased appetite and fatigue (50.0% each), stomatitis and vomiting (36.4% each), and dry mouth (27.3%). Clear dose relationships were not evident among the most commonly reported TEAEs

Nineteen patients (95.0%) in the Single-Agent QW Arm reported at least 1 TEAE. The most commonly reported (≥25%) preferred terms were fatigue and nausea (55.0% each), vomiting (45.0%), and diarrhea (30.0%). Clear dose relationships were not evident among the most commonly reported TEAEs (Investigator's Brochure, 2017).

Study C31001
Study C31001 is an ongoing phase 1b/2 study of the safety and efficacy of TAK- 228 + exemestane or fulvestrant when administered in women with ER+/HER2- advanced or metastatic breast cancer that has progressed on prior treatment with everolimus in combination with exemestane or fulvestrant. Patients in this study receive the same prior regimen of either exemestane or fulvestrant that they received prior to enrolling in this study; the only change is that they also receive TAK-228 as part of their continued

regimen.

As of the clinical data cutoff date (09 December 2016), 24 patients had received ≥1 TAK-228 dose in the phase 1 portion of the study and 68 patients had received ≥1 TAK-228 dose in the phase 2 portion of the study. Overall, most of the women enrolled as of the data cut were white (88% phase 1; 82% phase 2). At baseline, their median age was approximately58 years (range 32-83 years). As of data cutoff, 21 patients (2 in the phase 1 portion and 19 in the phase 2 portion) remained ongoing in the study.

As of the clinical data cutoff date, 10 treatment-emergent SAEs had been reported in 5 patients (21%) in the phase 1 portion of Study C31001. One Grade 5 (fatal) SAE of hepatic failure was reported. One Grade 4 SAE (dyspnea) was reported and resulted in study drug discontinuation.

As of the clinical cutoff date, 16 treatment-emergent SAEs had been reported in 11 patients (16%) in the phase 2 portion of Study C31001. One Grade 5 (fatal) SAE was reported (preferred term: death [not further specified]). No Grade 4 SAEs were reported; the remaining events were severity Grade 3 or lower.

Overall, across both phases of the study, commonly reported TEAEs included nausea (67% phase 1; 49% phase 2), fatigue (71% phase 1; 38% phase 2), diarrhea (67% phase 1; 28% phase 2), hyperglycemia (25% phase 1; 26% phase 2), pruritis (50% phase 1; 16% phase 2), vomiting (29% phase 1; 22% phase 2), and decreased appetite (33% phase 1; 19% phase 2). TEAEs that were considered severity ≥Grade 3 and were reported in more than 3 patients were hyperglycemia (8 patients), diarrhea (7 patients), nausea (5 patients), and fatigue (4 patients) (Investigator's Brochure, 2017).

Study C31002
Study C31002 is a phase 1 open label, single-arm, multicenter study to evaluate the effect of a single dose of 40 mg TAK-228 on the QT/QTc (QT interval corrected for heart rate) in patients with advanced solid tumors. The data cutoff date for the primary study report was 29 April 2016.

After completing the per-protocol PK/ECG assessments on Cycle 1 Day 3, patients could continue to receive TAK-228 if, in the opinion of the investigator, the patient was deriving clinical benefit, until they experienced disease progression. Patients continuing treatment received TAK-228 30 mg QW in 28-day cycles.

The most frequently observed TEAEs by preferred term (>25% of patients) after treatment with TAK-228 were nausea (35 [80%] patients), fatigue (27 [61%] patients), vomiting (25 [57%] patients), decreased appetite (20 [45%] patients), diarrhea (15 [34%] patients), weight decreased (14 [32%] patients), and urinary tract infection (12 [27%] patients).

PK Summary

PK data from Studies INK128-001, INK128-002, and INK128-003 indicate that TAK-228 exhibits fast oral absorption (t_{max} generally between 1-4 hours after dosing) and dose-linear PK with a mean plasma half-life of approximately 8 hours and does not accumulate meaningfully in plasma when dosed as frequently as once daily and under any of 4 tested dosing regimens. The PK data of TAK-228 were generally consistent, with no appreciable differences across 5 phase 1 studies, suggesting no appreciable difference in the PK of TAK-228 among patients with advanced solid tumors or patients with MM or WM.

There were no meaningful differences in the PK of TAK-228 when administered 24 hours after a 30-minute IV infusion of 80 mg/m^2 paclitaxel (Study INK128-003) compared with single-agent TAK-228 (Studies INK128-001 and INK128-002). The PK of paclitaxel also remained generally unaffected by TAK-228 co-administration, indicating the lack of a PK interaction between TAK-228 and paclitaxel.

There were no readily apparent differences in either the C_{max} or AUC of 4 mg TAK-228 unmilled or milled capsules when administered under fasted conditions.

Compared to the fasted state, when 4 mg of milled TAK-228 API was administered following a standard high-fat breakfast, there was an approximately 40% reduction in C_{max} and a delay in t_{max} (median t_{max} 6 hours [fed] vs. 2 hours [fasted]), but there was no meaningful change in AUC. The differences observed when TAK-228 was dosed under fed versus fasted conditions may help explain the different MTDs determined for TAK-228 QD dosing in Study INK128-001 compared with Study MLN0128-1004.

There were no readily apparent differences in the PK of TAK-228 when administered in conjunction with either 25 mg exemestane or with 500 mg fulvestrant.

Safety Issues

As of the clinical data cutoff (09December 2016), a total of 538 patients had received ≥ 1 dose of study drug across studies. A total of 25 deaths that occurred within 30 days of the last study drug dose had been reported to the clinical database as of the data cutoff; of these events, 1 (ventricular fibrillation and cardiac arrest; Study INK128-001) was considered related to TAK-228.
Across the studies and regardless of causality or dosing regimen, the most common TEAEs included nausea, fatigue, hyperglycemia, vomiting, diarrhea, stomatitis, and decreased appetite.

Due to the cardiac death on study INK128-001, study C31002, a phase 1 single-arm study to evaluate the effect of a single dose of 40 mg TAK-228 on the QT/QTc interval was initiated in patients with advanced solid tumors. After completing the per-protocol PK/ECG/cardiac contractility monitoring, the patients continued MLN0128 (TAK-228) 30 mg QW with continued cardiac monitoring. The study results showed that treatment with MLN0128 (TAK-228) was not associated with clinically meaningful effects on the overall electrocardiographic safety profile, and that ECHO/MUGA at screening was not

required.

Development of a Milled Formulation of MLN0128 (TAK-228)
In order to allow more predictable absorption of MLN0128 (TAK-228) after oral administration and to allow scale-up manufacturing of MLN0128 (TAK-228) capsules, Millenium/Takeda developed a new milled formulation of the agent. The physical milling step during the granulation process controls particle size distribution of MLN0128 (TAK-228). In order to observe whether this milling step altered the safety and PK profile of MLN0128 (TAK-228), the company performed in vivo studies with PK analysis of milled agent. These studies indicated that the milled formulation may result in faster absorption with possibly higher maximum concentration (Cmax), which could result in a different safety profile, compared to the previous unmilled API capsules.

Takeda developed new MLN0128(TAK-228) capsules containing milled active pharmaceutical ingredient (API) for clinical studies in 1 mg, 3 mg, and 5 mg strengths. Patients receiving the milled formulation were added onto ongoing studies C31001 and C31002, as well as a new study MLN0128-1004, with various treatment cohorts including daily and weekly administration of milled MLN0128(TAK-228).

The recommended dose of milled MLN0128 (TAK-228) was evaluated in 17 patients of MLN0128-1004, with PK, safety, and tolerability assessed. Six patients were given a 4 mg QD dose of milled MLN0128 (TAK-228) and 3 patients had observed DLT (rash, appetite loss and fatigue). A dose of 3 mg QD was given to 11 patients with only 1 DLT (decreased platelets) observed. The 3 mg QD dose of MLN0128 (TAK-228) was declared the RP2D, and was generally well tolerated and demonstrating objective responses in patients.

The significant difference in tolerability observed in the comparison of the MTDs between unmilled and milled MLN0128 (TAK-228) when administered QD may be possibly explained due to the effect of food on the safety/tolerability of unmilled drug in study IND128-001. The GastroPlus^{TM} simulation performed under fasting conditions on the trial demonstrated that unmilled and milled MLN0128 (TAK-228) administration result in comparable exposures to the drug; whereas in the fed state, milled MLN0128(TAK-228) resulted in higher C_{max} (1.5- to 2-fold higher) and earlier T_{max} than unmilled MLN0128(TAK-228) with comparable AUCs. Consequently, a dose of 3 mg QD was chosen as the RP2D of milled MLN0128 (TAK-228) dose in empty stomach conditions.

The RP2D for milled MLN0128(TAK-228) on a weekly schedule was determined to be 30 mg, the same weekly RP2D as seen for the older unmilled formulation. Six patients treated at 30 mg weekly with the milled formulation did not demonstrate any DLT, but the agent was not escalated further. No DLT had been demonstrated for milled MLN0128 (TAK-228) at the prior 20 mg QW dose as well.

TAK-228-1004, a phase I, open label study to evaluate the safety, tolerability, and pharmacokinetics of MLN0128 (TAK-228) in combination with paclitaxel in adult patients with advanced non-hematological malignancies-), with the new milled API was

used to determine the recommended phase 2 dose (RP2D) for MLN0128(TAK-228) QD×3days per week in combination with paclitaxel. The RP2D of milled MLN0128 (TAK-228), given 3 consecutive days weekly, in combination with weekly paclitaxel at 80 mg/m2, was 4 mg.

2.3 VEGF-Trap (Ziv-Aflibercept)

Ziv-aflibercept is also referred to as "AVE0005," "AVE0005 (VEGF Trap)," "VEGF Trap," "V-Trap," and "VGFT" in various clinical study protocols and other documents. However, it will be referred to by its international non-proprietary name (INN), ziv-aflibercept, throughout this protocol (Investigator's Brochure, 2017).

Ziv-aflibercept is a recombinant fusion protein consisting of human vascular endothelial growth factor (VEGF) receptor extracellular domains fused to the Fc portion of human immunoglobulin G1 (IgG1). Ziv-aflibercept contains portions of the extracellular domains of 2 different vascular endothelial growth factor receptors (VEGFRs): VEGFR1 (also known as Flt-1) and VEGFR2 (also known as KDR or Flk-1). Ziv-aflibercept binds VEGF in the picomolar (pmol/L) range, and also binds placental growth factor (PlGF), although with lower affinity. The affinity constants (K_D) for binding to 2 human isoforms of VEGF, $VEGF_{165}$ and $VEGF_{121}$, are 0.50 pmol/L and 0.36 pmol/L, respectively. The K_D for human PlGF2 is 39 pmol/L. The binding of ziv-aflibercept to its ligands in vivo is expected to block tumor angiogenesis and vascular permeability (Investigator's Brochure, 2017).

Ziv-aflibercept drug product is formulated as a sterile liquid for intravenous (IV) administration. Ziv-aflibercept has been found to be active with a broad pharmacological index against early and advanced stage disease in a variety of preclinical solid tumor models including sarcomas, and ovarian, prostate, mammary, colon, and gastric carcinomas when used as a single agent or in combination with cytotoxic agents. In mouse models of ascites formation with ovarian and renal cell carcinoma, ziv-aflibercept inhibited ascites formation and reduced tumor burden (Investigator's Brochure, 2017).

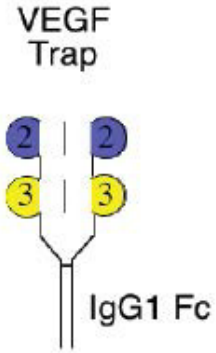
Two analytes were assayed in animal models specifically by enzyme linked immunosorbent assay (ELISA) methods: free aflibercept (compound not complexed to VEGF), and bound aflibercept (complexed aflibercept: VEGF [ratio 1/1]) (Investigator's Brochure, 2017).

Following IV administration in all animal species evaluated, free aflibercept was characterized by a low clearance (0.5 to 3 mL/hr/kg), a low volume of distribution (51 to 77 mL/kg), and a long apparent elimination half-life ($t_{1/2}$) of 48 to 98 hours. Based on the correlation between exposure and activity in non-clinical models, the target pharmacological exposure in humans is proposed to be a safely administered dose of ziv-aflibercept at which an excess of free aflibercept is sustained (Investigator's Brochure, 2017).

Mechanism of Action

VEGF is an angiogenic growth factor that plays a critical role during normal embryonic development and has also been implicated in the pathogenesis of a number of diseases, including cancer. The angiogenic growth factor family, comprised of 5 isoforms (VEGF- A, -B, -C, -D, -E, and placental growth factor [PlGF]), acts through 3 tyrosine kinase receptors,

VEGFR1, VEGFR2 and VEGF receptor 3 (VEGFR3). The diagram of VEGF Trap (below) shows the high affinity VEGF binding domain 2 from VEGFR1 coupled with the biologically more active VEGFR 2 domain 3 along with the IgG1 Fc domain. With a dissociation constant of 0.5 pM, the VEGF Trap has the highest binding affinity for VEGF described to date.



VEGF Trap is produced in Chinese hamster ovary cells as a dimeric glycoprotein with a molecular weight of 115 kDa (Holash *et al*., 2002). Unlike the humanized monoclonal antibody to VEGF, VEGF Trap is entirely comprised of human protein sequences, has a higher affinity for binding to VEGF, and binds to other VEGF family members, including PlGF. In addition, VEGF Trap has been shown to block VEGFR2 phosphorylation when added to cultured endothelial cells in the presence of exogenous VEGF (Holash *et al*., 2002).

Nonclinical Studies with Ziv-Aflibercept in Human Tumor Models

The toxicological profile of ziv-aflibercept was established via single and repeat dose toxicity studies in rats (general toxicity and safety pharmacology), monkeys (general toxicity and fertility) and rabbits (reproductive toxicity and local tolerance) (Investigator's Brochure, 2017).

Data from the general toxicity studies conducted in cynomolgus monkeys showed that most of the adverse effects of ziv-aflibercept were consistent with the inhibition of the VEGF pathway and were also reported in nonclinical studies conducted with other anti- VEGF drugs. Target organs identified in these studies included bone (interference with growth plate maturation of long bones and osteocartilaginous exostoses of vertebrae), kidney (frequently increased glomerular mesangial matrix, occasionally hyperplasia of parietal epithelium and periglomerular fibrosis), adrenals (decreased vacuolation with eosinophilia in the zona fasciculata), ovary (decreased number of maturing follicles, granulosa cells and/or theca cells), and nasal cavity (atrophy/loss of the septum and/or turbinates associated with necrotizing inflammation and various other epithelial, microvascular, cartilaginous and osseous findings). Other microscopic findings in the 6- month chronic toxicity study included vascular alterations in the choroid plexus and digestive tract, vascular degeneration and fibrosis in several tissues including the heart, and hepatic portal inflammation and periportal necrosis (Investigator's Brochure, 2017).

Ziv-aflibercept was shown to alter fertility parameters in sexually mature male and female monkeys treated for 6 months. These effects were noted at plasma exposures of free aflibercept that were close to exposures in patients at the recommended pharmacological doses and were considered to impact fertility in both males and females. Inhibition of the female reproductive function by anti-VEGF drugs is related to the key role of VEGF-mediated angiogenesis in follicular and luteal development. The effects of ziv-aflibercept on sexually mature male and female fertility were reversible (Investigator's Brochure, 2017).

The developmental effects of ziv-aflibercept were evaluated in an embryo-fetal study in rabbits. Ziv-aflibercept induced embryo-toxicity at maternotoxic doses and teratogenic effects at all doses tested. Ziv-aflibercept was locally well tolerated in rabbits receiving a single injection by the IV, intramuscular, and subcutaneous (SC) routes (Investigator's Brochure, 2017).

Ziv-Aflibercept Nonclinical Toxicology and PK studies

The safety pharmacology studies showed that ziv-aflibercept reduced vascular density in certain normal tissues in mice, increased blood pressure in rats and mice and delayed wound repair and healing in rabbits. Detectable effects on some of these parameters were noted at doses of ziv-aflibercept that were lower than the optimal pharmacological doses determined in tumor bearing mice. Maximal effects on wound healing and blood pressure were noted only at doses in the same range as pharmacological active doses. The increase in blood pressure reported in rodents was reversible and was reported in patients treated with ziv-aflibercept. The reversibility of the effect on wound repair and healing was not evaluated. These effects are related to the inhibition of the VEGF pathway. Ziv- aflibercept had no effects on respiratory function in rats or on thrombus formation in rabbits. Based on results obtained from toxicology studies, the absence of major effects on the central nervous system and cardiac function was established (Investigator's Brochure, 2017).

Summary of Ziv-Aflibercept Clinical Studies

As of 03 August 2016, the clinical development of ziv-aflibercept encompasses a total of 51 clinical oncology trials (31 Sanofi trials and 20 National Cancer Institute [NCI] trials) in more than 4385 cancer patients mostly with advanced solid malignancies as well as 2 clinical trials in 76 healthy subjects (PDY6655 and PDY6656). Doses have been administered up to 800 µg/kg SC twice weekly, 7 mg/kg IV every 2 weeks (q2w), and 9 mg/kg IV every 3 weeks (q3w) (Investigator's Brochure, 2017).

Ziv-aflibercept was initially administered SC in 2 single-agent dose-escalation safety and pharmacokinetic (PK) studies (TED6113/6114) in patients with advanced solid tumors or lymphomas. However, the SC route of administration was discontinued due to the high volume of injection required for the formulated ziv-aflibercept concentration of 25 mg/mL. Subsequently, only IV administration was used in all clinical studies (Investigator's Brochure, 2017).

Phase 1 single-agent dose escalation studies (TED6115/TED6116) evaluated safety, tolerability, PK and anti-tumor activity in patients with various solid tumor types. Phase 2 single-agent studies were designed to measure the effect of ziv-aflibercept in patients with ovarian cancers (ARD6122, ARD6772, and EFC6125), and non-small-cell lung adenocarcinoma ([NSCLA], ARD6123) (Investigator's Brochure, 2017).

Combination Phase 1 dose-escalation studies were conducted to evaluate safety, PK, and antitumor activity in patients with solid malignancies. The recommended Phase 2 dose (RP2D) of ziv-aflibercept was confirmed in these Phase 1 combination studies as 4 mg/kg every 2 weeks (q2w) when combined with chemotherapy administered every 2 weeks, as was initially determined in single-agent studies TED6115/TED6116.

Combination Phase 3 studies' primary objective was efficacy. In these studies, ziv-aflibercept was administered IV over 1 hour at 4 mg/kg q2w or 6 mg/kg q3w depending on the associated chemotherapy schedule (Investigator's Brochure, 2017).

In the q2w regimen in Phase 1 and Phase 3 combination studies, ziv-aflibercept was combined with standard doses of the following cytotoxic agents: oxaliplatin/5- fluorouracil/leucovorin (FOLFOX4), [TCD6117]), irinotecan/leucovorin/5-fluorouracil (irinotecan/LV5FU2, [TCD6118]), and gemcitabine or gemcitabine and erlotinib (TCD6121), gemcitabine (EFC10547/VANILLA), and irinotecan/5-FU/leucovorin (FOLFIRI, [EFC10262/VELOUR]). In the q3w regimen in Phase 1 and Phase 3 combination studies, ziv-aflibercept was combined with standard doses of docetaxel (Taxotere®)/cisplatin/5-fluorouracil (TCF, [TCD6119]), docetaxel or docetaxel/cisplatin or pemetrexed (TCD6120), and docetaxel (EFC10261/VITAL) (Investigator's Brochure, 2017).

Five Phase 3 combination studies have been conducted in the following indications: pancreatic (EFC10547/VANILLA), lung (EFC10261/VITAL) metastatic colorectal cancer ([MCRC], EFC10262/VELOUR and EFC11338/AFLAME), and prostate (EFC6546/VENICE). At the IB cutoff, all were completed (Investigator's Brochure, 2017).

In addition, 19 studies are being conducted with ziv-aflibercept under the sponsorship of the U.S. NCI cooperative group. Lastly, other studies have been conducted with a different formulation of aflibercept (VEGF-Trap Eye) in the ophthalmology indication (41 completed studies), under separate sponsorship. A biologics license application was authorized to Regeneron Pharmaceuticals for tradename EYLEA for treatment of patients with neovascular (wet) agerelated macular degeneration by the US FDA in November 2011(Investigator's Brochure, 2017).

Study EFC10262/VELOUR was a multinational, randomized, double-blind study, comparing the efficacy of ziv-aflibercept q2w versus placebo in patients with MCRC treated with irinotecan /5-FU combination (FOLFIRI) after failure of an oxaliplatin based regimen. The safety population consisted of 1216 MCRC patients who received either ziv-aflibercept plus FOLFIRI (611 patients) or placebo plus FOLFIRI (605 patients) (Investigator's Brochure, 2017).

The efficacy conclusion from EFC10262/VELOUR was that 4 mg/kg ziv-aflibercept administered as a 1-hour IV infusion q2w in combination with the FOLFIRI regimen demonstrated a statistically and clinically significant improvement in overall survival (OS), progression free survival (PFS) duration and a significantly higher overall response rate (ORR) in patients treated with ziv-aflibercept/FOLFIRI over those treated with placebo/FOLFIRI. These results were consistent across prespecified subgroups (gender, ethnic origin, age, body weight, creatinine clearance, Eastern cooperative oncology group [ECOG] performance status [PS] at baseline) and their robustness was confirmed by sensitivity analyses (Investigator's Brochure, 2017).

Overall, considering safety results from EFC10262/VELOUR, the addition of ziv-aflibercept to FOLFIRI gave no new toxicity signals but showed that: Some AEs of FOLFIRI were enhanced: These included grade 1 or 2 events such as diarrhea, asthenic conditions, decrease appetite, weight decrease, palmar plantar erythrodysesthesia syndrome, and hematological abnormalities (with the exception of anemia) and grade 3 or 4 events (>2% higher incidence in ziv-aflibercept/FOLFIRI arm compared to placebo/FOLFIRI arm) such as neutropenia,

hypertension, diarrhea, asthenic conditions, leukopenia, stomatitis and ulceration, infection, neutropenic complications and gastrointestinal (GI) and abdominal pains (Investigator's Brochure, 2017).

The risk of treatment-emergent adverse events (TEAEs) identified as possible anti-VEGF class specific AE (analyzed by grouped terms) were significantly increased and these were more frequently all grade hypertension and hemorrhage TEAEs. Considering analysis per prespecified subgroups during EFC10262/VELOUR, no unexpected clinical important difference was seen across the various analyzed subgroups (gender, race, age, of body weight, creatinine clearance, ECOG PS at baseline) with regard to incidences of TEAEs in patients treated with ziv-aflibercept/FOLFIRI. Similarly for the grouped terms of particular interest (hypertension, hemorrhage, venous thromboembolic events [VTEs], arterial thromboembolic events [ATEs], wound healing complications, renal failure events, acute drug reactions) regression analyses showed that interaction terms of treatment and the various intrinsic factors were not statistically significant indicating that the incidence of the selected grouped terms were not significantly different across the studied subgroups. The safety profile (either VEGF-blockade AEs or chemotherapy induced toxicities) in patients with renal or liver function impairment did not significantly differ when compared to that observed in patients with normal renal or liver function at baseline. Lastly, the safety profile of ziv-aflibercept combined with FOLFIRI was comparable between patients with or without prior bevacizumab (Investigator's Brochure, 2017).

Safety Issues

Overall, consistency was found between safety findings observed in the pivotal EFC10262/VELOUR trial and supporting safety data obtained from pooled analysis of single agent Phase 1 and Phase 2 studies (TED6115/6116, ARD6122, ARD6123, ARD6772, EFC6125), Combination Phase 1 studies analyzed side by side (TCD6117, TCD6118, TCD6119, TCD6120, TCD6121), and other Phase 3 studies analyzed individually (EFC10547/VANILLA, EFC10261/VITAL, and EFC6546/VENICE) (Investigator's Brochure, 2017).

In the pooled analysis of single-agent Phase 1 and Phase 2 studies for ziv-aflibercept, there was no apparent dose dependent relationship between 2 and the 4 mg/kg with regard to safety profile. The most frequently reported TEAEs by PT in both dose level groups were asthenia and fatigue, hypertension, headache, gastrointestinal pains (abdominal pain, abdominal pain upper), nausea, dysphonia, vomiting, arthralgia, decreased appetite, constipation, breathing abnormalities (cough, dyspnea), diarrhea, and arthralgia. All events occurred with an incidence of ≥20% overall, in the 2 and 4 mg/kg groups, except for dyspnea and cough in the 2 mg/kg group (17.0% and 11.6%, respectively), and for arthralgia in the 4 mg/kg group (18.2%) (Investigator's Brochure, 2017).

In combination studies for q2w and q3w regimens, in addition to the most commonly reported TEAEs in single-agent studies, the comparison of the rate of TEAEs (all grade) across Phase 1 combination studies (both regimens) showed the same categories of TEAEs (PT or HLT term ≥20%) than those observed in single-agent studies but with higher percentages when focusing on the expected background chemo-induced toxicities (such as diarrhea, nausea, vomiting,

decreased appetite, asthenic conditions [fatigue and anemia]). Infections percentage was also increased except in the gemcitabine and pemetrexed combinations. Other TEAEs were observed with an incidence ≥20% in Phase 1 combination studies in both regimen groups: Stomatitis and ulceration (HLT; except in the gemcitabine, pemetrexed and FOLFOX combinations), alopecia (only for combination with irinotecan/LV5FU in q2w regimen), pyrexia, palmar plantar erythrodysesthesia syndrome and weight decreased. In addition, some differences were observed between the 2 regimen groups depending on the background chemotherapy associated to ziv-aflibercept, such as dysgeusia, palmar-plantar erythrodysaesthesia, nail disorder in the q3w regimen studies (mainly in docetaxel-based combinations) specifically reported with an incidence ≥20% (Investigator's Brochure, 2017).

2.4 Rationale for Combination of MLN0128 (TAK-228) and VEGF-Trap (Ziv-Aflibercept)

Angiogenesis is one of the cardinal processes leading to invasion and metastasis of solid tumors. Synergistic effects of mTOR inhibitors combined with antiangiogenic agents have been widely researched and supported by extensive clinical and preclinical data. The inhibition of the mTOR pathway has been proven to exhibit antiangiogenic effects, further strengthening the capabilities of angiogenesis inhibitors such as ziv-aflibercept (Hanahan and Weinberg, 2011).

The angiogenic-signaling pathway may be triggered by the release of angiogenic promoters such as VEGF from tumor cells into the local microenvironment (Alvarez *et al*., 2012). VEGF is largely upregulated by the presence of hypoxia-inducible factor 1α (HIF-1α). HIF-1α mediates adaptive responses to hypoxic conditions. Increase in HIF-1α in response to a hypoxic environment is critical to the establishment and progression of many common cancers. Consequently, HIF-1-dependent activation of genes allows cancer cells to survive and metastasize.

Increased HIF-1α is associated with increased expression of VEGF, aggressive tumor growth, and poor patient prognosis. HIF-1α inhibition in combination with antiangiogenic therapy is a promising strategy for targeting tumor resistance (Naing, 2013). Agents that inhibit the mTOR pathway, like MLN0128 (TAK-228), have been shown to inhibit the activity of several angiogenic factors, including HIF-1α, which in turn results in decreased VEGF, thus decreased angiogenic activity (Majumder, *et al*., 2004). In a phase III study of everolimus in pancreatic neuroendocrine tumors, Yao *et al*. has demonstrated that mTOR inhibition may reduce circulating levels of sVEGFR1, PlGF, and bFGF (Yao *et al*., 2011). ***This important property of mTOR inhibitors, such as MLN0128 (TAK-228), makes them ideal candidates for combination therapy with ziv-aflibercept.***

Previous studies of combination bevacizumab and temsirolimus resulted in higher overall response rate; however, there were absolutely no differences in progression free survival and the toxicity profile of the combination of temsirolimus and bevacizumab. The full doses of each drug were much higher than anticipated and limited treatment continuation over time (Heng, 2011).

Our proposal of combining MLN0128 (TAK-228) and ziv-aflibercept have broader target

inhibition than bevacizumab/rapalog (TORC21, PlGF, etc.), and therefore the risk of toxicities could be higher. The advantage of our trial is it could prospectively define a few parameters for the measurement of tolerability, not just MTD. We will consider parameters such as rate of dose delay or dose reduction throughout treatment; rate of treatment discontinuation due to dose delay or dose reduction throughout treatment; and percentage of patients that can tolerate therapy through 2 months, 4 months, 6 months, etc.

Safety Issues

As of December 31, 2014, there had been five patients treated on this protocol. The first dose level explored was MLN0128 (TAK-228) 3 milligrams PO daily and ziv-aflibercept 3 milligram/kilogram intravenously every 2 weeks starting on Cycle 1 Day 1. The Grade1-2 possibly-related toxicities noted in the first 5 patients were as follows: arthralgia, mucositis, rash, hyperglycemia, hypercholesterolemia, hypertriglyceridemia, hypertension, abdominal pain, anorexia, weight loss, proteinuria, thrombocytopenia, elevated alanine aminotransferase, elevated aspartate aminotransferase, nausea, vomiting, dehydration, and diarrhea. The Grade 3 possibly-related events noted in the first 5 patients were pancreatitis (n=2) and rash (n=2). There have been 2 dose-limiting toxicities in the first dose level: rash maculo-papular and pancreatitis.

Based on internal data and discussion between CTEP and Millennium, MLN0128 (TAK-228) is better tolerated when given intermittently such as 3 days on and 4 days off, and the highest tolerated dose with that schedule has been 9 mg. Since we have seen dose-limiting toxicity on the first dose level, a more conservative approach has been implemented in the Amendment 06 dosing schema, in which patients will begin MLN0128 (TAK-228) on Day 2 of Cycle 1 and receive it for 3 days on and 4 days off. Ziv-aflibercept will continue to be given on Days 1 and 15 of each cycle.

2.5 Correlative Studies Background

Despite progress, the biologic activity of ziv-aflibercept may be difficult to assess because it may often seem primarily cytostatic rather than cytotoxic in function. When anti-VEGF agents are used in a monotherapeutic fashion, only a small number of patients have a major tumor response (Beaudry *et al*., 2005; Davis *et al*., 2003). To advance the clinical testing of ziv-aflibercept and the projected additional benefits of combination with MLN0128 (TAK-228), we plan to conduct biopsies. These studies are designed to determine if combining ziv-aflibercept and MLN0128 (TAK-228) will inhibit both the phosphorylation of effectors that are downstream of the targets of the drugs as well as potentiating anti- angiogenic activities.

For the expansion cohorts, we will be performing correlative studies. Our interventional radiologists will coordinate with our study radiologist. Our study radiologist will identify an optimal area for interventional radiologists to biopsy. Biopsies will be performed at baseline, on Cycle 1 Day 8, and at time of progression. All the treating investigators will be educated and trained to report to the principal investigator the first signs of progression.

Reverse Phase Protein Arrays (RPPA)

In collaboration with Dr. Funda Meric-Bernstam (Chair of the Department of Investigational Cancer Therapeutics; Professor, Department of Surgical Oncology, University of Texas MD Anderson Cancer Center), RPPAs will be performed on biopsy specimens, peripheral blood mononuclear cells (PBMC), and platelet-enriched plasma samples to quantitate protein expression, including phosphorylation, as well as markers of proliferation, cell cycle progression, angiogenesis and apoptosis. Expression/ phosphorylation of potential markers of response, including phospho-Akt (Thr308), phospho-Akt (Ser473), phospho-mTOR (Ser2448), total S6K1, phospho-S6K1 (Thr389), phospho-S6 (ser235/236), phospho-4E-BP1 (Thr70), phospho-4E-BP1 (ser65),; CD31, IGF-1R, insulin receptor will be assessed by RPPA. Array technology, robotics and robust algorithms are available to efficiently utilize the technologies as demonstrated in published work by Dr. Gordon Mills, Dr. Funda Meric-Bernstam, and their colleagues who have established and validated the technology at MD Anderson.

Immunohistochemistry (IHC) Analysis

In collaboration with Dr. Funda Meric-Bernstam, we will evaluate protein expression (both total and phosphorylated proteins) by IHC staining on slides from tumor samples. Expression of PI3K/mTOR signaling markers phospho-Akt (Ser473), phospho-S6 (ser235/236), phospho-4E-BP1 (ser65), as well as PTEN, SCD1, IGF-1R, and insulin receptor will be assessed (Figure 3). Microvessel density will also be evaluated in the tumor tissue using IHC and correlated with clinical tumor response and RPPA analyses. Pre- and post-treatment biopsies will be evaluated by H&E. Treatment-induced apoptosis will be assessed with IHC for cleaved caspase 3 and proliferation will be assessed by Ki- 67.

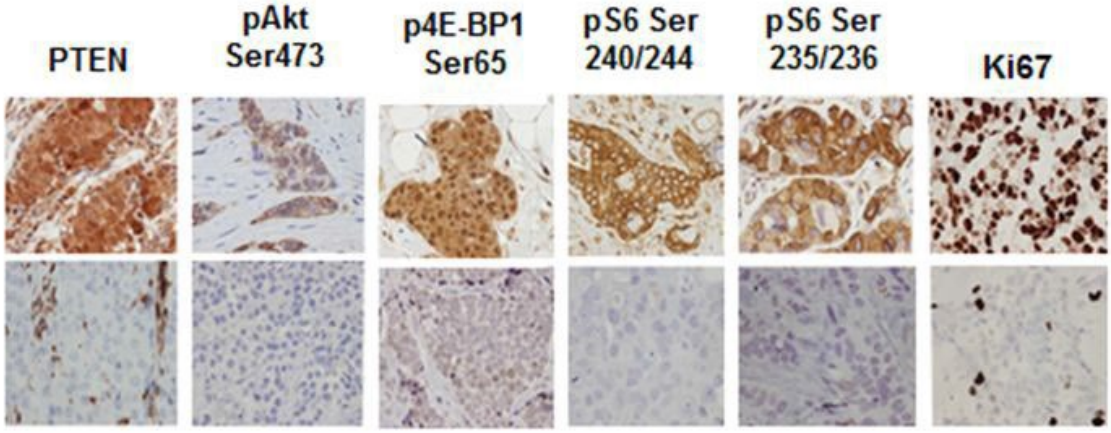


Figure 3. Examples of positive and negative staining PI3K/mTOR markers by IHC. IHC for PTEN, pAkt Ser473, p4E-BP1 Thr70, pS6 Ser235/236, pS6 Ser240/244 and Ki-67. Breast cancer samples with positive immunostaining are shown in the top panel, and samples with negative or low immunostaining are shown in the bottom panel. Data provided by Dr. Funda Meric-Bernstam.

In the event that tissue material is limited, we will give priority to RPPA analysis as RPPA is an extremely effective and sensitive method for measuring multiple proteins in cancer cells. This technique can be applied to minute amounts of material. Dr. Funda Meric-Bernstam and Dr. Gordon Mills have established this method, and we are confident that we can use this technology to measure all the markers of relevance to this study. However, when it is possible

to perform both RPPA and IHC for the same proteins, the results from one will validate the results from the other.

3. PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1 Patients with advanced or metastatic cancer that is refractory to standard therapy or relapsed after standard therapy. Patients must have histologically confirmed malignancy that is metastatic or unresectable and for which standard curative or palliative measures do not exist or are no longer effective.

3.1.2 Patients enrolled in the expansion cohort must have biopsiable disease. There will be preferential enrollment of patients with pancreatic neuroendocrine tumors, fibrolamellar hepatocellular carcinoma, or ovarian cancer during the dose expansion cohort.

3.1.3 Patients must be ≥4 weeks beyond treatment of any chemotherapy, other investigational therapy, hormonal, biological, targeted agents or radiotherapy, and must have recovered to ≤ grade 1 toxicity or previous baseline for each toxicity. Exception: Patients may have received palliative low dose radiotherapy to the limbs 1-4 weeks before this therapy provided pelvis, sternum, scapulae, vertebrae, or skull were not included in the radiotherapy field.

3.1.4 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of MLN0128 (TAK-228) in combination with ziv-aflibercept in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.5 ECOG performance status ≤1, see Appendix A.

3.1.6 Life expectancy of greater than 3 months.

3.1.7 Patients must have normal organ and marrow function as defined below:

- leukocytes ≥3,000/mcL
- absolute neutrophil count ≥1,500/mcL
- platelets ≥100,000/mcL
- hemoglobin ≥ 9 g/dL
- total bilirubin ≤1.5 × institutional upper limit of normal AST(SGOT)/ALT(SGPT) ≤2.5 × institutional upper limit of normal
- creatinine ≤1.5 × institutional upper limit of normal OR

- creatinine clearance ≥60 mL/min for patients with creatinine levels above institutional normal.
- Fasting serum glucose ≤ 130 mg/dL
- Fasting triglycerides ≤ 300 mg/dL
- HBA1C < 7.0%

3.1.8 Patients must have evaluable or measureable disease by RECIST 1.1.

3.1.9 Women of child-bearing potential MUST have a negative serum or urine pregnancy test within 7 days unless prior hysterectomy or menopause (defined as 12 consecutive months without menstrual activity). Patients should not become pregnant or breastfeed while on this study. The effects of MLN0128 (TAK-228) and ziv-aflibercept on the developing human fetus are unknown. For this reason, women of child-bearing potential must agree to use 1 highly effective method of contraception and 1 additional effective (barrier) method, at the same time, from the time of signing the informed consent through 90 days (or longer, as mandated by local labeling [eg; USPI, SmPC, etc;]) after the last dose of study drug; or agree to practice true abstinence.
Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.
Male patients, even if surgically sterilized (i.e., status post-vasectomy), who:

- Agree to practice highly effective barrier contraception during the entire study treatment period and through 120 days after the last dose of study drug, or
- Agree to completely abstain from heterosexual intercourse.

3.1.10 Ability to understand and the willingness to sign a written informed consent document.

3.1.11 Ability to swallow oral medications

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered to </= grade 1 adverse events due to agents administered more than 4 weeks earlier.

3.2.2 Patients who are receiving any other investigational agents.

3.2.3 Patients with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to MLN0128 (TAK-228) or ziv-aflibercept.

3.2.5 Uncontrolled intercurrent illness including active infection.

3.2.6 Pregnant women are excluded from this study because MLN0128 (TAK-228) and ziv-aflibercept are agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with MLN0128 (TAK-228) and ziv-aflibercept, breastfeeding should be discontinued if the mother is treated with MLN0128 (TAK-228) and ziv-aflibercept. These potential risks may also apply to other agents used in this study.

3.2.7 Patients with known human immunodeficiency virus infection are not to be enrolled in the study.

3.2.8 History of abdominal fistula, gastrointestinal perforation or intra-abdominal abscess within 28 days or manifestations of malabsorption due to prior gastrointestinal (GI) surgery or GI disease that may alter the absorption of MLN0128 (TAK-228).

3.2.9 New York Heart Association Class III or greater congestive heart failure within last 6 months or uncontrolled hyperlipidemia (cholesterol > 300 mg/dl; triglyceride 2.5XULN despite lipid lowering agent) within last 3 months, see Appendix B.

3.2.10 Uncontrolled diabetes (fasting serum glucose>130 mg/dl) despite best medical management or poorly controlled diabetes mellitus defined as HbA1c > 7%; subjects with a history of transient glucose intolerance due to corticosteroid administration are allowed in this study if all other inclusion/exclusion criteria are met.

3.2.11 History of uncontrolled hypertension, defined as blood pressure > 150/95 mmHg, or systolic blood pressure >180 mmHg when diastolic blood pressure < 90 mmHg, on at least 2 repeated determinations on separate days within 3 months prior to study enrollment.

3.2.12 Urine protein should be screened by dipstick or urine analysis. For proteinuria > 1+ or urine protein: creatinine ratio > 1.0, 24-hour urine protein should be obtained and the level should be < 2000 mg for patient enrollment.

3.2.13 Patients on anticoagulant therapy with unstable dose of warfarin and/or having an out-of- therapeutic range INR (>3) within the 4 weeks prior to drug administration.

3.2.14 Evidence of clinically significant bleeding diathesis or underlying coagulopathy, non- healing wound.

3.2.15 History of any of the following within the last 6 months prior to study entry:

- Ischemic myocardial event, including angina requiring therapy and artery revascularization procedures

- Ischemic cerebrovascular event, including TIA and artery revascularization procedures
- Requirement for inotropic support (excluding digoxin) or serious (uncontrolled) cardiac arrhythmia (including atrial flutter/fibrillation, ventricular fibrillation or ventricular tachycardia)
- Placement of a pacemaker for control of rhythm
- Pulmonary embolism

3.2.16 Significant active cardiovascular or pulmonary disease at the time of study entry, including:

- Uncontrolled high blood pressure (i.e., systolic blood pressure >150 mm Hg, diastolic blood pressure > 95 mm Hg)
- Pulmonary hypertension
- Uncontrolled asthma or O2 saturation < 90% by pulse oximetry on room air
- Significant valvular disease; severe regurgitation or stenosis by imaging independent of symptom control with medical intervention, or history of valve replacement
- Medically significant (symptomatic) bradycardia
- History of arrhythmia requiring an implantable cardiac defibrillator

3.2.17 Baseline prolongation of the rate-corrected QT interval (QTc) (e.g. repeated demonstration of QTc interval >480 milliseconds, or history of congenital long QT syndrome, or torsades de pointes)

3.2.18 Psychiatric illness/social situations that would limit compliance with study requirements

3.2.19 Have initiated treatment with bisphosphonates less than 30 days prior to the first administration of MLN0128 (TAK-228). Concurrent bisphosphonate use is only allowed if the bisphosphonate was initiated at least 30 days prior to the first administration of MLN0128 (TAK-228).

3.2.20 Patients who are taking proton pump inhibitor (PPI) within 7 days before receiving the first dose of study drug or who require treatment with PPIs throughout the trial or those who are taking H2 receptor antagonists within 24 hours of the first dose of study drug.

3.2.21 Patients with known history of hepatitis B surface antigen-positive, or known history or suspected active hepatitis C infection are not to be enrolled in the study.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

Not applicable to this study.

4.1 General Guidelines

Not applicable to this study.

4.2 Registration Process

Not applicable to this study.

5. TREATMENT PLAN

This is a phase I, single center, open-label, dose-escalation study of MLN0128 (TAK-228) and ziv-aflibercept administered in combination to patients with metastatic or locally advanced solid tumors. Additional sites may be added upon activation of NCI Early Experimental Therapeutics Network in consultation with CTEP.

This study will follow a standard 3+3 design. Three patients will be treated per dose level. If none of the patients experience a dose-limiting toxicity (DLT), the next cohort of three patients will be treated at the next higher dose level. If 1 of 3 patients treated at a dose experiences a DLT, that cohort will then be expanded to a total of six patients. If a DLT occurs in only 1 in 6 of the first dose level, 3 more patients may be enrolled at the next higher dose level (i.e., cohort 2). If 2 or more of 6 patients in any cohort experiences DLT, the MTD is considered to have been exceeded. No patients may be enrolled in the next dose level until 3 patients at the previous dose level have completed at least one cycle of therapy. Subjects enrolled in the dose escalation cohort who do not receive 75% of each drug in the first cycle of treatment, for reasons other than DLT, will be considered not evaluable for DLT and will be replaced. Those patients that are not likely to comply with the protocol in the future may be replaced.

Once the MTD is determined, or at the maximum tolerated dose level explored (Level 3) (if MTD is not reached at dose level 3), an additional 10 patients may enroll in the dose expansion cohort. Patients enrolled in this cohort must have biopsiable disease. We will be emphasizing enrollment of pancreatic neuroendocrine tumor (PNET), fibrolamellar hepatocellular carcinoma, and ovarian cancer patients.

5.1 Agent Administration

All protocol-specific criteria for administration of MLN0128 (TAK-228) must be met and documented before drug administration. The study drug will be administered only to eligible

patients under the supervision of the investigator or identified subinvestigator(s).

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

One cycle consists of 4 weeks of treatment (28 days). MLN0128 (TAK-228) will be taken orally for 3 days on and 4 days off starting on Cycle 1 Day 2 (and starting Day 2 of every cycle), and ziv-aflibercept will be given via intravenously (IV) infusion once every 2 weeks (days 1 and 15 for each cycle). Both study drugs will have a +/- 2 day window. If the ziv-aflibercept dosing is rescheduled, then the MLN0128 (TAK-228) dosing will be adjusted accordingly.

In Cycle 1, MLN1028 (TAK-228) and ziv-aflibercept will not be given on the same day. In Cycle 2 and beyond, every effort will be made so that MLN0128 (TAK-228) and ziv-aflibercept will not be given on the same day. The starting dose is Dose Level 1, which is MLN0128 (TAK-228) 4 mg oral for 3 days on and 4 days off and ziv-aflibercept 2 mg/kg every 2 weeks. If Dose Level 1 is beyond the MTD, the dose will be de-escalated to Dose Level -1. If Dose Level -1 is beyond the MTD, then the PI will discuss with NCI-CTEP.

Patients will be restaged at the end of every two cycles. Patients may be allowed to continue treatment after 2 cycles if there is continued clinical response or disease stabilization, and patients do not have significant toxicities. There is no maximum number of cycles a patient may receive if the patient is benefitting clinically.

Dose Escalation Schedule		
Dose Level	**Dose***	
	MLN0128 (TAK-228) (mg PO for 3 days on and 4 days off) Starting on Cycle 1 Day 2	**Ziv-Aflibercept (mg/kg IV Q 2 weeks) Starting on Cycle 1 Day 1**
Level -1	3	2
Level 1	4	2
Level 2	4	3
**Doses are stated as exact dose in units (*e.g.*, mg/m^2, mcg/kg, etc.) rather than as a percentage.* *As of Amendment 13/ Version 07, all newly enrolled patients will be given the milled formulation of MLN0128 (TAK-228), and a new dose escalation is being explored. Starting with Amendment 14/Version 08, the dose escalation will be re-started at Dose Level 1 with the updated dose level escalation.* *Both study drugs will have a +/- 2 day window. If the ziv-aflibercept dosing is rescheduled, then the MLN0128 (TAK-228) dosing will be adjusted accordingly.*		

Regimen Description

Agent	Premedications; Precautions	Dose	Route	Schedule	Cycle Length
MLN0128 (TAK-228)	Patient should refrain from eating 2 hours before and 1 hour after. May take with 5- HT3 antagonist for antinausea 30 min. prior to dosing (see Section 5.3 Guidelines for Supportive Care)	**Gelatin capsule	PO in the AM	3 Days on and 4 Days off, Starting on Day 2 of every cycle	28 days (4 weeks)
Ziv-Aflibercept	No premedications needed. *** Need 0.2 micron polyethersulfone filter (PVC with DEHP or polyurethane)	** in NS or D5W Conc. Between 0.6 – 8 mg/mL	IV over 60 minutes	Days 1 and 15	
***Doses as appropriate for assigned dose level.* ****May give premeds in subsequent doses after 1st dose if infusion related side effects (premeds may consist of diphenhydramine, steroids)*					

The patient will be requested to maintain a medication diary of each dose of medication (Appendix D). The medication diary will be returned to clinic staff at the end of each course.

MLN0128 (TAK-228) will be administered on an empty stomach. Patients should be instructed to refrain from eating and drinking (except for water and prescribed medications) for 2 hours before and 1 hour after each dose.

Patients should be instructed to take their study medication at approximately the same time on each scheduled dosing day and not to take more than the prescribed dose at any time. Patients should swallow the study medication whole and not chew it, open it, or manipulate it in any way before swallowing. If a patient does not take their MLN0128 (TAK-228) dose within the time frame specified (±24 hours of the QW scheduled dosing time, or +/- 12 hours of the QD×3 days per week schedule), then the dose should be skipped and considered a missed dose. Patients should record any missed doses in their diary and resume drug administration at the next scheduled time with the prescribed dosage. Under no circumstance should a patient repeat a dose or double-up doses.

5.1.1 MLN0128 (TAK-228)

MLN0128 (TAK-228) drug product will be supplied in the form of gelatin capsules and taken orally by mouth at approximately the same time every morning. Most commonly reported drug-related AEs included diarrhea (24%), fatigue (23%), vomiting (19%), rash (19%), dehydration (19%), hyperglycemia (19%), mucosal inflammation (16%), asthenia (15%), dysgeusia (13%), thrombocytopenia (11%), stomatitis (10%) and blood creatinine increased (8%).

Patients should take MLN0128 (TAK-228) with 8 ounces of water and should refrain from eating (except water and prescribed medications) for 2 hours before and 1 hour after each dose. Patients may also use 5-HT3 antagonist such as ondansetron, phenothiazines such as promethazine or prochlorperazine, benzodiazepines such as lorazepam as premedications for MLN0128 (TAK-228) per supportive care guideline under Section 5.3. Patients will be counseled to take medication with full glass of water and to keep well hydrated during the day

to prevent hyperglycemic episodes and prevent renal impairment. MLN0128 (TAK-228) is metabolized primarily via CYP1A2, with minor contribution from CYP3A4. Strong CYP1A2 inhibitors and CYP inducers should be administered with caution and at the discretion of the investigator during the study.

In cases where a subject misses dosing at his/her dosing time, the subject may still take the dose within 12 hours of the regular dosing time (subjects should not take 2 consecutive daily doses within 12 hours of each other). Subjects who vomit after receiving MLN0128 (TAK-228) will not receive a replacement dose within 24 hours. If confirmed that the study drug has been vomited, the dose should be noted as having been missed.

If severe emesis or mucositis prevents the patient from taking scheduled doses, that dose will be skipped. If emesis occurs after study medication ingestion, the dose will not be readministered, and patients should resume dosing at the next scheduled time with the prescribed dosage. Patients should record the occurrence of the emesis in their dosing diaries. Under no circumstance should a patient repeat a dose or double-up doses.

5.1.2 Ziv-Aflibercept

Ziv-Aflibercept solution for injection is available as a 200 mg vial (25 mg/mL). It is compatible in either 0.9% sodium chloride or 5% dextrose solutions and is not to be mixed with other drugs. Ziv-Aflibercept solutions at 0.6 to 8 mg/mL are stable for up to 24 hours under refrigerated conditions (2 to 8°C) or up to 8 hours at ambient temperature (approximately 25°C) in polypropylene syringes or in infusion bags made of polyvinyl chloride (PVC) containing di(2-ethylhexyl)phthalate (DEHP) or polyolefin (PVC free DEHP free). Diluted solutions of ziv-aflibercept should be administered using infusion tubing made of PVC containing DEHP, DEHP free PVC containing tris(2-ethylhexyl) trimellitate (TOTM), polypropylene, polyethylene lined PVC, or polyurethane. The infusion sets must contain a 0.2 μm polyethersulfone inline filter. Polyvinylidene fluoride (PVDF) filters and Nylon filters should not be used. Infusion can be conducted by gravity, with an IV infusion pump, or with a syringe pump using administration sets made of the above materials. The infusion should not exceed two hours at room temperature (approximately 25°C).

The most common treatment-emergent adverse events reported on IV VEGF Trap treatment were fatigue, pain, and constipation. Potentially drug-related severe adverse events encountered to date have included hypertension, fatigue, dyspnea, nausea/vomiting, headache, arthralgia, hoarseness, and lower extremity deep vein thrombosis.

There are no premedications recommended for ziv-aflibercept. However, if patients experience first dose infusion related side effects, subsequent doses may be given with antihistamines, H2 antagonists, and steroids, per institutional standards for drug infusion reactions.

5.2 Definition of Dose-Limiting Toxicity

Management and dose modifications associated with the above adverse events are outlined in Section 6.

Dose escalation will proceed within each cohort according to the following scheme. Dose-limiting toxicity (DLT) is defined below.

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

Definition of DLT

CTCAE version 5.0 will be utilized. DLTs will be based on events occurring during the first cycle of study drug administration (DLT window is 1 cycle= 28 days). If multiple toxicities are seen, the presence of DLT shall be based on the most severe toxicity experienced.

Patients who experience an AE that meets the definition of a DLT during or after completing Cycle 1 should have their study drug treatment interrupted. If the event resolves to grade 1 or baseline values within 3 weeks of interrupting planned therapy, and in the opinion of the investigator the benefits of continuing treatment outweigh the risks posed by the toxicity, patients may continue study treatment with MLN0128 (TAK-228) dose reduction.

DLTs are defined as adverse events (AEs) related to study agents with an attribution of possible, probably, or definite and fulfilling one of the following criteria:

Hematologic

- Grade 4 neutropenia lasting > 7 days
- Febrile neutropenia (defined as absolute neutrophil count [ANC] < 1.0 x 10^9/L and fever ≥ 38.5º C) or documented grade ≥ 3 infection with ANC ≤ 1.0 x 10^9/L.
- Platelet count < 25,000/mm lasting > 7 days

- Delay of treatment > 7 days due to hematologic toxicity

Non-hematologic

- Any toxicity ≥ grade 3 that persists for > 7 days, **except:**
  1. nausea/vomiting, diarrhea and electrolyte imbalances;
  2. grade 3 lab abnormalities that are asymptomatic and responsive to supportive measures and that are without clinical consequence; and
  3. grade 3 hyperglycemia or grade 3 diabetes that can be stably controlled.
- Delay of treatment > 14 days due to non-hematologic toxicity

EXCEPTION:
Patients who experience grade 3 hypertension that resolves within 14 days with medical management will not be defined as a DLT.

Patients who experience proteinuria that resolves to <2 g within 14 days will not be defined as a DLT.

Hypersensitivity/Allergic reactions with expected severity and presentation will not be considered a DLT

Patients who withdraw from the study prior to completion of the first cycle of study treatments for reasons other than treatment-related adverse events will be replaced.

Patients can have treatment withheld for up to 1 cycle (28 days) for reasons other than toxicity (i.e. social reasons).

5.3 General Concomitant Medication and Supportive Care Guidelines

Subjects must not receive any other investigational drugs or any other antineoplastic therapy including radiotherapy while participating in this study. Supportive treatment such as antiemetics, antidiarrheals, analgesics, blood products, etc., may be used at the investigator's discretion. Concurrent palliative radiation may be permitted after discussing with CTEP.

5.3.1 Concomitant Medications

The PI or pharmacist or PI delegate will go over and check the list of medication for individual patients.

Because there is a potential for interaction of MLN0128 (TAK-228) with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes. Appendix C presents guidelines for identifying medications/substances that could potentially interact with the study agent(s).

Strong CYP1A2 inhibitors and CYP inducers should be administered with caution, at the discretion of the investigator. Alternative treatments, if available, should be considered. Histamine H2 receptor antagonists may be allowed, if needed provided that the histamine H2 receptor antagonist is not taken within 12 hours before and within 6 hours after study drug administration. Patients receiving histamine H2 receptor antagonists before enrollment must stop using these medications for at least 24 hours before their first dose of study drug. Examples of histamine H2 receptor antagonists include ranitidine, famotidine, and nizatidine. Cimetidine, a moderate cytochrome P450 (CYP)1A2 inhibitor, is not recommended as a first choice H2 receptor antagonist. Strong CYP1A2 inhibitors and CYP inducers should be administered with caution, at the discretion of the investigator (see Appendix C). Alternative treatments, if available, should be considered.

The use of live vaccines and close contact with those who have received live vaccines should be avoided during treatment with study drug. Examples of live vaccines are: intranasal influenza, measles, mumps, rubella, oral polio, Bacille Calmette-Guerin, yellow fever, varicella, and TY21a typhoid vaccines.

Systemic corticosteroids (either IV or oral steroids, excluding inhalers) should be excluded unless necessary for treatment a TAK-228-related AE (eg, rash) as recommended by the treating physician.

Neutralizing antacid preparations (acid neutralizers) and calcium supplements are not permitted during Cycle 1 on study drug administration days in the phase 1/1b portion of the study, but may be taken as needed on non-TAK-228 administration days. However, for all other cycles, administration of neutralizing antacids and calcium preparations is permitted except from 4 hours before until 2 hours after TAK-228 administration. Some anti-gas preparations may also have antacid properties, and should also not be permitted from 4 hours before until 2 hours after study drug administration.

5.3.2 Supportive Care

- **Hypersensitivity reactions with any dose**: Stop infusion of ziv-aflibercept for 30-60 minutes. Medicate with diphenhydramine 25 – 50 mg IV (or a similar antihistamine) and steroids such as hydrocortisone 50-100 mg IV or dexamethasone 10-20 mg IV approximately 30 minutes before re- starting the ziv-aflibercept. If the subject re-develops a hypersensitivity reaction despite treatment with diphenhydramine and steroids, the infusion should be stopped for another 30 – 60 minutes, depending upon the severity of the reaction. The infusion may be resumed by administering a histamine H2-receptor antagonist approximately 30 minutes before restarting the ziv-aflibercept infusion and meperidine 25-50 mg IV if patient still had chills and rigors. Famotidine 20 mg IV or ranitidine 50 mg IV are recommended rather than cimetidine because of the lack of likely metabolic/pharmacologic interactions with the former drugs. The rate of the ziv-aflibercept infusion may also be slowed from 60 minutes to 2 hours. Also, it is permissible to premedicate overnight with steroids such as dexamethasone or prednisone. All subjects should be monitored while receiving the ziv-aflibercept infusion and emergency medical equipment and health care personnel must be readily available to respond to hypersensitivity reactions or other medical emergencies.

- Nausea/vomiting induced by study agents should be treated with a serotonin receptor antagonist (5HT3) ondansetron 8 mg PO 30 min. prior to dosing then Q 8 hours prn, or using phenothiazine derivatives such as prochlorperazine 10 mg PO Q 8 hours prn or promethazine 25 mg PO Q 6 hours prn as breakthrough. If this is inadequate, a benzodiazepine such as lorazepam or corticosteroid such as dexamethasone (except with MLN0128 (TAK-228)) or prednisone should be added until acute nausea is controlled. After acute nausea has resolved, consideration should be given to initiation of prophylactic antiemetic therapy. If nausea recurs despite reasonable medical intervention (as outlined above), dose reduction will be needed.

- Diarrhea should be managed with loperamide: 4 mg at first onset, then 2 mg every 2-4 hours until diarrhea is controlled (maximum = 16 mg loperamide/day). Diphenoxylate/Atropine (Lomotil™) may be added and alternate with loperamide.

- Hyperlipidemia may be treated with appropriate lipid lowering agents.

- Management of Hyperglycemia
  On the basis of the clinical experience in TAK-228 trials, most episodes of hyperglycemia observed occurred within the first 60 days after initiation of treatment with TAK-228 and have been either Grade 1 or Grade 2, and have responded quickly to oral metformin. Hyperglycemia has not been dose-limiting since the institution of a standard regimen for early treatment of hyperglycemia.

  All patients developing hyperglycemia during the study should have their glucose closely monitored by study staff and will be referred to our endocrinologist collaborator for management. The investigator may choose to continue close monitoring of patients who develop Grade 1 hyperglycemia (fasting glucose >ULN ≤160 mg/dL) or, alternatively, consider initiating treatment with an oral hypoglycemic agent, such as metformin. All patients with ≥Grade 2 hyperglycemia (fasting glucose >160 mg/dL) must be treated aggressively with oral hypoglycemic agents and/or insulin as clinically indicated. The investigator should consult an endocrinologist, if needed, to aid in optimizing the patient's hyperglycemia treatment plan.

  It is recommended that patients with elevated fasting blood glucose be initially treated with a fast acting insulin sensitizer such as metformin at 500 mg orally QD, and titrate up to a maximum of 1000 mg orally BID as needed. Concurrent addition to metformin of DPP-4 inhibitors (eg, sitagliptin or vildagliptin) and/or insulin should also be considered. Oral sulfonylureas (eg, glipizide or glyburide) should be used with caution, due to the higher risk of inducing hypoglycemia in patients. The dose of oral hypoglycemic agents should be adjusted in patients with renal insufficiency. In addition, patients should be encouraged to follow a low carbohydrate diet once hyperglycemia is first observed.
  If any fasting serum glucose reading performed at the site indicates hyperglycemia (>ULN or ≥110 mg/dL), the study staff should first confirm that the patient was fasting at the time of blood specimen collection (ie, nothing by mouth for at least 8 hours before collection).

- In-Home Daily Fasting Blood Glucose (FBG) Monitoring
  In addition to obtaining fasting glucose levels at the clinic visits as outlined in the Schedule of Events, all patients will be given a glucometer to monitor their daily FBG levels at home. The level should be collected daily, predose on dosing days, and at approximately the same time each day.

  On Cycle 1 Day 1, the patient will be provided an in-home glucometer. Patients should be trained on proper use of the glucometer and instructed to collect a daily FBG level every morning (predose on dosing days), starting on Cycle 1 Day 2. Patients will be instructed to bring the glucometer with them to each study visit so that the data collected can be reviewed and recorded in the source documents (Appendix E). Investigators will be responsible for reviewing the home glucose monitoring logs for hyperglycemia. The patient will be instructed to contact the site immediately if the value is abnormal (ie, ≥150 mg/dL) for further instructions on the management of their hyperglycemia. Hyperglycemia observed during home glucose monitoring should be confirmed in the clinic.

  If no irregularities in the fasting blood glucose level are observed during a minimum of 2 consecutive months, then the frequency of in-home fasting blood glucose testing can be reduced to a minimum frequency of once weekly, depending on the investigator's judgment and approval. Patients will continue to notify the investigator of fasting blood glucose levels that exceed 150 mg/dL and, if blood glucose levels are not well controlled, or if the patient requires either oral hypoglycemic agents or insulin to control blood glucose levels, then the frequency of in-home testing of FBG levels will be reinstated to daily.

- Suggested management of mucositis:
  - Xyloxylin (1:1:1 ratio) (diphenhydramine, Maalox, lidocaine) 10 mL swish/swallow QID as needed
  - Caphosol (sodium phosphate) 15 mL swish/spit every 4 hours as needed Valacyclovir 500 mg po TID for treatment
  - Biotene MW every 4 hours as needed
  - Carafate 1 Gm/10 mL 10 mL swish/swallow or spit QID as needed
  - Glutamine swish/swallow BID as needed (1 scoop (4 grams) mixed with water)

- Routine supportive measures for cancer patients such as erythropoietin, analgesics, blood transfusions, antibiotics, bisphosphonates, and hematopoietic colony stimulating factors for treatment of cytopenias are permitted.

- The administration of anticancer therapies, other investigational agents, or prophylactic use of hematopoietic colony stimulating factors are not permitted during Cycle 1; however, Hematopoietic colony stimulating factors may be used in subsequent cycles per guidelines and PI discretion. Accepted standards such as the American Society of Clinical Oncology (ASCO) Guidelines on the Use of Hematopoietic Colony-Stimulating Factors should be followed.

5.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression per RECIST criteria,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.5 Duration of Follow Up

Patients will be followed for 4 weeks (+/- 7 days) after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. Every attempt will be made to adhere to 4 weeks; however, the follow-up visit will depend on the patient's ability to return and at the physician's discretion.

5.6 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

Subjects will be carefully instructed to notify the Principle Investigator or study coordinator at the first appearance of any toxicity. The study coordinator will speak with subjects on clinic visit days to determine the degree and duration of any toxicity. Toxicity will be evaluated using the CTCAE version 5.0. Subject data will be analyzed for evidence of cumulative toxicity with repeated courses of therapy.

Please note, per the physician's discretion, a patient may continue MLN0128 (TAK-228) alone if the patient is receiving clinical benefit, but has to stop ziv-aflibercept for toxicity reasons.

Dose modifications for toxicity (in individual patients) relative to **MLN0128 (TAK-228) in Dose Level 1 and 2:**

MLN0128 (TAK-228) (mg PO 3 days on and 4 days off starting Day 2 of every Cycle)
3 mg
Discontinue

If patients in MLN0128 (TAK-228) Dose Level -1 require dose modification, those patients will have drug discontinuation.

Dose modifications for toxicity (in individual patients) relative to **ziv-aflibercept in Dose Level 3 through Dose Level 1:**

Ziv-Aflibercept (mg/kg IV Q 2 weeks)
2
Discontinue

If patients in ziv-aflibercept Dose Level -1 require dose modification, those patients will have drug discontinuation.

Intra-patient dose (re-)escalation (after toxicity induced reduction) will be allowed on a case-by-case basis and after discussion and approval by CTEP.

Treatment Modification (in individual patients):

Nausea/Vomiting	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**

≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose. Maximize anti-emetic therapy. Consider IV fluid hydration.	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Maximize anti-emetic therapy. Initiate tube feeding, IVF or TPN. If experienced for ≤72 hours, hold TAK-228 until ≤Grade 1, then resume TAK-228 without dose modification. If experienced for >72 hours despite optimal therapy, hold TAK-228 until ≤ Grade 1, then resume treatment with the dose of TAK-228 reduced by 1 level.	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Maximize anti-emetic therapy. Initiate tube feeding, IVF or TPN. If experienced for ≤72 hours, hold TAK-228 until ≤Grade 1, then resume TAK-228 without dose modification. If experienced for >72 hours despite optimal therapy, hold TAK-228 until ≤ Grade 1, then resume treatment with the dose of TAK-228 reduced by 1 level.	Off protocol therapy
*Patients requiring a delay of >2 weeks should discontinue ziv-aflibercept, but may continue MLN0128 (TAK-228). **Patients requiring > two dose reductions should go off protocol therapy.		
Prevention/Prophylaxis: Prophylactic use of anti-emetic, antinausea, and antidiarrheal medications are encouraged and may be used before each TAK-228 dosing as needed throughout the study. IV=intravenous, IVF=intravenous fluids, TPN=total parental nutrition.		

Diarrhea	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.		
Recommended management: Loperamide antidiarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used.		

Neutropenia	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**

≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.		

Thrombocytopenia	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.		

Mucositis/ Stomatitis	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at one dose level lower.	Hold until ≤ Grade 1. Resume at one dose level lower.
Grade 3*	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4*	Off protocol therapy	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.		
Recommended management: See Section 5.3 for supportive care and suggested management of mucositis.		

Hypertension	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
≤ Grade 1	No change in dose***	No change in dose***
Grade 2	No change in dose***	No change in dose***
Grade 3*	If hypertension is controlled within 2 weeks of the delay, resume MLN0128 (TAK-228) at same dose level.	Delay the administration of ziv-aflibercept for a maximum of 2 weeks, until recovery to blood pressure (BP) ≤ 150/100 or to systolic BP < 180 if diastolic BP < 90 for patients with known history of isolated systolic hypertension: •If BP is controlled within 2 weeks delay: -First episode: re-administer ziv-aflibercept at one dose level lower. - Second episode: re-administer ziv-aflibercept with reduced to two dose levels lower. -Third episode, discontinue ziv-

		aflibercept. •In case of re-occurrence of grade 3 BP despite dose reduction of ziv-aflibercept, or if BP is still uncontrolled despite 1 omission of administration of ziv-aflibercept, the patients will be permanently discontinued from ziv-aflibercept. MLN0128 (TAK-228) will be continued if the investigator thinks the patient is benefiting from it.
Grade 4*	If hypertension is controlled within 2 weeks of the delay, resume MLN0128 (TAK-228) at same dose level.	Seek cardiologist opinion, and permanently discontinue.
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy. ***Initiate antihypertensive drug therapy and close monitoring of blood pressure, as needed.		

Proteinuria	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
UPCR*** [0-1]	No change in dose	No change in dose
UPCR [1-2] Absence of Hematuria	No change in dose	Perform 24h urine: • If ≤3.5g/24h: o Resume at one dose level lower o >2g/24h prior current dosing then omit dosing and repeat 24h urine, if: ▪ ≤2g/24h prior current dosing, resume at one dose level lower ▪ >2g/24h prior current dosing, discontinue • If >3.5g/24h and previous 24h urine was: o ≤2g/24h prior current dosing then resume at one dose level lower o >2 ≤ 3.5g/24h prior current dosing then omit dosing and repeat 24h urine, if: ▪ ≤2g/24h prior current dosing, resume at one dose level lower ▪ >2g/24h prior current dosing, discontinue o >3.5g/24h prior current dosing then discontinue
UPCR [1-2] Presence of Hematuria OR UPCR >2	No change in dose	Omit dosing ziv-aflibercept then perform 24h urine • Full nephrologic work-up and seek nephrologist opinion • TMA*** not suspected and ≤2g/24h prior current dosing o Dose ziv-aflibercept

Proteinuria	Management/Next Dose for MLN0128 (TAK-228)	Management/Next Dose for Ziv-Aflibercept
		○ If second occurrence, resume at one dose level lower • TMA not suspected and >2 ≤ 3.5g/24h prior current dosing, omit dosing and perform 24h urine, if: ○ ≤2g/24h prior current dosing, then resume at one dose level lower ○ >2g/24h prior current dosing, then discontinue • TMA not suspected and >3.5g/24h prior current dosing****, discontinue • TMA diagnosed, discontinue and seek nephrologist opinion
Nephrotic Syndrome	If proteinuria is controlled within 2 weeks of the delay, resume MLN0128 (TAK-228) at same dose level	Off protocol therapy
Note: Patients requiring a delay of >2 weeks should discontinue ziv-aflibercept, but may continue MLN0128 (TAK-228). Patients requiring > two dose reductions should go off protocol therapy. ***UPCR: Urinary protein creatinine ratio; TMA: Thrombotic micro-angiopathy ****When patient is already treated at lowest dose level, ziv-aflibercept should be discontinued.		

Hyperglycemia	Management/Next Dose for MLN0128 (TAK-228)	Management/Next Dose for Ziv-Aflibercept
≤ Grade 1	No change in dose; Continue close monitoring of blood sugars. Initiate oral hypoglycemic agent.	No change in dose
Grade 2	No change in dose; Initiate oral hypoglycemic agent and/or insulin if not well controlled on oral agent	No change in dose
Grade 3	Hold drug until ≤ Grade 2. Resume MLN0128 (TAK-228) based on timing of recovery: ≤ 1 week: resume at same dose and schedule; >1 but ≤ 2 weeks: reduce TAK-228 by 1 dose level > 2 weeks: stop MLN0128 (TAK-228) and discontinue subject from the study.	No change in dose.
Grade 4	Off protocol therapy	If hyperglycemia is controlled within 2 weeks of the delay, resume ziv-aflibercept at same dose level
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy. Note: The investigator will work closely with the endocrinologist, and metformin will be the recommended oral hyperglycemic agent. Prevention/Prophylaxis:		

- Follow fasting glucose levels during clinic visits.
- Monitor home glucometer test results.
- Check HbA1c levels every 3 months during therapy.
- Recommend life-style modifications, as appropriate (balanced diet, limited alcohol consumption, increased physical activity).
- Most episodes of Grade 1 or 2 hyperglycemia respond quickly to oral metformin. Early initiation of therapy at the lowest therapeutic dose is recommended to prevent higher grade hyperglycemia.
- Fasting blood glucose levels ≥150 mg/dL by glucometer should be followed by closer monitoring of serum glucose and possible intervention.

Pneumonitis and Non-Infectious Pneumonitis	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
≤ Grade 1	No change in dose; Rule out infection and closely monitor	No change in dose
Grade 2	Rule out infection and consider treatment with corticosteroids until symptoms improve to ≤ Grade 1. Interrupt MLN0128 (TAK-228) treatment: When symptoms ≤ Grade 1, re-initiate MLN0128 (TAK-228) treatment at a Dose reduced by 1 level. If no recovery within 4 weeks, then discontinue MLN0128 (TAK-228).	No change in dose.
Grade 3	Rule out infection and consider treatment with corticosteroids until symptoms improve to ≤ Grade 1. Interrupt MLN0128 (TAK-228) treatment until symptoms resolve to ≤ Grade 1. At the physician's discretion, consider re-initiating MLN0128 (TAK-228) treatment at a dose reduced by 1 level. If toxicity recurs at Grade 3, discontinue MLN0128 (TAK-228) treatment. Obtain PFTs and pulmonary consultation.	If pneumonitis improves within 2 weeks of the delay, resume ziv-aflibercept at same dose level.**
Grade 4	Off protocol therapy	If pneumonitis improves within 2 weeks of the delay, resume ziv-aflibercept at same dose level.**
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.		

Hyperlipidemia	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Treat hyperlipidemia according to standard guidelines.- Maintain dose if	No change in dose

	tolerable Triglycerides ≥ 500 mg/dl should be treated urgently due to risk of pancreatitis- If toxicity becomes intolerable, interrupt MLN0128 (TAK-228) dosing until recovery to ≤ Grade 1. Reinitiate at same dose.	
Grade 3	Treat hyperlipidemia according to standard guidelines. Hold* until < Grade 2. Resume at one dose level lower, if indicated.**	If hyperlipidemia is controlled within 2 weeks of the delay, resume ziv-aflibercept at same dose level
Grade 4	Off protocol therapy	If hyperlipidemia is controlled within 2 weeks of the delay, resume ziv-aflibercept at same dose level
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.		

Rash	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose. Symptomatic treatment. Consider treatment with topical steroid cream/ointment and/or oral anti-histamines or antibiotics.	No change in dose
Grade 3	Dermatology consultation. Consider treatment with topical steroid cream/ointment and/or oral anti-histamines or antibiotics. Hold* until </= Grade 2. Resume MLN0128 (TAK-228) based on timing of recovery: ≤ 3 weeks: Reduce TAK-228 by 1 dose level; > 3 weeks: stop MLN0128 (TAK-228) and discontinue subject from the study	If rash improves within 2 weeks of the delay, resume ziv-aflibercept at same dose level.**
Grade 4	Patients who develop Grade 4 rash should permanently discontinue study treatment, unless they derive clinical benefit, in which case they may be retreated at a reduced dose level after recover to ≤ Grade 1 severity. Grade 4 rash is defined as rash acneifom/papulopustular with papules and/or pustules covering any % body surface area, which may or may not be associated with symptoms of pruritus or tenderness, and are associated with extensive superinfection with intravenous (IV) antibiotics indicated; life threatening consequences (NCI CTCAE Version 5.0).	If rash improves within 2 weeks of the delay, resume ziv-aflibercept at same dose level.**
*Patients requiring a delay of >2 weeks should go off protocol therapy.		

Rash	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
**Patients requiring > two dose reductions should go off protocol therapy. Prevention/Prophylaxis: • Rash should be managed aggressively. The investigator should consider consulting a dermatologist or other specialist, if needed. • A skin biopsy at the site of rash should be considered as soon as possible after the initial episode.		

QTc Prolongation	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Evaluate for other possible causes (eg, electrolyte disturbance, concomitant medication, etc.) Continue MLN0128 (TAK-228) at the same dose and schedule.	No change in dose.
Grade >/=3*	Evaluate for other possible causes (eg, electrolyte disturbance, concomitant medication)[a]; Consider a formal consult by a cardiologist; Notify the study doctor; Additional ECGs may be performed at intervals that the treating physician deems clinically appropriate until repeated QTc measurements fall or are below the threshold interval that triggered the repeat measurement. Notify the study doctor; Additional ECGs may be performed at intervals that the treating physician deems clinically appropriate until repeated QTc measurements fall or are below the threshold interval that triggered the repeat measurement. Hold TAK-228 Patients who experience persistent symptomatic Grade 3 or Grade 4 QTc prolongation without another cause should permanently discontinue study treatment.	If QTc prolongation improves within 2 weeks of the delay, resume ziv-aflibercept at same dose level.**
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy. ECG=electrocardiogram, IV=intravenous, msec=milliseconds, QTc=QT interval corrected for heart rate. (a) A list of medications known to prolong QTc can be found at https://www.crediblemeds.org/new-drug-list/		

Other Non-Hematologic Toxicities (Including	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**

Asthenia, Weakness, and Fatigue)		
≤ Grade 1	Initiate appropriate medical therapy and monitor. If tolerable, then no adjustment is required.	No change in dose
Grade 2	Initiate appropriate medical therapy and monitor. If tolerable, no adjustment required. If toxicity becomes intolerable, hold TAK-228 until recovery to ≤Grade 1, then reinitiate at same dose.	No change in dose
Grade >/=3*	Hold TAK-228 until recovery to ≤ Grade 1. Reinitiate TAK-228 at dose reduced by 1 level Patients who develop Grade 4 nonhematological toxicities (with the exception of isolated non-clinically significant laboratory values) should permanently discontinue study treatment, unless they derive clinical benefit, in which case they may be retreated at a reduced dose level after recovery to ≤ Grade 1 severity.	No change in dose.
*Patients requiring a delay of >2 weeks should go off protocol therapy.		

Aspartate Aminotransferase/ Alanine Aminotransferase Elevations	**Management/Next Dose for MLN0128 (TAK-228)**	**Management/Next Dose for Ziv-Aflibercept**
≤ Grade 1	No change in dose.	No change in dose
Grade 2	No change in dose; Closely monitor LFTs at least weekly or more frequently as indicated. Assess patient for other causes of transaminitis (eg, past medical history, concomitant medications).	No change in dose
Grade 3*	Closely monitor LFTs at least weekly or more frequently as indicated. Assess patient for other causes of transaminitis (eg, past medical history, concomitant medications). Hold TAK-228 until ≤Grade 1; Restart TAK-228 at the same dose. Permanently discontinue study treatment if in combination with Grade 2 total bilirubin elevation when alternative causes cannot be identified (ie, Hy's Law).	No change in dose.

Grade 4*	Closely monitor LFTs at least weekly or more frequently as indicated. Assess patient for other causes of transaminitis (eg, past medical history, concomitant medications). Stop TAK-228 and discontinue patient from the study. Permanently discontinue study treatment if in combination with Grade 2 total bilirubin elevation when alternative causes cannot be identified (ie, Hy's Law).	No change in dose.
*Patients requiring a delay of >2 weeks should go off protocol therapy. Prevention/Prophylaxis: Ensure proper screening of patients for study participation. LFTs=liver function tests, ULN=upper limit of normal.		

Management of Patients with Possible Cardiac Instability

For patients showing signs of cardiac instability after MLN0128 (TAK-228) administration, additional monitoring onsite before clinic discharge should be considered.

Management of Specific Adverse Events Related to Ziv-Aflibercept

Discontinue treatment for any of the following:

- Severe hemorrhage
- Gastrointestinal perforation
- Compromised wound healing
- Fistula formation
- Hypertensive crisis or hypertensive encephalopathy
- Arterial thromboembolic events
- Nephrotic syndrome or thrombotic microangiopathy (TMA)
- Reversible posterior leukoencephalopathy syndrome.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting (via CTEP Adverse Event Reporting System (CTEP-AERS)) **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform

presentation of events by body system. In addition to the comprehensive list, a subset of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. The SPEER is a list of events that are protocol-specific exceptions to expedited reporting to NCI via (CTEP-AERS) (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm for further clarification.

NOTE: The highest grade currently reported is noted in parentheses next to the AE in the SPEER. Report **ONLY** AEs higher than this grade expeditiously via CTEP-AERS. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

7.1.1 CAEPRs for CTEP IND Agent(s)

7.1.1.1 CAEPR for MLN0128 (TAK-228) (NSC# 768435)

Version 2.3, July 28, 2019[1]

Adverse Events with Possible Relationship to MLN0128 (TAK-228) (CTCAE 5.0 Term) [n= 390]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
CARDIAC DISORDERS			
		Cardiac arrest	
		Ventricular fibrillation	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
Constipation			***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 2)***
	Dry mouth		***Dry mouth (Gr 2)***
	Dyspepsia		
Mucositis oral			***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	General disorders and administration site conditions - Other (mucosal inflammation)		***General disorders and administration site conditions - Other (mucosal inflammation) (Gr 2)***
INFECTIONS AND INFESTATIONS			
	Urinary tract infection		***Urinary tract infection (Gr 2)***

Adverse Events with Possible Relationship to MLN0128 (TAK-228) (CTCAE 5.0 Term) [n= 390]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
INVESTIGATIONS			
	Creatinine increased		***Creatinine increased (Gr 2)***
		Electrocardiogram QT corrected interval prolonged	
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	Weight loss		***Weight loss (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 2)***
	Dehydration		***Dehydration (Gr 2)***
Hyperglycemia			***Hyperglycemia (Gr 3)***
	Hypokalemia		***Hypokalemia (Gr 2)***
	Hypomagnesemia		***Hypomagnesemia (Gr 2)***
	Hypophosphatemia		***Hypophosphatemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Back pain		***Back pain (Gr 2)***
	Pain in extremity		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Dysgeusia		***Dysgeusia (Gr 2)***
	Headache		***Headache (Gr 2)***
PSYCHIATRIC DISORDERS			
	Insomnia		
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 2)***
	Oropharyngeal pain		
		Pneumonitis	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Pruritus			***Pruritus (Gr 2)***
Rash maculo-papular			***Rash maculo-papular (Gr 2)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

Adverse events reported on MLN0128 (TAK-228) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that MLN0128 (TAK-228) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other

(hyperviscosity syndrome); Blood and lymphatic system disorders - Other (Raynaud's phenomenon); Febrile neutropenia
CARDIAC DISORDERS - Heart failure; Pericardial effusion; Sinus tachycardia; Ventricular arrhythmia
EYE DISORDERS - Blurred vision; Eye pain; Photophobia; Vision decreased
GASTROINTESTINAL DISORDERS - Abdominal distension; Colitis; Dysphagia; Esophagitis; Gastritis; Gastroesophageal reflux disease; Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (intestinal perforation); Gastrointestinal disorders - Other (salivary hypersecretion); Hemorrhoids; Ileus; Oral pain; Pancreatitis; Small intestinal obstruction; Small intestinal perforation; Toothache
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (groin pain); Malaise; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Gallbladder obstruction
IMMUNE SYSTEM DISORDERS - Allergic reaction
INFECTIONS AND INFESTATIONS - Abdominal infection; Infections and infestations - Other (cystitis); Infections and infestations - Other (lower respiratory tract infection); Infections and infestations - Other (mucosal infection); Infections and infestations - Other (parotid gland); Kidney infection; Lung infection; Papulopustular rash; Sepsis; Skin infection; Upper respiratory infection
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall; Fracture; Injury, poisoning and procedural complications - Other (accidental overdose); Injury, poisoning and procedural complications - Other (postoperative fever); Injury, poisoning and procedural complications - Other (subdural hemorrhage); Tracheal obstruction
INVESTIGATIONS - Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Blood lactate dehydrogenase increased; Cholesterol high; GGT increased; Lipase increased; Lymphocyte count decreased; Neutrophil count decreased; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperkalemia; Hypernatremia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia; Metabolism and nutrition disorders - Other (severe chronic malnutrition); Metabolism and nutrition disorders - Other (vitamin D deficiency)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Chest wall pain; Flank pain; Generalized muscle weakness; Muscle cramp; Myalgia
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (non-hodgkin lymphoma); Treatment related secondary malignancy
NERVOUS SYSTEM DISORDERS - Ataxia; Intracranial hemorrhage; Lethargy; Nervous system disorders - Other (carotid artery occlusion); Nervous system disorders - Other (neuropathy peripheral); Paresthesia; Radiculitis; Stroke; Tremor
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Psychiatric disorders - Other (mental status changes)
RENAL AND URINARY DISORDERS - Dysuria; Hematuria; Proteinuria; Renal and urinary disorders - Other (strangury)
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Vaginal hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchopulmonary hemorrhage; Epistaxis; Hiccups; Hypoxia; Nasal congestion; Pleural effusion; Pleuritic pain; Pneumothorax; Postnasal drip; Productive cough
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Rash acneiform; Urticaria
VASCULAR DISORDERS - Flushing; Hypertension; Hypotension; Thromboembolic event

Note: MLN0128 (TAK-228) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.1.1.2 CAEPR for Ziv-Aflibercept (VEGF-Trap, NSC # 724770)

Version 2.8, April 17, 2019[1]

Adverse Events with Possible Relationship to Ziv-aflibercept (VEGF-Trap, AVE 0005) (CTCAE 5.0 Term) [n= 941]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
		Febrile neutropenia	
		Hemolytic uremic syndrome	
		Thrombotic thrombocytopenic purpura	
CARDIAC DISORDERS			
		Cardiac disorders - Other (intracardiac thrombus)	
		Chest pain - cardiac	
		Myocardial infarction	
		Restrictive cardiomyopathy	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Constipation		***Constipation (Gr 2)***
	Diarrhea		***Diarrhea (Gr 2)***
		Gastrointestinal fistula[2]	
		Gastrointestinal perforation[3]	
	Mucositis oral		
Nausea			***Nausea (Gr 2)***
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		***Edema limbs (Gr 2)***
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	Pain		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Wound complication		
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased		
	Creatinine increased		

Adverse Events with Possible Relationship to Ziv-aflibercept (VEGF-Trap, AVE 0005) (CTCAE 5.0 Term) [n= 941]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
	Neutrophil count decreased		***Neutrophil count decreased (Gr 4)***
	Platelet count decreased		***Platelet count decreased (Gr 4)***
	Weight loss		
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
	Dehydration		
	Hyponatremia		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 2)***
	Myalgia		***Myalgia (Gr 2)***
NERVOUS SYSTEM DISORDERS			
Headache			***Headache (Gr 3)***
		Ischemia cerebrovascular	***Ischemia cerebrovascular (Gr 2)***
		Reversible posterior leukoencephalopathy syndrome	
		Transient ischemic attacks	
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
		Nephrotic syndrome	
Proteinuria			***Proteinuria (Gr 3)***
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
	Genitourinary system fistula[4]		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 3)***
	Rhinorrhea		
Voice alteration			***Voice alteration (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		
	Palmar-plantar erythrodysesthesia syndrome		
	Skin hyperpigmentation		
VASCULAR DISORDERS			
		Arterial thromboembolism	
Hypertension			***Hypertension (Gr 3)***
	Thromboembolic event		***Thromboembolic event (Gr 3)***

Adverse Events with Possible Relationship to Ziv-aflibercept (VEGF-Trap, AVE 0005) (CTCAE 5.0 Term) [n= 941]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Vascular disorders - Other (hemorrhage)[5]		***Vascular disorders - Other (hemorrhage) (Gr 4)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIG.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Gastrointestinal fistulas may include: Anal fistula, Colonic fistula, Duodenal fistula, Esophageal fistula, Gastric fistula, Gastrointestinal fistula, Rectal fistula, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[3]Gastrointestinal perforation may include: Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Ileal perforation, Jejunal perforation, Rectal perforation, and Small intestinal perforation under the GASTROINTESTINAL DISORDERS SOC.

[4]Genitourinary fistulas may include: female genital tract fistula, uterine fistula, and vaginal fistula.

[5]The majority of hemorrhage events were mild. Major events, defined as symptomatic bleeding in a critical area or organ (e.g., eye, GI hemorrhage, GU hemorrhage, respiratory hemorrhage), and nervous system [including fatal intracranial hemorrhage and cerebrovascular accident] have been reported.

[6]Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on ziv-aflibercept (VEGF-Trap, AVE 0005) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that ziv-aflibercept (VEGF-Trap, AVE 0005) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Hemolysis
CARDIAC DISORDERS - Cardiac disorders - Other (left ventricular diastolic dysfunction); Heart failure; Left ventricular systolic dysfunction; Pericarditis; Supraventricular tachycardia
EAR AND LABYRINTH DISORDERS - Tinnitus; Vertigo
ENDOCRINE DISORDERS - Hyperthyroidism; Hypothyroidism
EYE DISORDERS - Blurred vision; Extraocular muscle paresis; Eye disorders - Other (blindness transient); Eye disorders - Other (diplopia); Vitreous hemorrhage
GASTROINTESTINAL DISORDERS - Abdominal distension; Anal mucositis; Belching; Colitis; Dental caries; Dry mouth; Dyspepsia; Dysphagia; Esophageal pain; Flatulence; Gastritis; Gastrointestinal disorders - Other (early satiety); Gastrointestinal disorders - Other

(gastrointestinal necrosis); Gastrointestinal disorders - Other (hiatal hernia); Gastrointestinal disorders - Other (intestinal ischemia); Gastrointestinal disorders - Other (peritonitis); Gastrointestinal disorders - Other (pneumatosis intestinalis); Gingival pain; Hemorrhoids; Ileus; Oral pain; Rectal mucositis; Rectal ulcer; Small intestinal mucositis; Small intestinal obstruction
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Edema trunk; Facial pain; Injection site reaction; Non-cardiac chest pain
HEPATOBILIARY DISORDERS - Cholecystitis
IMMUNE SYSTEM DISORDERS - Allergic reaction
INFECTIONS AND INFESTATIONS - Infection[6]
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Biliary anastomotic leak; Gastric anastomotic leak; Infusion related reaction; Vascular access complication; Wound dehiscence
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood bilirubin increased; Blood lactate dehydrogenase increased; Cardiac troponin I increased; Ejection fraction decreased; GGT increased; Hemoglobin increased; Lipase increased; Serum amylase increased
METABOLISM AND NUTRITION DISORDERS - Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypoalbuminemia; Hypocalcemia; Hypoglycemia; Hypokalemia; Hypomagnesemia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Avascular necrosis; Back pain; Chest wall pain; Generalized muscle weakness; Head soft tissue necrosis; Joint range of motion decreased; Muscle cramp; Muscle weakness upper limb; Musculoskeletal and connective tissue disorder - Other (musculoskeletal stiffness); Myositis; Neck pain; Osteonecrosis; Osteonecrosis of jaw; Pain in extremity; Pelvic soft tissue necrosis; Rotator cuff injury
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor hemorrhage
NERVOUS SYSTEM DISORDERS - Amnesia; Ataxia; Cognitive disturbance; Dizziness; Dysgeusia; Encephalopathy; Extrapyramidal disorder; Leukoencephalopathy; Memory impairment; Paresthesia; Peripheral sensory neuropathy; Seizure; Syncope; Vagus nerve disorder
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Insomnia; Psychiatric disorders - Other (mental status change); Psychosis
RENAL AND URINARY DISORDERS - Hematuria
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Irregular menstruation
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Hoarseness; Hypoxia; Laryngeal mucositis; Nasal congestion; Pharyngeal mucositis; Pharyngolaryngeal pain; Pleural effusion; Pneumonitis; Pneumothorax; Pulmonary fibrosis; Respiratory, thoracic and mediastinal disorders - Other (nasal dryness); Respiratory, thoracic and mediastinal disorders - Other (septal perforation); Respiratory, thoracic and mediastinal disorders - Other (throat swelling); Tracheal fistula; Tracheal mucositis
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Dry skin; Hyperhidrosis; Nail loss; Rash acneiform; Rash maculo-papular; Skin and subcutaneous tissue disorders - Other (hyperemia); Skin ulceration
VASCULAR DISORDERS - Hematoma; Hypotension; Peripheral ischemia; Phlebitis

Note: Ziv-aflibercept (VEGF-Trap, AVE 0005) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the

combination may result in events never previously associated with either agent.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized until March 31, 2018 for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.3.4.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse **Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention** [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must also be reported in routine study data submissions.**

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agents administered in this study can be found in Section 7.1.

8.1 CTEP IND Agent(s)

8.1.1 MLN0128 (TAK-228) (NSC #768435)

Other Names: TAK-228, INK128
Chemical Name: 3-(2-amino-1,3-benzoxazol-5-yl)-1-(propan-2-yl)-1Hpyrazolo [3, 4-d] pyrimidin-4-amine

Classification: mTOR inhibitor, TORC1/2

CAS Registry Number: 1224844

Molecular Formula: $C_{15}H_{15}N_7O$ **M.W.:** 309.3

Approximate Solubility: MLN0128 (TAK-228) exhibits a pH-dependent aqueous solubility:
At physiological pH, the solubility is approximately 0.1 mg/mL;
At or below pH 3, the solubility is greater than 15 mg/mL.

Mode of Action: MLN0128 (TAK-228) is a non-rapamycin analog mTOR (mechanistic target of rapamycin) kinase inhibitor. The mTOR kinase regulates cell growth, translational control, angiogenesis, and cell survival by integrating nutrient and hormonal signals. The mTOR complex (TORC) is an intracellular point of convergence for a number of cellular signaling pathways. ML0128 is a potent and selective adenosine tri-phosphate (ATP)-competitive inhibitor of mTOR complex 1 and 2 (TORC1/2).

Description: White to off-white, crystalline powder.

How Supplied: MLN0128 (TAK-228) is supplied by Millennium Pharmaceuticals, Inc. and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as size 2 hard gelatin capsules in the following strengths: 1 mg (white opaque color) and 3 mg (orange opaque color) The 5 mg (gray opaque color) will not be utilized in the protocol. The composition of the drug product consists of a blend of MLN0128 drug substance, microcrystalline cellulose, and magnesium stearate. **Milled** formulations will have a white label with a large watermark of the strength on the label. As of Amendment 13/Version 07, all newly enrolled patients will be given the milled formulation of MLN0128 (TAK-228).

MLN0128 capsules are packaged in 30-count, 60-cc high-density polyethylene (HDPE), white, opaque, round, tamper- and child-resistant bottles.

Storage: Capsules are to be stored in the original package between 15°C to 30°C, with allowed short-term excursions between 2°C and 40°C.

Route of Administration: Orally, given 1 hour before or 2 hours after a meal. Do not chew, open or manipulate the capsule in any way prior to swallowing. Each dose should be taken with 8 ounces (240 ml) of water.

Potential Drug Interactions: Multiple human metabolizing enzymes are involved in themetabolism of MLN0128 (TAK-228). Recently completed in vitro metabolism experiments in human hepatocytes using ^{14}C-labeled TAK-228 suggest that TAK-228 is metabolized primarily via CYP1A2 (approximately 31%-40%), with a minor contribution from CYP3A4 (approximately 11%-22%). These data suggest that TAK-228 is also metabolized by direct glucuronidation (approximately 22%) and an unidentified non-uridine diphosphate glucuronosyl transferase pathway (approximately 18%). The new data differ from the previous in vitro CYP phenotyping data obtained using recombinant CYP enzymes, which suggested the involvement of CYP2C9 (approximately 35%), CYP2C19 (approximately 28%), and CYP3A4 (approximately 28%) in TAK-228 metabolism. In addition, physiologically based PK modeling and simulation using the new metabolism data for TAK-228 suggest that the risk for a metabolism-based drug-drug interaction with TAK-228 appears to be low.

Patient Care Implications:
Women of childbearing potential should use effective methods of contraception during and through 90 days after the last dose of MLN0128.

Men should use effective methods of contraception and not donate sperm during and through 120 days after the last dose of MLN0128.

Availability
MLN0128 (TAK-228) is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.3).

NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

In general, sites may order initial agent supplies when a subject is being screened for enrollment onto the study.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing

(OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB's website for specific policies and guidelines related to agent management.

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: PMBRegPend@ctep.nci.nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx
- CTEP Identity and Access Management (IAM) account: https://eapps-ctep.nci.nih.gov/iam/
- CTEP Associate Registration and IAM account help: ctepreghelp@ctep.nci.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET).

8.1.2 Ziv-Aflibercept (NSC #724770)

Other Names: aflibercept, AVE0005, ziv-aflibercept

Classification: Recombinant humanized fusion protein (Chinese hamster ovary source).

M.W.: 115 kDa

Approximate Solubility: at least 100mg/ml water (with 5 mM Na phosphate, 5 mM Na citrate, 100 mM NaCl, 0.004-0.08% Polysorbate 20) at 5°C and 25°C.

Mode of Action: The cytokine VEGF binds to and activates VEGFR1 and VEGFR2 on the vascular endothelium, promoting new vessel formation. VEGF Trap is a soluble recombinant decoy receptor that binds and inactivates extravascular and hematologic VEGF. It reduces tumor vasculature density, available nutrient supply, and tissue matrix components escaping from leaky tumor vessels.

Description: The fusion protein VEGF Trap is 2 portions of human VEGF receptors

extracellular domains, VEGFR1 and VEGFR2, fused to the Fc portion of human IgG1.

How Supplied: VEGF Trap is supplied by Sanofi-Aventis Pharmaceuticals and distributed by the CTEP, DCTD, NCI. VEGF Trap is a sterile, nonpyrogenic, colorless to pale yellow solution in vials of 100 mg (4 mL) or 200 mg (8 mL) at a concentration of 25 mg/mL. The solution contains the following excipients: sucrose, sodium chloride, sodium citrate dihydrate, citric acid monohydrate, polysorbate 20, sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate, and water for injection. The pH of VEGF Trap is 6.2. The product is packaged in a type 1, clear borosilicate glass vial closed with a flanged cap with tear-off lid and inserted sealing disc, Flurotec® (PTFE) coated.

Preparation: Prior to infusion, the VEGF Trap dosage form must be diluted directly into infusion bags of 0.9% sodium chloride solution or 5% dextrose. The concentration of the diluted solution can range between 0.6 and 8 mg/mL. The pH of the diluted solution is about 6.2.

Storage: Store intact vials in the refrigerator (2^{o} to 8^{o} C).

Stability: Shelf life stability studies of intact vials are ongoing. VEGF Trap's provisional shelf-life is up to 36 months at 2^{o} to 8°C.

Caution: The sterile single use vials contain no antibacterial preservatives. Discard remaining agent 8 hours after initial entry.

VEGF Trap diluted to a concentration of 0.6 to 8 mg/mL in 0.9% NaCl or 5% dextrose has demonstrated chemical and physical stability for up to 24 hours under refrigerated conditions (2 to 8°C) or up to 8 hours at ambient temperature (approximately 25°C) in infusion bags made of polyvinyl chloride (PVC) containing di(2-ethylhexyl)phthalate (DEHP) or polyolefin (PVC free DEHP free).

Route of Administration: Intravenous

Method of Administration: : Administer VEGF Trap intravenously over 1 hour into a peripheral vein or central venous catheter using gravity, an infusion pump, or syringe pump. The infusion should not exceed two hours at room temperature (approximately 25°C).

VEGF Trap may be administered using infusion tubing made of PVC containing DEHP, polyethylene lined PVC, DEHP free PVC containing tris (2-ethylhexyl) trimellitate (TOTM), polypropylene, or polyurethane.

The infusion set must contain a 0.2 micron polyethersulfone inline filter. Polyvinylidene fluoride (PVDF) filters or Nylon filters should **not** be used.

Availability

Ziv-Aflibercept is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Ziv-Aflibercept is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.3).

8.1.3 Agent Ordering and Agent Accountability

8.1.3.1 NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://eapps- ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.3.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Biologic activity of ziv-aflibercept may be difficult to assess because it may often seem primarily cytostatic rather than cytotoxic in function. When anti-VEGF agents are used in a monotherapeutic fashion, only a small number of patients have a major tumor response (Beaudry *et al*., 2005; Davis *et al*., 2003). To advance the clinical testing of ziv-aflibercept and the projected additional benefits of combination with MLN0128 (TAK-228), biopsies will be performed. These studies are designed to determine if combining ziv-aflibercept and MLN0128 (TAK-228) will inhibit both the phosphorylation of effectors that are downstream of the targets of the drugs as well as potentiating anti-angiogenic activities.

For the expansion cohorts, we will be performing correlative studies in 10 patients. There will be discussion between the PI and NCI-CTEP to further expand with additional patients. Our interventional radiologists will coordinate with our study radiologist. Our study radiologist will identify an optimal area for interventional radiologists to biopsy. Biopsies will be performed at

baseline, on Cycle 1 Day 8, and at time of progression. All the treating investigators will be educated and trained to report to the principal investigator the first signs of progression. For the Biomarker Assay Characterization for RPPA and IHC, please see Appendix F.

9.1 Immunohistochemistry (IHC)

Dr. Funda Meric-Bernstam's (Chairman of the Department of Investigational Cancer Therapeutics at M. D. Anderson) laboratory will evaluate protein expression (both the total and phosphorylated proteins) by immunohistochemical (IHC) staining on slides from fresh tumor samples as well as archival tissue material (if available). Multiple predictive biomarkers relevant to the mechanism of action of mTOR inhibitors and VEGF-trap inhibitors will be evaluated in the tumor specimens.

Expression/phosphorylation of potential markers of response (including but not limited to AKT, pAKTSer473, pS6 and PTEN) and markers of angiogenesis (including CD31) will be detected in the tumors using immunohistochemistry and correlated with clinical tumor response. IHC will also be used to access microvessel density. Archival tissue and pre- treatment/post-treatment biopsies will be evaluated by H&E. Treatment-induced apoptosis will be assessed with IHC for cleaved caspase (Meric-Bernstam *et al.*, 2004).
The reason we chose IHC as a tool to evaluate response to new drug before and after therapy is because most biological processes and functions occur at the protein level. Ultimately, protein functional status and its expression level in cancer may provide the best assessment for therapeutic development.

The current standard method in diagnostic pathology is a conventional semiquantitative visual analysis of brown stain immunohistochemical staining (DAB based) with a light microscope.

In the event that tissue material is limited, we will give priority to RPPA analysis as RPPA is an extremely effective and sensitive method for measuring multiple proteins in cancer cells and this technique can be applied to tiny amounts of material. Dr. Funda Meric-Bernstam and Dr. Gordon Mills' laboratory at MD Anderson Cancer Center has established this method, and we are confident that we can use this technology to measure all the markers of relevance to this study. However, when it is possible to perform both RPPA and IHC for the same proteins, the results from each platform will validate each other.

9.1.1 Outcome measure

Total and phosphorylated expression measurements including (but not limited to): pAKTSer473, pS6, PTEN, CD31.

9.1.2 Assessment

IHC measurement will be based on percent of positive staining cells under microscope and intensity of positive cells. Intensity will be ranked as 0: no staining, 1: weak staining, 2: moderate staining 3: strong staining. The intensity is multiplied by the percentage of positive

cells. This is called H-score, which will correlate to therapeutic response.

Timing of Assessment

Samples will be collected at baseline, Day 8 of Cycle 1, and at progression.

After all samples have been collected, tissue samples will be processed no later than 3 months from the time of collection at Dr. Funda Meric-Bernstam's laboratory. Basic data analysis will be performed no later than 2 months after the time of slide staining.

9.1.3 Data recording

The slide will be read by the following day after IHC and imaging data will be transferred to computer after read.

9.1.4 Collection of specimens

Please refer to Appendix G.

9.1.5 Site performing correlative study

The University of Texas MD Anderson Cancer Center in Houston, Texas, in the Division of Cancer Medicine (Departments of Investigational Cancer Therapeutics and Systems Biology) will be performing the IHC.

9.2 Reverse Phase Protein Assay (RPPA)

Dr. Funda Meric-Bernstam's (Chairman of the Department of Investigational Cancer Therapeutics at M. D. Anderson) laboratory will perform Reverse Phase Protein Arrays (RPPAs) on peripheral blood mononuclear cells (PBMCs), platelet-enriched plasma samples, and tumor biopsies to quantitate protein expression/phosphorylation, as well as proliferation, cell cycle progression, and apoptosis markers. Our priority will be looking at molecular alterations in the mTOR signaling pathway (including but not limited to AKT, pAKTSer473, pS6, and PTEN) and markers of angiogenesis.

A number of blood vessel-specific markers will be quantified using RPPA analysis. CD31 is a particularly useful marker for quantifying angiogenesis by RPPA. Decreased CD31 levels can provide a surrogate for inhibition of angiogenesis. Other blood vessel-specific markers that can serve as surrogates for angiogenesis and can be quantified using RPPA include VEGF receptors VEGFR1 and VEGFR2. We have identified and validated antibodies that detect CD31, VEGFR1 and VEGFR2 for RPPA. The arrayers, robotics and robust algorithms are available to efficiently utilize the technologies.

Assessment of MLN0128 (TAK-228) alone, ziv-aflibercept alone and MLN0128 (TAK-228) + ziv-aflibercept combination administration will be assayed by RPPA on patient PBMCs and tumor biopsies. Specimens will be analyzed for (1) effect on target proteins or proteins

associated with the target protein upon administration of each drug alone or combination of drugs (2) effect on biological markers (e.g., proliferation, apoptosis, etc.) (3) potential off-target effects (4) association of protein levels (basal or treated) or post-translational modifications with patient outcome.

9.2.1 Outcome Measure

Total and phosphorylated expression measurements including (but not limited to): pAKTSer473, pS6, PTEN, CD31.

9.2.2 Assessment

Method of Assessment
Flash-frozen tumor tissues (obtained as outlined in Appendix G) will be quickly cut (if necessary) and weighed; tumor samples with a mass range of 10-40 mg are placed in ice-cold lysis buffer (1% Triton X100, 50mM Hepes, pH 7.4, 150mM NaCl, 1.5mM $MgCl_2$, 1mM EGTA, 100mM NaF, 10mM NaPPi, 10% glycerol, freshly added Roche Complete tablet, freshly added 1mM Na_3VO_4) at 1mL/40mg tissue, and the sample will be homogenized on ice. PBMC pellets (obtained as outlined in Appendix H) will be lysed in a similar fashion. Protein estimation of supernatants collected from lysed tissue will be determined by bicinchroninic acid assay. Proteins in sample will be denatured and linearized upon addition of SDS sample buffer (35% Glycerol, 8% SDS, 0.25M Tris-HCL, pH 6.8, 10% 2-mercaptoethanol) and boiling.

Sample concentrations will be adjusted to ~1mg/ml using dilution buffer (3 parts lysis buffer: 1 part SDS buffer). Samples will be serially diluted 2-fold at least 5 times, and spotted onto nitrocellulose-coated microscope (FAST) slides. Relative levels of proteins or protein modifications will be detected by amplifying signal with streptavidin-biotin and tyramide and colorimetric development by diaminobenzidine. Stained slides will be scanned for quantitation by a 24+-bit, CCD flatbed scanner at 600+ dpi in grayscale and saved as a 16-bit tiff image. Relative levels of total or post- translationally modified proteins will be quantitated by software developed for RPPA (MicroVigene and supercurve script written for R). Other analyses will be performed as appropriate, including supervised and unsupervised hierarchical clustering of samples and proteins.

Timing of Assessment
Samples will be collected at baseline, Day 8 of Cycle 1, and at progression.

After all samples have been collected, tissue samples will be processed no later than 3 months from the time of collection at Dr. Funda Meric-Bernstam's laboratory. RPPA sample dilution, slide printing and staining will be performed no later than 3 months after the time of tissue processing. Basic data analysis will be performed no later than 2 months after the time of slide staining.

9.2.3 Data Recording

Method of Recording
All files created (xls, tif, txt, ppt) are currently stored (password protected) in the Systems Biology server, which is backed up in designated space within the institution.

Timing of Recording
Data will be recorded in the server no later than one week after data has been acquired.

9.2.4 Collection of Specimen(s)

Please refer to Appendix G and Appendix H.

9.2.5 Handling of Specimen(s)

Please refer to Appendix G and Appendix H.

9.2.6 Shipping of Specimen(s)

The specimens will not be shipped to another location. All correlative studies will be performed at The University of Texas MD Anderson Cancer Center in Houston, Texas, in the Division of Cancer Medicine (Departments of Investigational Cancer Therapeutics and Systems Biology).

9.2.7 Site(s) Performing Correlative Study

The University of Texas MD Anderson Cancer Center in Houston, Texas, in the Division of Cancer Medicine (Departments of Investigational Cancer Therapeutics and Systems Biology) will be performing the IHC.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks (28 days) prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. Routine lab studies, vital signs, weight, height, and performance status must be done within 14 days of Day 1 and will have a flexibility window of +/- 2 days. Any re-staging/disease assessment criteria will have a flexibility window of +/- 3 days. Dosing can be given up to 2 days after physical exam as long as it has not been less than 7 days from last dose. However, this will not be routine practice and will only occur in special circumstances as deemed appropriate by PI. There will be a +/-2 day flexibility window for assessments on Day 1, Day 8, Day 15, and Day 22 of each cycle. Both study drugs will have a +/- 2 day window. If the ziv-aflibercept dosing is rescheduled, then the MLN0128 (TAK-228) dosing will be adjusted accordingly.

	Screening			Cycle 1				Cycle 2				Cycle 3 and Beyond				Off Study[f]
	Within 28 Days	Within 14 Days	Within 7 Days	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22	
MLN0128 (TAK-228)				A---------- 3 Days On and 4 Days Off Oral Starting Day 2 of Every Cycle----------A												
Ziv-Aflibercept				B		B		B		B		B		B		
Informed consent	X															
Demographics	X															
Medical history	X															
Concurrent meds	X			X---------- X												
Physical exam	X			X		X		X		X		X		X		X
Vital signs		X		X		X		X		X		X		X		X
Height		X														
Weight		X		X		X		X		X		X		X		X
Performance status		X		X		X		X		X		X		X		X
CBC w/diff, plts		X		X	X	X	X	X	X	X	X	X		X		X
Serum chemistry[a]		X		X	X	X	X	X	X	X	X	X		X		X

	Screening			Cycle 1				Cycle 2				Cycle 3 and Beyond				Off Study[f]
	Within 28 Days	Within 14 Days	Within 7 Days	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22	
Amylase/Lipase[a]		X		X	X	X	X	X		X		X		X		X
Coagulation (PT/PTT/INR)[a]		X		X				X				X				X
Fasting Lipid Panel[a]		X		X	X	X	X	X		X		X		X		X
Fasting Serum Glucose[a]		X		X				X				X				X
HbA1c[a]		X										X				
B-HCG[b]			X[b]	X				X				X				
In-Home Glucose Monitoring[h]				X----------Daily----------X				X----------Daily----------X				X[h]----------Daily----------X				
Urinalysis[c]		X		X		X		X		X		X		X		X
EKG[g]	X															X
ECHO/MUGA	X															
Adverse event evaluation				X---------- X												X
Tumor measurements[d]	X			Tumor measurements are repeated every 8 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.												X
Radiologic evaluation	X			Radiologic measurements should be performed every 8 weeks.												X
Biopsy[e]		X			X											X
PBMC Sample[e]		X			X											X

A: MLN0128 (TAK-228): Dose as assigned; 3 days on and 4 days off oral starting on Day 2 of every cycle

B: Ziv-Aflibercept: Dose as assigned; Administered intravenously every 2 weeks (Days 1 and 15 of every cycle)

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, magnesium, total protein, SGOT [AST], SGPT [ALT], sodium will be conducted at baseline and every visit. PT, PTT/INR will be collected at Day 1 of every cycle and off study visit. The fasting lipid panel will be collected at baseline, weekly during Cycle 1, Day 1 and Day 15 of Cycle 2 and beyond and off study visit. HbA1c will be collected at screening, Cycle 3 Day 1 and every 3 cycles thereafter. Fasting serum glucose will be conducted on Day 1 of all Cycles.
Amylase/lipase will be obtained at screening, weekly during Cycle 1, every 2 weeks during Cycle 2 and beyond, and Off Study visit.

	Screening			Cycle 1				Cycle 2				Cycle 3 and Beyond				Off Study[f]
	Within 28 Days	Within 14 Days	Within 7 Days	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22	
b: Serum pregnancy test (women of childbearing potential) within 7 days. Serum or urine pregnancy test is required on Day 1 of each cycle. If screening pregnancy test is negative, then a Cycle 1 Day 1 pregnancy is not required.																
c: Assess urine protein creatinine (UPC) ratio. If UPC ratio is > 1.0, 24-hour urine collection for protein and creatinine clearance should be obtained.																
d. Tumor assessments will be performed after every two cycles beginning with Cycle 2 and will include CT or MRI and/or PET scan. Whichever modality is chosen for tumor assessment, the same modality should be used for serial evaluations of the patient throughout the trial.																
e: Biopsies, and PBMC samples will be taken in the dose expansion cohort only. Baseline biopsy, and PBMC will be obtained within 8 days of C1D1.																
f: Off-study evaluation to be performed within 30 days (+/- 7 days) after discontinuation of study drugs. In addition, for patients who experience significant toxicities on study, follow-up should continue until toxicities resolve or are deemed irreversible.																
g. EKG: Performed at screening, as clinically indicated during the study, and during the Off Study Visit.																
h In-home daily fasting glucose monitoring: Patients will be given a glucometer to monitor fasting glucose levels at home collected daily predose on dosing days, at approximately the same time each day and will be instructed to notify the study clinician any time the fasting glucose is abnormal (i.e. ≥150mg/dL). In-home glucose monitoring is not required on days when fasting glucose is measured in the clinic. For further instruction see Section 5.3.																

11. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated every 8 weeks. In addition to a baseline scan, confirmatory scans will also be obtained 8 weeks following initial documentation of an objective response.

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 8 (not less than 4) weeks following initial documentation of objective response. Scans will include CT or MRI and/or PET scan. Whichever modality is chosen for tumor assessment, the same modality should be used for serial evaluations of the patient throughout the trial. However, PET CT may be alternated with a CT if needed or indicated in a given patient. If a subject has been on study for 12 months the staging interval may be increased to re-evaluate for response every 12 weeks (3 cycles).

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) (Eisenhauer *et al*., 2009). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with MLN0128 (TAK-228) and/or ziv-aflibercept.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray or as ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. *If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol*.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non- cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non- nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

11.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray **Lesions** on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published (Gordon *et al*., 2004; Bubley *et al.*, 1999; Scher *et al*., 2008). In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer (Vergote *et al*., 2000).

Cytology, Histology These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.2 Antitumor Effect – Hematologic Tumors

Hematologic tumors will not be evaluated in this study.

11.3 Other Response Parameters

Other response parameters will not be evaluated in this study.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application.

Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/reporting/cdus.html).

Note: If your study has been assigned to CDUS-Complete reporting, **all** adverse events (both routine and expedited) that have occurred on the study and meet the mandatory CDUS reporting guidelines must be reported via the monitoring method identified above. If your study has been assigned to CDUS-Abbreviated reporting, no adverse event reporting (routine or expedited) is required to be reported via CDUS.

12.1.2 Responsibility for Data Submission

Study participants are responsible for submitting CDUS data and/or data forms to either the Coordinating Center or to the Lead Organization on the study quarterly. The date for submission to the Coordinating Center or to the Lead Organization will be set by them. CDUS does not accept data submissions from the participants on the study. When setting the dates, allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP by the quarterly deadlines (see Section 12.1.1). For trials monitored by CTMS, a quarterly report of data will be provided by Theradex to the Coordinating Center.

12.2 CTEP Multicenter Guidelines

Not applicable to this study.

12.3 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study.
   Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

   a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

   b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

   c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative

Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected.
   Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release.
   Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

Dr. Suyu Liu (Biostatistics at UT MD Anderson Cancer Center) will perform all statistical analyses. The primary objective of this study is to determine the maximum tolerated dose (MTD) of the combination of MLN0128 (TAK-228) and ziv-aflibercept in patients with advanced cancers refractory to standard therapy.

A standard 3+3 dose-escalation design will be implemented for this phase I portion of the study. There will be no intra-patient dose escalation, and no patients will be enrolled in the next dose level until the toxicity is fully assessed after the completion of 1 cycle and at least 3 patients enrolled at the previous dose level.

Three patients will be treated per dose level. If none of the patients experience a dose-limiting toxicity (DLT), the next cohort of three patients will be treated at the next higher dose level. If 1 of 3 patients treated at a dose experiences a DLT, that cohort will then be expanded to a total of six patients. If a DLT occurs in only 1 in 6 of the first dose level, 3 more patients may be enrolled at the next higher dose level (i.e., cohort 2). If 2 or more of 6 patients in any cohort experiences DLT, the MTD is considered to have been exceeded. No patients may be enrolled in the next dose level until 3 patients at the previous dose level have completed at least one cycle of therapy.

The software used to compute the statistical designs is Multc Lean v2.1.

Primary Endpoints
The primary endpoints are safety, tolerability, and determination of DLT and MTD.

Secondary Endpoints
The secondary endpoints are to determine changes in parameter values compared to baseline, at early scans, restaging, and at time of progression will be assessed.

For the expansion cohort only, the secondary endpoints are to determine changes in analyses performed on PBMCs and biopsies, and changes of phosphorylation levels of AKT (Ser473) and pS6 as well as other biomarkers.

<u>MTD Expansion Cohort</u>
Once the MTD is determined, the expansion cohort may be opened. The MTD is defined as the highest dose level at which no more than 1 of 6 evaluable patients has had a DLT. At the MTD, or at Dose Level 3 if no DLTs are observed, six patients should be treated before the dose is declared as MTD.

Up to 10 additional patients may be entered at the MTD once it is determined for the purpose of collecting additional information on toxicity, tolerability, and biologic effect at that dose. These additional 10 patients will be monitored for toxicity. We will emphasize enrollment of pancreatic neuroendocrine tumors (pNET) and ovarian carcinoma. There will be discussion between the PI

and NCI-CTEP to further expand with additional patients.

Toxicity monitoring for the expansion cohort

We will consider stopping the expansion cohort for excessive toxicity if, among the additional 10 patients in the expansion cohort (beyond the initial 6), there are > 3/3, 4/5 or 5/9 patients with DLT. This would yield approximately 90% likelihood of declaring excessive toxicity for a true DLT rate of 0.7 and only approximately 10% likelihood of declaring excessive toxicity for a true rate of 0.3.

Analysis of Primary Endpoints

Data from all patients who receive one or more doses of drug will be incorporated into the final safety analysis. To assess clinical safety, all adverse experiences, vital sign measurements, clinical laboratory information, radiographic studies and concomitant illnesses will be summarized by visit. Adverse experiences and laboratory results will be summarized by severity grade (NCI CTCAE version 4.0 until March 31, 2018, and version 5.0 beginning April 1, 2018).

All patients who receive any amount of the study drug will be evaluable for toxicity. The MTD is defined as the highest dose level at which no more than 1 of 6 evaluable patients has had a DLT.

13.2 Sample Size/Accrual Rate

Twenty-nine patients were treated in the initial dose escalation dosing schemas. As of Amendment 13/ Version 07, three dose levels are included in the revised dose escalation with a 3+3 design, and there will be a maximum of 18 patients enrolled in the revised dose escalation cohorts. At the MTD, an additional 10 patients will be enrolled in the expansion cohort. Prior to Amendment 13/ Version 07, there were 29 patients enrolled in the dose escalation. Therefore, the maximum accrual is 57 patients (29 + 18 + 10).

Accrual rate: 1-3 patients per month

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	3	+	6	=	9
Not Hispanic or Latino	28	+	20	=	48
Ethnic Category: Total of all subjects	31	+	26	=	57

Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	1	+	1	=	2
Black or African American	2	+	2	=	4

Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	28	+	23	=	51
Racial Category: Total of all subjects	31	+	26	=	57

13.3 Stratification Factors

None

13.4 Analysis of Secondary Endpoints

Tumor Response
The secondary endpoints are to determine changes in parameter values compared to baseline, at early scans, restaging, and at time of progression will be assessed. Tumor response will be assessed by RECIST (Version 1.1) (Section 11.0).

Evaluation of Tumor Size
Secondary objectives include getting an early indication of efficacy by assessing tumor size. The sum of the largest diameters of the index lesions will be used for tumor size. Tumor size will be measured at baseline and at time of best response. For each patient, the % change in tumor size from baseline to best response will be computed. We will construct a waterfall plot of these values.

Correlative Studies
For the expansion cohort only, the correlative endpoints are to assess the biological effect of the treatment. Due to the relatively small number of patients, the correlative studies should be viewed as exploratory in nature.

Reverse Phase Protein Array (RPPA)
RPPA will be performed on the tumor biopsies performed at baseline and on Day 8 of cycle 1. Total and phosphorylated expression will be determined for several proteins including (but not limited to): pAKTSer473, pS6, PTEN, and CD31. The biomarker of primary interest is phospho-AKT.

Immunohistochemistry (IHC):
IHC will be performed on the tumor biopsies performed at baseline and on Day 8 of cycle 1. Total and phosphorylated expression will be determined for several proteins including (but not limited to): pAKTSer473, pS6, PTEN, and CD31. The biomarker of primary interest is phospho-AKT.

Analyses
For each marker above, we will assess changes from baseline to cycle 1 day 8 using a paired t-test unless the data are clearly not normally distributed in which case we will use a Wilcoxon signed rank test. With 10 patients, we can detect a 1-SD mean difference (where SD is the standard deviation of the differences) as significant with 80% power using a two-sided 5% alpha.

Changes in BF and phospho-AKT are of primary interest and assessment of changes in other biomarkers will be considered exploratory.

We will compute the Pearson correlation coefficient between the percent change between baseline and cycle 1 day 8 for BF and tumor size. If the data are not normally distributed, we will use Spearman's rank correlation coefficient. With 10 patients, we can detect a correlation coefficient of 0.8 or higher as significantly different from zero with 80% power using a two-sided 5% alpha. Assessments of correlation between tumor size and changes in other biomarkers and among the various biomarker changes will be considered exploratory.

REFERENCES

Alvarez EA, Brady WE, Walker J, Rotmensch J, Zhou XC, Kendrick JE, Yamada SD, Schilder JM, Cohn D, Harrison CR, Moore, KN, Aghajanian C. Phase II trial of combination of aflibercept and temsirolimus in the treatment of recurrent or persistent endometrial carcinoma: a gynecologic oncology group study. Gynecologic Oncology. 2012.

Beaudry P FJ, Naumov GN, Wang A, Baker CH, Ryan A, Soker S, Johnson BE, Folkman J, Heymach JV. Differential effects of vascular endothelial growth factor receptor-2 inhibitor ZD6474 on circulating endothelial progenitors and mature circulating endothelial cells: implications for use as a surrogate marker of antiangiogenic activity. Clinical Cancer Research. 2005; 11:3514-3522.

Bilen MA, Naing A, Hong DS, Falchook GS, Fu S, Wen S, Wheler JJ, Durand JB, Ewer M, Kurzrock R. Association between elevated blood pressure and clinical response in patients treated with anti-vascular endothelial growth factor (anti-VEGF) therapy in phase I clinical trials. J Clin Oncol 30, 2012 (suppl; abstr e13506).

Bubley GJ, Carducci M, Dahut W, Dawson N, et al. Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: recommendations from the prostate- specific antigen working group. J Clin Oncol 1999; 3461-3467

Chiang GG, Abraham RT. Targeting the mTOR signaling network in cancer. Trends in Molecular Medicine 2007;13(10):433-42.

Dancey JE. Molecular targeting: PI3 kinase pathway. Ann Oncol 2004; 15(Suppl 4):233-239.

Davis DW MD, Abbruzzese, Herbst RS. Surrogate markers in antiangiogenesis clinical trials. British Journal of Cancer. 2003; 89:8-14.

Eisenhauer, EA, Therasse P, Bogaerts J, Schwartz LH, et al., New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Ca 2009; 45(2): 228-247.

Fasolo A and Sessa C. mTOR inhibitors in the treatment of cancer. Expert Opin Invest Drugs 2008; 17(11):1717-1734

Georgakis GV and Younes A. From Rapa Nui to rapamycin: targeting PI3K/Akt/mTOR for cancer therapy. Expert Rev Anticancer Ther 2006; 6(1):131-140.

Gordon J, Rustin S, Quinn M, Thigpen T, et al., Re: new guidelines to evaluate the response to treatment in solid tumors (ovarian cancer). JNCI J Natl Cacner Inst 2004; 96(6): 487-488.

Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646-674.

Heng DYC. Combination therapy in metastatic renal cell carcinoma. The Lancet Oncology

2011; 12(7): 613-614.

Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M, Boland P, Leidich R, Hylton D, Burova E, Ioffe E, Huang T, Radziejewski C, Bailey K, Fandl JP, Daly T, Wiegand SJ, Yancopoulos GD, Rudge JS. VEGF-Trap: a VEGF blocker with potent antitumor effects. Proc Natl Acad Sci U S A 2002; 99(17):11393-8.

Investigator's Brochure (VEGF TRAP, AVE0005- Aflibercept), Edition 15 (2017). Sanofi R&D, Centre de Recherche Vitry Alfortville, France.

Investigator's Brochure (MLN0128 (TAK-228)), Edition 10 (2017). Millennium Pharmaceuticals, Inc., Cambridge, MA.

Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR, Loda M, Lane HA, Sellers WR. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med. 2004 Jun; 10(6):594-601. Epub 2004 May 23.

Meric-Bernstam F and Mills GB. Mammalian target of rapamycin. Semin Oncol 2004; 31(Suppl 16):10-17

Naing A, Kurzrock R, Burger A, Gupta S, Lei X, Busaidy N, Hong D, Chen HX, Doyle LA, Heilbrun LK, Rohren E, Ng C, Chandhasin C, LoRusso P. Phase I trial of cixutumumab combined with temsirolimus in patients with advanced cancer. Clin Cancer Res. 2011 Sep 15; 17(18):6052-60.

Naing A, LoRusso P, Fu S, Hong DS, Anderson P, Benjamin RS, Ludwig J, Chen HX, Doyle LA, Kurzrock R. Insulin growth factor-receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with refractory Ewing's sarcoma family tumors.
Clin Cancer Res. 2012 May 1; 18(9):2625-31.

Naing A, LoRusso P, Fu S, Hong D, Doyle LA, Phan AT, Habra MA, Kurzrock R. Insulin growth factor receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with metastatic adrenocortical carcinoma. British Journal of Cancer 2013, 1-5. (in print)

Naing A. Overcoming resistance to mTOR inhibition for enhanced strategies in clinical trials. Expert Opin Investig Drugs. 2013 Apr 30

Scher HI, Halabi S, Tannock I, Morris M, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the prostate cancer clinical trials working group. J Clin Oncol 2008; 26(7): 1148-1159.

Vergote I, Gordon J, Rustin S, Eisenhauer EA et al. Re: new guidelines to evaluate the response to treatment in solid tumors (ovarian cancer). JNCI J Natl Cancer Inst 2000; 92(18):

1534-1535.

Yao J, Tsuchihashi Z, Paneerselvam A, Winkler R, Bugarini R, Pavel M. Effect of everolimus treatment on markers of angiogenesis in patients with advanced pancreatic neuroendocrine tumors (pNET): results from the phase III RADIANT-3 study [abstract]. Ann Oncol 2011; 22.

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B NEW YORK HEART ASSOCIATION (NYHA) CLASSIFICATIONS

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

APPENDIX C INFORMATION ON POSSIBLE DRUG INTERACTIONS

Appendix C: List of Relevant Cytochrome P450 Inhibitors and Inducers

Moderate CYP1A2 Inhibitors		
Cimetidine	methoxsalen	
Strong CYP1A2 Inhibitors		
Fluvoxamine	ciprofloxacin	
Clinically Significant Enzyme Inducers		
Carbamazepine	rifabutin	St. John's Wort
Phenobarbital	rifampin	Phenytoin
rifapentine		
Source: fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ ucm093664.htm. Note that these lists are not exhaustive.		

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team

The patient_____is enrolled on a clinical trial using the experimental agent MLN0128 (TAK-228) and Ziv-Aflibercept. This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.

MLN0128 (TAK-228) interacts with many drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements such as St. John's wort.

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet**. These are the things that you and they need to know:

MLN0128 (TAK-228) interacts with (a) certain specific enzyme(s) in your liver.

- The enzymes in question are strong CYP1A2 inhibitors and CYP inducers.
- MLN0128 (TAK-228) must be used very carefully with other medicines that need these liver enzymes to be effective or to be cleared from your system.
- Other medicines may also affect the activity of the enzyme.
    - Substances that increase the enzyme's activity ("inducers") could reduce the effectiveness of the drug, while substances that decrease the enzyme's activity ("inhibitors") could result in high levels of the active drug, increasing the chance of harmful side effects.
    - Substances that increase the enzyme's activity ("inducers") could result in high levels of the active drug, increasing the chance of harmful side effects, while substances that decrease the enzyme's activity ("inhibitors") could reduce the effectiveness of the drug.
    - MLN0128 (TAK-228) is considered an "inducer/inhibitor" of the enzyme, meaning that it can affect the levels of other drugs that are processed by that enzyme. This can lead to harmful side effects and/or reduce the effectiveness of those medications.
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong CYP1A2 inhibitors and CYP inducers".
- Your prescribers should look at this web site http://medicine.iupui.edu/clinpharm/ddis/table.aspx or consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid.

- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.
- Be careful:
    - If you take acetaminophen regularly: You should not take more than 4 grams a day if you are an adult or 2.4 grams a day if you are older than 65 years of age. Read labels carefully! Acetaminophen is an ingredient in many medicines for pain, flu, and cold.
    - If you take herbal medicine regularly: You should not take St. John's wort while you are taking MLN0128 (TAK-228).

Other medicines can be a problem with your study drugs.

- Concomitant administration of any proton pump inhibitor (PPI) is not permitted during the study. Patients receiving PPI therapy before enrollment must stop using the PPI for 7 days before their first dose of study drugs. Examples of PPIs include omeprazole, esomeprazole, pantoprazole, lansoprazole, and rabeprazole.
- See restrictions that apply to the use of histamine H2 receptor antagonists, neutralizing antacids, calcium and anti-gas preparations.
- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is _____

  and he or she can be contacted at _____

INFORMATION ON POSSIBLE DRUG INTERACTIONS

You are enrolled on a clinical trial using the experimental agent **_____.** This clinical trial is sponsored by the NCI. _____ interacts with drugs that are processed by your liver. Because of this, it is very important to:

- Tell your doctors if you stop taking regular medicine or if you start taking a new medicine.
- Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) that you are taking part in a clinical trial.
- Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

_____ interacts mainly with a specific liver enzyme called CYP1A2 and additionally with CYP3A4, and must be used very carefully with other medicines that interact with this enzyme.

- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors of CYP3A4, 2C9, or 2C19."
- Before prescribing new medicines, your regular prescribers should go to http://medicine.iupui.edu/clinpharm/ddis/table.aspx for a list of drugs to avoid, or contact your study doctor.
- Your study doctor's name is _____

  and can be contacted at _____**.**

APPENDIX D PATIENT'S MEDICATION DIARY - AGENT MLN0128 (TAK-228)

Today's date _____
Patient Name_____(*initials acceptable)* **Patient Study ID** _____
INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle of treatment.
2. Your dose = ____ mg. Take ____ 1 mg and/or ____ 3 mg capsules. You will take MLN0128 (TAK-228) capsules via mouth for 3 days on and 4 days off starting on Day 2 of every cycle. Follow the dose selected by your physician. You should take MLN0128 (TAK-228) with a full glass of water. You should not eat for 2 hours before and 1 hour after you take MLN0128 (TAK-228)
3. Record the date, the number of capsules that you took, and when you took them
4. If you have any comments, please record them in the Comments column and notify the study coordinator or research nurse.
5. Please bring this form and your bottles of MLN0128 (TAK-228) capsules when you return for each appointment.
6. If you miss a dose, take it as soon as you remember. If it is almost time for your next dose, skip the missed dose and take the medicine at the next regularly scheduled time. Do not double the doses to make up the missed dose.
7. If you vomit after taking MLN0128 (TAK-228), please record in the comments section and do not take another dose until the next day's dose.
8. Please record your daily glucose reading and daily blood pressure (BP).

Day	Date	Time of dose	# of Capsules taken		Glucose	BP	Comments
			1 mg	3 mg			
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

Day	Date	Time of dose	# of Capsules taken		Glucose	BP	Comments
			1 mg	3 mg			
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							
27							
28							

Patient's signature: _____ Date: _____

Physician's Office will complete this section:

1. Date patient started protocol treatment_____
2. Date patient was removed from study_____
3. Patient's planned total daily dose_____
4. Total number of capsules taken this month_____
5. Physician/Nurse/Data Manager's Signature _____

APPENDIX E GLUCOSE MONITORING INSTRUCTIONS

In-Home Daily Fasting Glucose Monitoring for 2013-0665

- The level should be collected **daily**, **predose on dosing days**, and at approximately the **same time each day**.

- Collect **FASTING** in the am, prior to taking your MLN1028 pill.

- **Record the value in your drug diary**.
  - If >150, call the triage nurse (713- 563-1930)
  - If the triage nurse is not available, proceed to your local ER.

- **Bring your glucometer back** with your **drug diary** when you see the MD.

- Collect daily, for 2 months, at the same time. After 2 months your doctor will decide if the frequency can be reduced to once weekly.

Patients will continue to notify the clinic **(Triage nurse 713- 563-1930)** of fasting blood glucose levels that exceed 150 mg/dL and, if blood glucose levels are not well controlled, or if the patient requires either oral hypoglycemic agents or insulin to control blood glucose levels, then the frequency of in-home testing of FBG levels will be reinstated to daily

APPENDIX F BIOMARKER ASSAY CHARACTERIZATION

Study Checklist for CTEP-Supported Early Phase Trials with BIOMARKER ASSAYS

INSTRUCTIONS: For INTEGRAL assay, respond to Items 1-7. For INTEGRATED assay, respond to Items 1-3 and 5-7. (In lieu of completing items 5-6, the CTEP assay templates available at http://ctep.cancer.gov/protocolDevelopment/default.htm#ancillary_correlatives may be utilized.)

Please submit a response to each of the criteria below and complete one Study Checklist for each Biomarker endpoint.

1. Markers:

Primary markers: Akt, phospho-Akt (p-Akt) (Thr308), p-Akt (Ser473), CD31, and PTEN.

Secondary markers: p-4E-BP1 (Thr37/46), p-4E-BP1 (Ser65), GSK3alpha/beta (Ser21/9), IGF-1Rbeta, IRS1, p-MAPK (Thr202/204), p-MEK1/2 (Ser217/221), p-mTOR (Ser2448), p-p27 (Thr157), p-p27 (Thr198), p-p38 MAPK (Thr180/182), PCNA, PI3K p85, PI3K p110 alpha, p-S6 (Ser235/236), p-S6 (Ser240/244), S6K1, p-S6K1 (Thr389), and p-TSC2 (Thr1462).

2. For an integral or integrated assay, indicate the role(s) of the biomarker assay in the trial: N/A
   A. Eligibility criterion – N/A
   B. Assignment to treatment – N/A
   C. Stratification variable – N/A
   D. Risk classifier or score – N/A
   E. Other (describe in detail):

3. Identify the specific individual(s) and laboratory(ies) who are being considered for conducting the assay(s) for the trial.

Specimen collection and handling: Argun Akcakanat, MD, PhD. RPPA analysis: MD Anderson Cancer Center RPPA Core Facility.

4. Integral laboratory assays used for clinical decision-making must be performed in a CLIA-certified facility. Provide the lab's CLIA number that is performing the integral biomarker study(ies) and the expiration date of the certificate. **N/A**

5. Describe the assay:

   A. Specify the analyte(s), technical platform, and sources of assay components (e.g., reagents, chips, and calibrators).

RPPA arrays will be performed according to SOP at MD Anderson RPPA Core Facility.

Frozen samples will be thawed at room temperature and heated at 50°C for 15-20 minutes. Tumor lysates will be diluted in five 2-fold serial dilutions (1:1, 1:2, 1:4, 1:8, and 1:16) in dilution buffer (lysis buffer containing 1% SDS) in 96-well plates. Equal volumes of 80% glycerol and 2x PBS will be added to each well. Samples will be transferred to 384-well master plates for printing. Plates will be sealed and stored at -80°C until use.

An Aushon 2470 Arrayer (Aushon BioSystems) will create 1056 spot arrays on nitrocellulose-coated slides (Grace Biolab) from serial dilutions. Total array spots will be arranged on each slide including the spots corresponding to positive and negative controls prepared from mixed cell lysates or dilution buffer, respectively.

Every antibody in the panel was subjected to a stringent validation procedure. Only antibodies with a single or dominant band and no nonspecific binding on western blotting were further assessed by direct comparison to RPPA. Antibodies that recognized known cell characteristics, such as size variants due to cleavage, mutations or deletions were acceptable. To validate antibodies against phosphorylated epitopes, cell lines were stimulated (e.g. growth factors) or inhibited by specific inhibitors to generate phosphorylated and nonphosphorylated forms of a protein. Also, cell lines that were genomically altered, such as DNA transfected and overexpressing or short interfering RNA transfected and inhibited, could be used for validation. If antibodies passed the above criteria, results of RPPA and western blotting had to be parallel. Only antibodies with a Pearson correlation coefficient greater than 0.7 between RPPA and western blotting as well as wide dynamic range will be used.

Staining will be accomplished by a Dako - Autostainer Link 48 (Dako North America, Inc., Carpinteria, CA). Slides will be precleared with ReBlot Plus Mild Antibody Stripping Solution (EMD Millipore, Billerica, MA) in PBS containing 0.1% Tween-20 for 30 minutes. The blocking and amplification reagents are provided in Catalyzed Signal Amplification System (CSA, Dako). Following TBS-T rinse, slides will be blocked for peroxidase, avidin, biotin, and proteins 5 minutes each and followed by TBS-T washes. Slides will be incubated with primary antibody (Appendix 1) for 20 minutes, TBS-T washed and incubated with the secondary antibody (Vector Laboratories, Inc., Burlingame, CA) for 20 minutes. Antibodies will be diluted in Antibody Diluent with Background Reducing Components (Dako). After TBS-T wash, slides will be subjected to amplification reagents, strepavidin-biotin complex, amplification reagent and streptavidin-HRP. For signal detection, diaminobenzidine (DAB) will be added to slide and after 3 minutes of incubation, staining reaction will be stopped by washing the slide with distilled water. The slides will then be air-dried.

The slides will be scanned on a Canon flatbed scanner at 1200 dpi in grayscale, and saved as a 16-bit tiff file. Spot intensities will be determined as mean net values, in which the local background intensity will be subtracted, by reading tiff images on Array-Pro Analyzer software (Media Cybernetics, Inc., Rockville, MD). A representative natural logarithmic value of each sample curve on the slide (curve average) was then used as a relative quantification of the amount of each protein in each sample.

Each dilution curve will be fitted with a logistic model written in the language R (Supercurve Fitting, developed by the Department of Bioinformatics and Computational Biology in MD Anderson Cancer Center, "http://bioinformatics.mdanderson.org/OOMPA"). Briefly, this fits a single curve using all the spot intensities of all the samples on a slide with the signal intensity as the response variable and the dilution steps are independent variable. Supercurve predicts that each antibody has unique binding properties. The fitted curve is plotted with the signal intensities – both observed and fitted - on the y-axis and the log2-concentration of proteins on the x-axis for diagnostic purposes. This fitted logistic curve is called "supercurve." Goodness of fit of supercurve is determined by calculating a mean R^2 of the linear portion of the curve (trimmed mean) and color scale mapping of residuals R^2 for each spot intensity. In addition to visual inspection of slides for evenness of staining, sufficient signal intensity, and titration of diluted spots, quantitation data provides strict measures. Good quality slides have a trimmed mean R^2 over 0.5 and a residuals R^2 map of at least 50% of spots in the acceptable range.

At the final step, the protein concentrations of each set of slides will then be normalized by median polish, which was corrected across samples by the linear expression values using the median expression levels of all antibody experiments to calculate a loading correction factor for each sample.

B. Describe the specimens, and anticipated methods for specimen acquisition, fixation or stabilization, and processing. Provide justification for the timing of specimen collection.

Sample acquisition and initial processing: Whole blood will be collected by Investigational Cancer Therapeutics (ICT) personnel, and patients will be transferred to the Department of Interventional Radiology. Whole blood for extraction of peripheral mononuclear cells (PBMC) will be collected in an 8 ml CPT (cell preparation tube) with sodium citrate (BD, Becton, Dickinson and Company). Whole blood for preparation of platelet rich plasma will be collected in CTAD (buffered sodium citrate theorphylline adenosine dipyridamole) tube. The transfer of CPT and CTAD tubes to Dr. Funda Meric-Bernstam's lab and initiation of sample processing will begin within 30 minutes. Detailed protocols for PBMCs and platelet rich plasma are provided as Appendices 2 and 3, respectively. Once preparation is completed, at the final step both PBMC and platelet rich plasma samples will be snap- frozen in liquid nitrogen and stored at -80°C. Tumor biopsies will be done by interventional radiology and biopsies will be snap-frozen in liquid nitrogen according to the protocol in Appendix G. After being transferred to Dr. Funda Meric-Bernstam's lab, tumor samples will also be stored at -80°C until use.

Sample processing for RPPA: PBMC pellets will be resuspended in RPPA lysis buffer (1% Triton X100, 50 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM EGTA, 100 mM NaF, 10 mM NaPPi, 10% glycerol, 1 mM Na_3VO_4, cOmplete Protease Inhibitor Cocktail Tablets (Roche Diagnostics Corporation, Indianapolis, IN) containing 0.25% sodium deoxycholate.

Samples will be lysed on ice and following centrifugation for 10 min at 4C at 16000 xg in a microcentrifuge, supernatant will be transferred to a clean microfuge tube. Protein concentration will be determined by bicinchroninic acid (BCA) assay (Pierce Biotechnology, Rockford, IL). Protein samples will be denatured by adding 4x SDS sample buffer (35% glycerol, 8% SDS, 250 mM Tris-HCl, pH 6.8, 10% 2-mercaptoethanol). Final PBMC sample concentration will be adjusted to 3 µg/µl. Lysates will be boiled, aliquoted and stored at -80°C until use.

Platelet rich plasma pellets will be resuspended in RPPA lysis buffer. Samples will be lysed on ice and following centrifugation for 10 min at 4°C at 16000 x g in a microcentrifuge, supernatant will be transferred to a clean microfuge tube. Protein concentration will be determined by BCA assay. Protein samples will be denatured by adding 4x SDS sample buffer. Final PBMC sample concentration will be adjusted to 2 µg/µl. Lysates will be boiled, aliquoted and stored at -80°C until use.

Tumor samples will be transferred to a ceramic bead tube and RPPA lysis buffer will be added. Samples will be homogenized using a table top tissue homogenizer (Precellys 24, Bertin Technologies, Montigny-le-Bretonneux, France) at 4°C. Following centrifugation for 10 min at 4°C at 16000 x g in a microcentrifuge, supernatant will be transferred to a clean microfuge tube. Protein concentration will be determined by BCA assay. Protein samples will be denatured by adding 4x SDS sample buffer. Final PBMC sample concentration will be adjusted to 1.5 µg/µl. Lysates will be boiled, aliquoted and stored at -80°C until use.

C. Describe the scoring procedures and type of data to be acquired:

Samples are quantitative/continuously distributed in log2 and fold change from baseline will be reported as the primary endpoint for signaling markers indicated in the protocol.

6. Provide data on the analytical performance of the assay.

A. For *in vitro* tests, describe the current status of studies defining the accuracy, precision, reportable range, reference ranges/intervals (normal values), turn-around time and failure rate of the assay as it is to be performed in the trial. Describe the use of positive and negative controls, calibrators, and reference standards for clinical assays. Describe any critical preanalytic variables. For guidance on regulatory requirements for laboratory assays please visit:

RPPA is a well-established assay, supported by several published articles. Appendix 4 lists selected studies where samples were processed in MD Anderson RPPA Core Facility. This list also includes studies completed in Meric-Bernstam lab.

The relatively slow growing component of the assay is the antibody list. This is because of the stringent conditions defined to validate RPPA quality antibodies. The validated antibodies are defined as: only antibodies with a single or dominant band and no nonspecific binding on western blotting were further assessed by direct comparison to RPPA (Figure 1). Antibodies that recognized known cell characteristics, such as size

variants due to cleavage, mutations or deletions were acceptable. To validate antibodies against phosphorylated epitopes, cell lines were stimulated (e.g. growth factors) or inhibited by specific inhibitors to generate phosphorylated and nonphosphorylated forms of a protein. Also, cell lines that were genomically altered, such as DNA transfected and overexpressing or short interfering RNA transfected and inhibited, could be used for validation. If antibodies passed the above criteria, results of RPPA and western blotting had to be parallel. Only antibodies with a

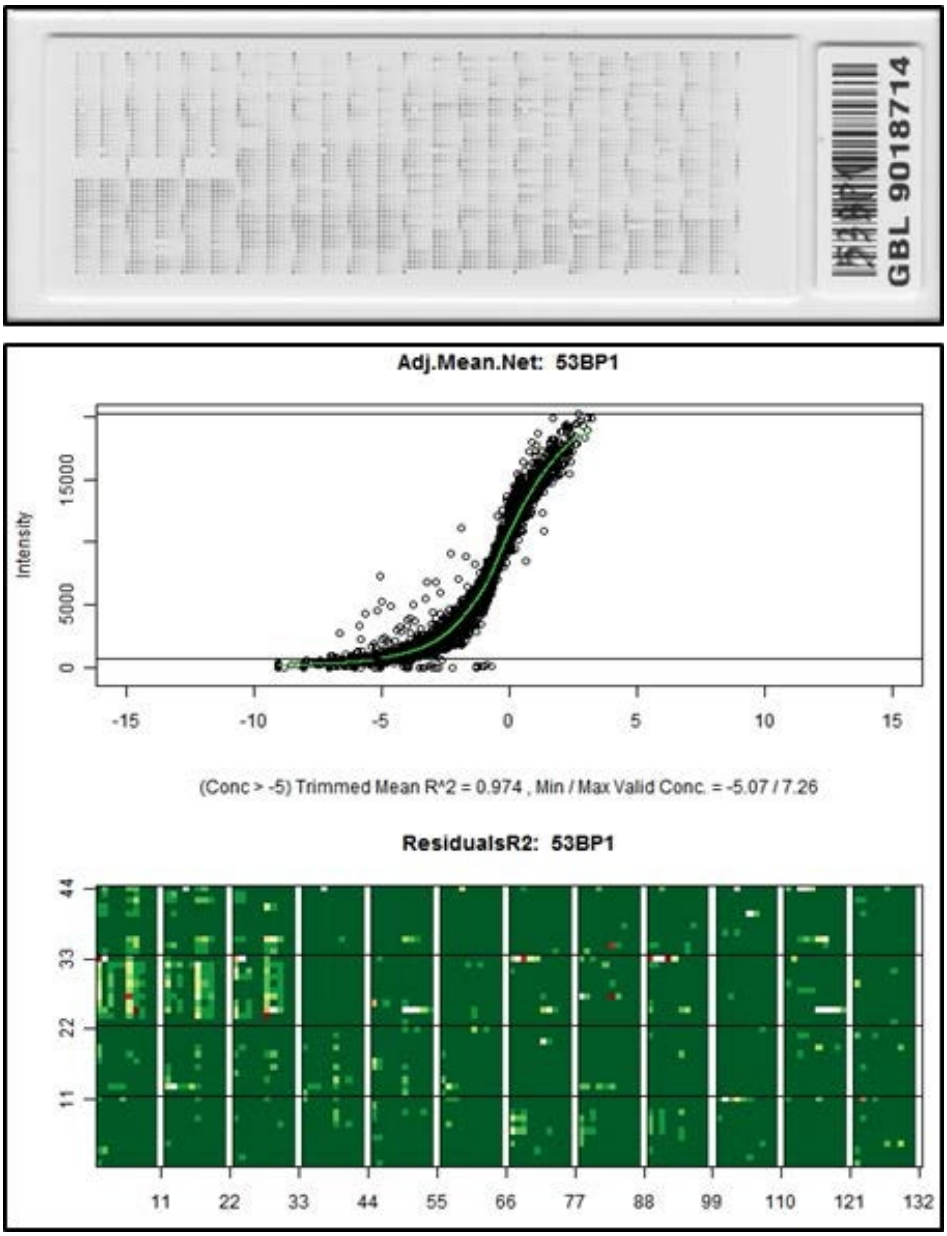


Figure 1. RPPA slide probed with validated 53BP1 antibody (upper panel), supercurve fitting and trimmed mean R2 (middle panel), and color scale mapping (lower panel).

Pearson correlation coefficient greater than 0.7 between RPPA and western blotting as

well as wide dynamic range will be used. As of October 3, 2013, there are 204 antibodies included in the standard antibody list of the RPPA Core Facility. The validation data of the antibodies listed in "1. Markers," are provided in Appendix 5. RPPA antibody panel is dynamic and some antibodies are removed from this panel in time, when they no longer meet the expected criteria. The major reasons to stop using an antibody are: (1) the antibody is no longer available, it is discontinued by the company, (2) lot number is changed and the new lot does not pass validation, (3) red blob antibodies are removed from the standard list for tissue samples. 'Red blob antibody' recognizes unidentified 'damaged' components in addition to its specific protein. The 'damaged' components were observed only in certain tissue samples causing very high positive signals to interfere with the network display.

The RPPA Core Facility has a two Aushon 2470 arrayers for printing of up to 100 slides per run with several automated runs feasible in a day. There are two Dako - Autostainer Link 48 staining systems that probes each slide with a different antibody. Each autostainer is capable of staining approximately 60 slides per day under conditions that are specific for each individual antibody.

Lysates will be serial diluted to define the linear range of each antigen-antibody reaction. Protein extraction methods of tumor tissue, PBMC and platelet rich plasma lysates show a couple of minor differences. First, preliminary studies showed that adding 0.25% sodium deoxycholate to RPPA lysis buffer for PBMCs increased the signal intensity in western blotting, whereas it had no significant effect on the signal intensity in RPPA. Second, final protein concentration is different for these three samples. If we describe the level of blood contamination of these three types of samples, the ranking would be PBMC > platelet rich plasma > tumor. Protein quantitation assays are colorimetric and contaminants result in a false increase in optic density readings. Thus result in a discrepancy between reported and real protein concentrations. Preliminary studies comparing signal intensities in western blotting and RPPA experiments showed that a correction in the final protein concentration was the most easy and reliable way to overcome this discrepancy.

Data will be collected and analyzed using quantification software Array-Pro Analyzer. This software has multiple features including automated spot identification, background correction, controlling for location, serial dilution-signal intensity curve construction and concentration determination. The values derived from the slope and intercept will be expressed relative to standard control cell lysates or control peptides on the array. Lastly, Supercurve application is collaboration between the RPPA Core Facility and the Department of Bioinformatics, which has updated several times to answer the needs of the users, particularly providing more reliable data.

B. If the assay will be performed at more than one site, describe how inter-laboratory variability in the measurements listed in 5A above will be assessed. Describe how these sources of variation will be minimized to maintain performance at all sites within acceptable limits and to prevent drift or bias in assay. ***N/A***

7. Provide data on the clinical utility of the integral/integrated assay as it will be used in the trial:
   A. Provide background information that justifies the use of this assay result as a marker for this trial. State the hypothesis and rationale for utilizing the biomarker, with supporting preclinical and clinical data, when available. For example, if the integral marker will be used as a stratification or treatment-determining variable, data supporting its prognostic or predictive association with a main trial endpoint should be described or referenced.
   B. Describe the expected distribution of the biomarker in the study population. Justify the number of patients and specimens to determine feasibility and to demonstrate that the studies are likely to produce interpretable results.
   C. If cutpoints will be used, specify the cutpoint(s) and describe how these will be used in the trial. Provide the rationale for the cutpoint(s) selected. What proportion of subjects is expected to have values above and below the proposed assay value cutpoints? What magnitude of effect (e.g., treatment benefit) or outcome (e.g., prognosis) is expected for patients with assay results above and below the proposed cutpoint(s)?
   D. Describe the conditions under which treating physicians and or patients will be able to access the biomarker assay results.

In the context of this study, patients in dose expansion will undergo serial biopsies of tumors to characterize the effects that MLN0128 (TAK-228) and Aflibercept has upon the biology of both the tumor and the stroma. Tumor biopsies will be obtained through Interventional Radiology if considered minimal surgical risk. The timing of biopsies is defined to allow analysis of the effects of MLN0128 (TAK-228) and Aflibercept upon the tumor and stroma in dose expansion before treatment, after treatment with MLN0128 (TAK-228) and Aflibercept, and upon progression on both agents. This plan will allow the discovery of possible predictive biomarkers that can be queried in future trials with this combination.

In collaboration with Dr. Funda Meric-Bernstam, we will evaluate protein expression (both total and phosphorylated proteins) by RPPA and IHC staining on slides from tumor samples. Expression of PI3K/mTOR signaling markers and angiogenesis will be assessed (Figure 1). Microvessel density will also be evaluated in the tumor tissue using IHC and correlated with clinical tumor response and RPPA analyses. Pre- and post-treatment biopsies will be evaluated by H&E. Treatment-induced apoptosis will be assessed with IHC for cleaved caspase 3 and proliferation will be assessed by Ki-67.

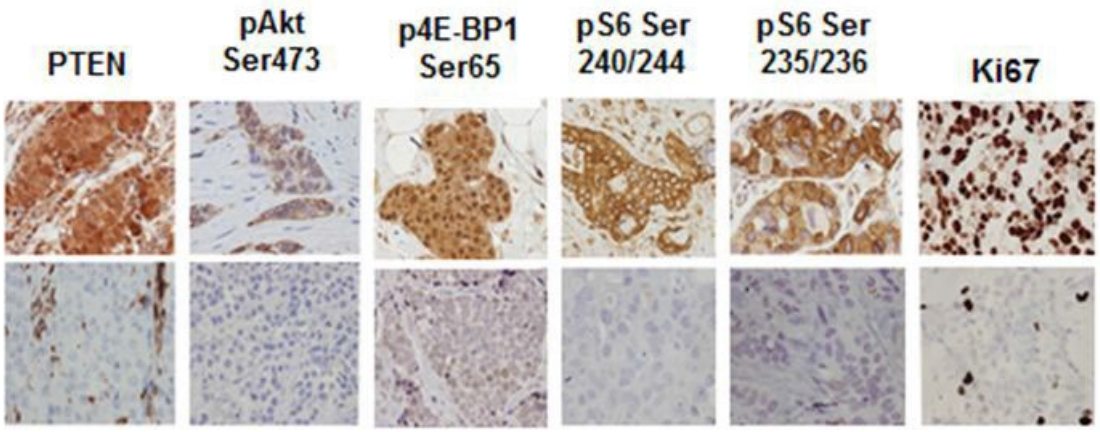


Figure 1. Examples of positive and negative staining PI3K/mTOR markers by IHC. IHC for PTEN, pAkt Ser473, p4E-BP1 Thr70, pS6 Ser235/236, pS6 Ser240/244 and Ki-67.
Breast cancer samples with positive immunostaining are shown in the top panel, and samples with negative or low immunostaining are shown in the bottom panel. Data provided by Dr. Funda Meric-Bernstam.

In the event that tissue material is limited, we will give priority to RPPA analysis as RPPA is an extremely effective and sensitive method for measuring multiple proteins in cancer cells. This technique can be applied to minute amounts of material. Dr. Funda Meric- Bernstam and Dr. Gordon Mills have established this method, and we are confident that we can use this technology to measure all the markers of relevance to this study. However, when it is possible to perform both RPPA and IHC for the same proteins, the results from one will validate the results from the other.

Reverse phase protein array (RPPA) is a recently developed quantitative assay that analyzes nanoliter amounts of sample for potentially hundreds of proteins. This antibody-based assay determines levels of protein expression, as well as protein modifications such as phosphorylation, cleavage, and fatty acid modification. RPPA allow concordant interrogation of multiple signaling molecules and their functional status. In essence, the RPPA study has major strengths in identification and validation of cellular targets, characterization of signaling pathways and networks, as well as determination of on and off target activity of novel drugs. The integrated information will display the potential therapeutic targets or biomarkers to accurately predict or rapidly define intracellular signaling networks and functional outcomes affected by therapeutics. Thus, the RPPA approach provides a method to assess multiple markers in a global manner. Linking robust pathway mapping approaches to molecular therapeutics should provide an expanding repertoire of validated biomarkers and targeted therapeutics for clinical evaluation. Others have shown the benefit of measuring a functional subset of proteins using a RPPA in an exploratory manner in the expansion cohort of a trial using the combination of sorafenib and bevacizumab[2]. This subset included proteins in the canonical PI3K/AKT/mTOR pathway as well as other signaling proteins. We hypothesize that inhibition of the PI3K/AKT/mTOR pathway at a protein level will be predictive of response to therapy with MLN0128 (TAK-228). Patients in stage 2 will undergo serial biopsies prior to initiation of therapy and after two weeks of single agent

MLN0128 (TAK-228) to characterize the effects that MLN0128 (TAK-228) has upon the biology of both the tumor and the stroma. Current validated antibodies in the RPPA include MEK1/2 (S217/221), AKT (S473, T308), PI3K, p70S6K (T389), MAPK1/2 (T202/204), GSK3beta(S21/S9), p27 (T157, T198), p38MAPK (T180/182), rS6K (S235/236,
S240/244), and 4E-BP1 (S65). On-study biopsy (D1) can occur up to one week prior to initiation of therapy. We estimate that 10 patients per arm will provide power for exploratory translational analyses examining target presence, activation, modulation, and relationship to clinical outcome. This plan will allow the discovery of possible predictive biomarkers that can be queried in future trials with this combination. To be conservative, the sample size required was calculated under the assumption that a Bonferroni adjustment is required for the 10 primary evaluations to be performed. Thus, requiring a two-tailed p-value <0.005 (=0.05/10) for each test to be considered statistically significant will be used to estimate the desired number of subjects. In practice, a Hochberg adjustment will be used to evaluate the significance of the multiple comparisons, which is not as stringent as a Bonferroni adjustment, but will still maintain an overall 0.05 significance level for the comparisons performed. Furthermore, since this study is mainly pilot in intent, the findings will be reported in that context. With 10 patients in a cohort, for each such comparison after single and combination drug administration, there is 80% power to detect a difference from baseline equal to 1.5 standard deviation of the change from baseline for each of the 10 parameters, with an overall alpha=0.05 as stated above. This calculation assumes that a paired t-test will be used to test the difference in time for each parameter. If these changes are not normally distributed (p<0.05 by Shapiro-Wilks test), then a Wilcoxon signed rank test will be used instead of a t-test. In addition, a paired t-test or Wilcoxon signed rank test will be used to evaluate the significance of changes for each correlative end point.
Previous clinical trials have demonstrated the feasibility of proteomics analysis on clinical samples associated with targeted agents in these populations. We expect to identify 1-2 predictive markers to help design a biomarker driven phase 2 trial at the completion of this study. There are potential pitfalls to this study including failure to identify a predictive marker from our primary endpoint measurements. We will analyze more than 100 proteins in excess of those identified as our primary endpoints in the specific aims. While the statistics were powered for analysis of these primary endpoints, deeper analysis of the proteomic datasets in an exploratory fashion may provide other markers of interest to future trials. Given the datasets available from previous clinical trials that demonstrated some efficacy for TORC1 inhibition or single agent bevacizumab, a comparative analysis with the data obtained in this trial might yield important clues as to inherent resistance to the combination.

#	Ab Name	Gene Name	Company	Catalog #	Internal Ab ID	Species	Validation Status*
1	14_3_3_beta	YWHAB	Santa Cruz	sc-628	882	Rabbit	Validated
2	14_3_3_epsilon	YWHAE	Santa Cruz	sc-23957	913	Mouse	Use with Caution
3	14_3_3_zeta	YWHAZ	Santa Cruz	sc-1019	883	Rabbit	Validated
4	4E BP1	EIF4EBP1	CST	9452	2	Rabbit	Validated
5	4E BP1_pS65	EIF4EBP1	CST	9456	3	Rabbit	Validated
6	4E BP1_pT37/T46	EIF4EBP1	CST	9459	6	Rabbit	Validated
7	53BP1	TP53BP1	CST	4937	985	Rabbit	Validated
8	A Raf	ARAF	CST	4432	1217	Rabbit	Validated
9	ACC_pS79	ACACA ACACB	CST	3661	13	Rabbit	Validated
10	ACC1	ACACA	Epitomics	1768-1	14	Rabbit	Under Evaluation
11	ACVRL1	ACVRL1	Abcam	ab108207	1086	Rabbit	Use with Caution
12	ADAR1	ADAR1	Abcam	ab88574	1198	Mouse	Validated
13	Akt	AKT1 AKT2 AKT3	CST	4691	1084	Rabbit	Validated
14	Akt_pS473	AKT1 AKT2 AKT3	CST	9271	230	Rabbit	Validated
15	Akt_pT308	AKT1 AKT2 AKT3	CST	2965	1154	Rabbit	Validated
16	AMPK_alpha	PRKAA1	CST	2532	39	Rabbit	Use with Caution
17	AMPK_pT172	PRKAA1	CST	2535	40	Rabbit	Validated
18	Annexin I	ANXA1	BD Biosciences	610066	1208	Mouse	Validated
19	Annexin VII	ANXA7	BD Biosciences	610668	1142	Mouse	Validated
20	AR	AR	Epitomics	1852-1	756	Rabbit	Validated
21	ARHI	DIRAS3	MDACC Laboratory	Bast Lab	1273	Mouse	Use with Caution
22	ATP5H	ATP5H	Abcam	ab110275	1252	Mouse	Use with Caution
23	B Raf	BRAF	Santa Cruz	sc-5284	96	Mouse	Use with Caution
24	B Raf_pS445	BRAF	CST	2696	94	Rabbit	Validated
25	Bad_pS112	BAD	CST	9291	63	Rabbit	Validated
26	Bak	BAK1	Epitomics	1542-1	71	Rabbit	Use with Caution
27	BAP1	BAP1	Santa Cruz	sc-28383	1207	Mouse	Validated
28	Bax	BAX	CST	2772	73	Rabbit	Validated
29	Bcl 2	BCL2	Dako	M0887	80	Mouse	Validated
30	Bcl xL	BCL2L1	CST	2762	85	Rabbit	Validated

31	Beclin	BECN1	Santa Cruz	sc-10086	87	Goat	Use with Caution
32	Beta_catenin	CTNNB1	CST	9562	75	Rabbit	Validated
33	Beta_catenin_pT41/S45	CTNNB1	CST	9565	1170	Rabbit	Validated
34	Bid	BID	Abcam	ab32060	88	Rabbit	Use with Caution
35	Bim	BCL2L11	Abcam	ab32158	90	Rabbit	Validated
36	BRcA2	BRCA2	CST	9012	761	Rabbit	Use with Caution
37	c Jun_pS73	JUN	CST	9164	155	Rabbit	Validated
38	c Kit	KIT	Abcam	ab32363	157	Rabbit	Validated
39	c Met_pY1235	MET	CST	3129	727	Rabbit	Validated
40	c Myc	MYC	Santa Cruz	sc-764	1143	Rabbit	Use with Caution
41	c Raf	RAF1	Millipore	04-739	1201	Rabbit	Validated
42	c Raf_pS338	RAF1	CST	9427	179	Rabbit	Validated
43	Caspase 7_cleavedD198	CASP7	CST	9491	109	Rabbit	Use with Caution
44	Caveolin 1	CAV1	CST	3238	114	Rabbit	Validated
45	CD29	CD29	BD Biosciences	610467	1206	Mouse	Validated
46	CD31	PECAM1	Dako	M0823	127	Mouse	Validated
47	CD49b	ITGA2	BD Biosciences	611016	937	Mouse	Validated
48	CDK1	CDC2	CST	9112	1007	Rabbit	Validated
49	CDKN2A/p16INK4a	CDKN2A	Abcam	ab81278	1231	Rabbit	Validated
50	Chk1	CHEK1	CST	2360	1203	Mouse	Use with Caution
51	Chk1_pS345	CHEK1	CST	2348	903	Rabbit	Use with Caution
52	Chk2	CHEK2	CST	3440	146	Mouse	Validated
53	Chk2_pT68	CHEK2	CST	2197	147	Rabbit	Use with Caution
54	cIAP	BIRC2	Millipore	07-759	930	Rabbit	Use with Caution
55	Claudin 7	CLDN7	Novus	NB100-91714	852	Rabbit	Validated
56	Collagen VI	COL6A1	Santa Cruz	sc-20649	171	Rabbit	Validated
57	Complex II_Subunit 30	MED30	Invitrogen	459230	1069	Mouse	Validated
58	Cox 2	CMC2	CST	4842	1218	Rabbit	Use with Caution

59	Cox IV	PTGS3	Abcam	ab14744	1253	Mouse	Use with Caution
60	Cyclin B1	CCNB1	Epitomics	1495-1	192	Rabbit	Validated
61	Cyclin D1	CCND1	Santa Cruz	sc-718	194	Rabbit	Validated
62	Cyclin E1	CCNE1	Santa Cruz	sc-247	201	Mouse	Validated
63	Cyclophilin F	PPIF	Abcam	ab110324	1257	Mouse	Validated
64	DJ 1	PARK7	Abcam	ab76008	891	Rabbit	Validated
65	Dvl 3	DVL3	CST	3218	940	Rabbit	Validated
66	E cadherin	CDH1	CST	3195	1099	Rabbit	Validated
67	E2F1	E2F1	Santa Cruz	sc-251	1261	Mouse	Validated
68	eEF2	EEF2	CST	2332	1060	Rabbit	Use with Caution
69	eEF2K	EEF2K	CST	3692	1061	Rabbit	Validated
70	EGFR	EGFR	CST	2232	1120	Rabbit	Validated
71	EGFR_pY1068	EGFR	CST	2234	217	Rabbit	Use with Caution; also sees pHer2
72	EGFR_pY1173	EGFR	Abcam	ab32578	221	Rabbit	Validated
73	eIF4E	EIF4E	CST	9742	722	Rabbit	Validated
74	eIF4G	EIF4G1	CST	2498	1124	Rabbit	Use with Caution
75	ER alpha	ESR1	Lab Vision	RM-9101-S	238	Rabbit	Validated
76	ER alpha_pS118	ESR1	Epitomics	1091-1	241	Rabbit	Validated
77	ETS 1	ETS1	Bethyl	A303-501A	1200	Rabbit	Validated
78	FAK	FAK	CST	3285	1228	Rabbit	Under Evaluation
79	FAK_pT397	PTK2	CST	3283	1227	Rabbit	Validated
80	FASN	FASN	Cell Signaling	3180	1156	Rabbit	Validated
81	Fibronectin	FN1	Epitomics	1574-1	262	Rabbit	Validated
82	Fox03a	FOXO3	CST	2497	1122	Rabbit	Use with Caution
83	FoxM1	FOXM1	CST	5436	1123	Rabbit	Validated
84	FoxO3a_pS318/S321	FOXO3	CST	9465	270	Rabbit	Use with Caution
85	G6PD	G6PD	Santa Cruz	sc-373887	1155	Mouse	Validated
86	Gab2	GAB2	CST	3239	943	Rabbit	Validated
87	GAPDH	GAPDH	Ambion	AM4300	274	Mouse	Use with Caution
88	GATA3	GATA3	BD Biosciences	558686	764	Mouse	Validated
89	GCN5L2	KAT2A	CST	3305	1263	Rabbit	Validated
90	GPBB	PYGM	SDI / Novus	NBP1-32799	1248	Rabbit	Validated
91	GSK3_alpha/beta	GSK3A GSK3B	Santa Cruz	sc-7291	284	Mouse	Validated
92	GSK3_alpha/beta_pS21/S9	GSK3A GSK3B	CST	9331	285	Rabbit	Validated
93	GSK3_pS9	GSK3A GSK3B	CST	9336	1082	Rabbit	Validated
94	GYS	GYS	CST	3886	1035	Rabbit	Validated
95	GYS_pS641	GYS	CST	3891	1036	Rabbit	Validated
96	HER2	ERBB2	Lab Vision	MS-325-P1	1038	Mouse	Validated
97	HER2_pY1248	ERBB2	R&D Systems	AF1768	1075	Rabbit	Use with Caution; likely sees pEGFR
98	HER3	ERBB3	Santa Cruz	sc-285	911	Rabbit	Validated
99	HER3_pY1289	ERBB3	CST	4791	728	Rabbit	Use with Caution
100	Heregulin	NRG1	CST	2573	890	Rabbit	Validated
101	Histone H3	HIST3H3	Abcam	ab1791	1250	Rabbit	Validated
102	IGF1R	IGF1R	CST	3018	1220	Rabbit	Validated
103	IGFBP2	IGFBP2	CST	3922	335	Rabbit	Validated
104	INPP4B	INPP4B	CST	4039	1065	Rabbit	Validated
105	IRS1	IRS1	Millipore	06-248	802	Rabbit	Validated
106	JNK_pT183/Y185	MAPK8	CST	4668	888	Rabbit	Validated
107	JNK2	MAPK9	CST	4672	380	Rabbit	Use with Caution
108	Lck	LCK	CST	2752	397	Rabbit	Validated
109	MAPK_pT202/Y204	MAPK1 MAPK3	CST	4377	405	Rabbit	Validated
110	Mcl 1	MCL1	CST	5453	1222	Rabbit	Validated
111	MDM2_pS166	MDM2	CST	3521	1164	Rabbit	Validated
112	MEK1	MAP2K1	Epitomics	1235-1	417	Rabbit	Validated
113	MEK1_pS217/S221	MAP2K1	CST	9154	1076	Rabbit	Validated
114	MEK2	MAP2K2	CST	9125	1243	Rabbit	Validated
115	MIG 6	ERRFI1	Sigma Aldrich	WH0054206M1	1062	Mouse	Validated
116	MSH2	MSH2	CST	2850	905	Mouse	Validated

117	MSH6	MSH6	SDI / Novus	2271.00.02	1063	Rabbit	Use with Caution
118	mTOR	FRAP1	CST	2983	444	Rabbit	Validated
119	mTOR_pS2448	FRAP1	CST	2971	446	Rabbit	Use with Caution
120	MYH11	MYH11	SDI / Novus	21370002	1139	Rabbit	Validated
121	Myosin IIa_pS1943	MYH9	CST	5026	1160	Rabbit	Validated
122	N cadherin	CDH2	CST	4061	452	Rabbit	Validated
123	N Ras	NRAS	Santa Cruz	sc-31	1136	Mouse	Validated
124	Napsin	NAPSA	Abcam	ab129189	1274	Rabbit	Use with Caution
125	NDRG1_pT346	NDRG1	CST	3217	1126	Rabbit	Validated
126	NF2	NF2	SDI	2271.00.02	1046	Rabbit	Use with Caution
127	NFkB p65_pS536	NFKB1	CST	3033	457	Rabbit	Use with Caution
128	Notch1	NOTCH1	CST	3268	1064	Rabbit	Validated
129	p21	CDKN1A	Santa Cruz	sc-397	470	Rabbit	Validated
130	p27	CDKN1B	Abcam	ab32034	897	Rabbit	Validated
131	p27_pT157	CDKN1B	R&D	AF1555	842	Rabbit	Use with Caution
132	p27_pT198	CDKN1B	Abcam	ab64949	878	Rabbit	Validated
133	p38 alpha MAPK	MAPK1	CST	9228	1175	Mouse	Validated
134	p38 MAPK	MAPK14	CST	9212	478	Rabbit	Validated
135	p38_pT180/Y182	MAPK14	CST	9211	479	Rabbit	Validated
136	p53	TP53	CST	9282	481	Rabbit	Use with Caution
137	p70S6K	RPS6KB1	Epitomics	1494-1	493	Rabbit	Validated
138	p70S6K_pT389	RPS6KB1	CST	9205	494	Rabbit	Validated
139	p90RSK	RPS6KA1	CST	9347	759	Rabbit	Use with Caution
140	PAI 1	PAI1	BD Biosciences	612024	499	Mouse	Validated
141	Paxillin	PXN	Epitomics	1500-1	505	Rabbit	Use with Caution
142	PCNA	PCNA	Abcam	ab29	511	Mouse	Use with Caution
143	PDCD 1L1	PDCD1	Santa Cruz	sc-19090	1234	Goat	Use with Caution
144	PDCD4	PDCD4	Rockland	600-401-965	816	Rabbit	Use with Caution
145	PDGFR_beta	PDGFR	CST	3169	1225	Rabbit	Validated
146	PDK1	PDK1	CST	3062	515	Rabbit	Validated
147	PDK1_pS241	PDK1	CST	3061	516	Rabbit	Validated
148	PEA15	PEA15	CST	2780	1017	Rabbit	Validated
149	PEA15_pS116	PEA15	Invitrogen	44-836G	1018	Rabbit	Validated
150	PI3K_p110_alpha	PIK3CA	CST	4255	808	Rabbit	Use with Caution
151	PI3K_p85	PIK3R1	Millipore	06-195	523	Rabbit	Validated
152	PKC_alpha	PRKCA	Millipore	05-154	529	Mouse	Validated
153	PKC_alpha_pS657	PRKCA	Millipore	06-822	530	Rabbit	Use with Caution
154	PKC_delta_pS664	PRKCD	Millipore	07-875	932	Rabbit	Validated
155	PKCpan BetaII_pS660	PKC	CST	9371	1137	Rabbit	Validated
156	PMS2	PMS2	SDI / Novus	22510002	1246	Rabbit	Validated
157	PR	PGR	Abcam	ab32085	549	Rabbit	Validated
158	PRAS40_pT246	AKT1S1	Biosource	441100G	739	Rabbit	Validated
159	PREX1	PREX1	Abcam	ab102739	1204	Rabbit	Validated
160	PTEN	PTEN	CST	9552	566	Rabbit	Validated
161	Rab11	RAB11A RAB11B	CST	3539	1083	Rabbit	Under Evaluation
162	Rab25	RAB25	CST	4314	1150	Rabbit	Validated
163	Rad50	RAD50	Millipore	05-525	987	Mouse	Validated
164	Rad51	RAD51	CST	8875	1262	Rabbit	Validated
165	Raptor	RPTOR	CST	2280	1128	Rabbit	Validated
166	Rb_pS807/811	RB1	CST	9308	557	Rabbit	Validated
167	RBM15	RBM15	SDI / Novus	21390002	1138	Rabbit	Validated
168	Rictor	RICTOR	CST	2114	1129	Rabbit	Use with Caution
169	Rictor_pT1135	RICTOR	CST	3806	1130	Rabbit	Validated
170	S6_pS235/236	RPS6	CST	2211	600	Rabbit	Validated
171	S6_pS240/244	RPS6	CST	2215	601	Rabbit	Validated
172	SCD1	SCD1	Santa Cruz	sc-58420	1127	Mouse	Validated
173	SDHA	SDHA	Abcam	ab14715	1255	Mouse	Validated
174	SF2	SFRS1	Invitrogen	32-4500	1131	Mouse	Validated
175	SHC_pY317	SHC1	CST	2431	1031	Rabbit	Validated
176	Smad1	SMAD1	Epitomics	1649-1	922	Rabbit	Validated
177	Smad3	SMAD3	Abcam	ab40854	796	Rabbit	Validated
178	Smad4	SMAD4	Santa Cruz	sc-7966	920	Mouse	Validated
179	Src	SRC	Millipore	05-184	621	Mouse	Validated

180	Src_pY416	SRC	CST	2101	623	Rabbit	Use with Caution
181	Src_pY527	SRC	CST	2105	626	Rabbit	Validated
182	STAT3_pY705	STAT3	CST	9131	637	Rabbit	Validated
183	STAT5_alpha	STAT5A	Abcam	ab32043	638	Rabbit	Validated
184	Stathmin	STMN1	Abcam	ab52630	718	Rabbit	Validated
185	Syk	SYK	Santa Cruz	sc-1240	1033	Mouse	Validated
186	TAZ	WWTR1	CST	2149	777	Rabbit	Validated
187	TIGAR	C12ORF5	Abcam	ab137573	1107	Rabbit	Validated
188	Transglutaminase	TGM2	Lab Vision	MS-224-P1	908	Mouse	Validated
189	TRFC	TRFC	SDI / Novus	22500002	1140	Rabbit	Validated
190	TSC1	TSC1	CST	4906	1125	Rabbit	Use with Caution
191	TTF1	TTF1	Abcam	ab76013	1081	Rabbit	Validated
192	Tuberin	TSC2	Epitomics	1613-1	670	Rabbit	Validated
193	Tuberin_pT1462	TSC2	CST	3617	671	Rabbit	Validated
194	TYRO3	TYRO3	CST	5585	1080	Rabbit	Validated
195	UBAC1	UBAC1	Sigma Aldrich	HPA005651	1270	Rabbit	Validated
196	UGT1A	UGT1A	Santa Cruz	sc-271268	1267	Mouse	Validated
197	UQCRC2	UQCRC2	Abcam	ab14745	1256	Mouse	Use with Caution
198	VDAC1/Porin	VDAC1	Abcam	ab14734	1254	Mouse	Validated
199	VEGFR2	KDR	CST	2479	688	Rabbit	Validated
200	XRCC1	XRCC1	CST	2735	906	Rabbit	Use with Caution
201	YAP	YAP1	Santa Cruz	sc-15407	780	Rabbit	Under Evaluation
202	YAP_pS127	YAP1	CST	4911	782	Rabbit	Under Evaluation
203	YB 1	YBX1	SDI / Novus	1725.00.02	700	Rabbit	Validated
204	YB 1_pS102	YBX1	CST	2900	835	Rabbit	Validated

Validation Status*
Valid = RPPA and WB correlation > 0.7
Use with caution = RPPA and WB correlation < 0.7
Under Evaluation = Antibody has given mixed results and / or evaluated by another lab; We are in the process of (re)validating

Selected References

1. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61-70.
2. Agarwal R, Gonzalez-Angulo AM, Myhre S, Carey M, Lee JS, Overgaard J, et al. Integrative analysis of cyclin protein levels identifies cyclin b1 as a classifier and predictor of outcomes in breast cancer. Clin Cancer Res 2009;15:3654-62.
3. Amit I, Citri A, Shay T, Lu Y, Katz M, Zhang F, et al. A module of negative feedback regulators defines growth factor signaling. Nat Genet 2007;39:503-12.
4. Ashour AA, Abdel-Aziz AA, Mansour AM, Alpay SN, Huo L, Ozpolat B. Targeting elongation factor- 2 kinase (eEF-2K) induces apoptosis in human pancreatic cancer cells. Apoptosis 2013.
5. Bartholomeusz C, Gonzalez-Angulo AM, Kazansky A, Krishnamurthy S, Liu P, Yuan LX, et al. PEA- 15 inhibits tumorigenesis in an MDA-MB-468 triple-negative breast cancer xenograft model through increased cytoplasmic localization of activated extracellular signal-regulated kinase. Clin Cancer Res 2010;16:1802-11.
6. Byers LA, Diao L, Wang J, Saintigny P, Girard L, Peyton M, et al. An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. Clin Cancer Res 2013;19:279-90.
7. Byers LA, Sen B, Saigal B, Diao L, Wang J, Nanjundan M, et al. Reciprocal regulation of c-Src and STAT3 in non-small cell lung cancer. Clin Cancer Res 2009;15:6852-61.
8. Byers LA, Wang J, Nilsson MB, Fujimoto J, Saintigny P, Yordy J, et al. Proteomic profiling identifies dysregulated pathways in small cell lung cancer and novel therapeutic

targets including PARP1. Cancer Discov 2012;2:798-811.

9. Creighton CJ, Fu X, Hennessy BT, Casa AJ, Zhang Y, Gonzalez-Angulo AM, et al. Proteomic and transcriptomic profiling reveals a link between the PI3K pathway and lower estrogen receptor (ER) levels and activity in ER+ breast cancer. Breast Cancer Res 2010;12:R40.
10. Davies MA, Fox PS, Papadopoulos NE, Bedikian AY, Hwu WJ, Lazar AJ, et al. Phase I study of the combination of sorafenib and temsirolimus in patients with metastatic melanoma. Clin Cancer Res 2012;18:1120-8.

11. Fu S, Hennessy BT, Ng CS, Ju Z, Coombes KR, Wolf JK, et al. Perifosine plus docetaxel in patients with platinum and taxane resistant or refractory high-grade epithelial ovarian cancer. Gynecol Oncol 2012;126:47-53.

12. Gao M, Liang J, Lu Y, Guo H, German P, Bai S, et al. Site-specific activation of AKT protects cells from death induced by glucose deprivation. Oncogene 2013.

13. Gonzalez-Angulo AM, Hennessy BT, Meric-Bernstam F, Sahin A, Liu W, Ju Z, et al. Functional proteomics can define prognosis and predict pathologic complete response in patients with breast cancer. Clin Proteomics 2011;8:11.

14. Gonzalez-Angulo AM, Liu S, Chen H, Chavez-Macgregor M, Sahin A, Hortobagyi GN, et al. Functional proteomics characterization of residual breast cancer after neoadjuvant systemic chemotherapy. Ann Oncol 2013;24:909-16.

15. Gonzalez-Angulo AM, Meric-Bernstam F, Chawla S, Falchook G, Hong D, Akcakanat A, et al. Weekly nab-Rapamycin in Patients with Advanced Nonhematologic Malignancies: Final Results of a Phase I Trial. Clin Cancer Res 2013;19:5474-84.

16. Gopal YN, Deng W, Woodman SE, Komurov K, Ram P, Smith PD, et al. Basal and treatment- induced activation of AKT mediates resistance to cell death by AZD6244 (ARRY-142886) in Braf- mutant human cutaneous melanoma cells. Cancer Res 2010;70:8736-47.

17. Grote T, Siwak DR, Fritsche HA, Joy C, Mills GB, Simeone D, et al. Validation of reverse phase protein array for practical screening of potential biomarkers in serum and plasma: accurate detection of CA19-9 levels in pancreatic cancer. Proteomics 2008;8:3051-60.

18. Hayashi N, Iwamoto T, Gonzalez-Angulo AM, Ferrer-Lozano J, Lluch A, Niikura N, et al. Prognostic impact of phosphorylated HER-2 in HER-2+ primary breast cancer. Oncologist 2011;16:956-65.

19. Hennessy BT, Gonzalez-Angulo AM, Carey MS, Mills GB. A systems approach to analysis of molecular complexity in breast cancer. Clin Cancer Res 2009;15:417-9.

20. Hennessy BT, Lu Y, Gonzalez-Angulo AM, Carey MS, Myhre S, Ju Z, et al. A Technical Assessment of the Utility of Reverse Phase Protein Arrays for the Study of the Functional Proteome in Non- microdissected Human Breast Cancers. Clin Proteomics 2010;6:129-51.

21. Hennessy BT, Lu Y, Poradosu E, Yu Q, Yu S, Hall H, et al. Pharmacodynamic markers of perifosine efficacy. Clin Cancer Res 2007;13:7421-31.

22. Holder AM, Gonzalez-Angulo AM, Chen H, Akcakanat A, Do KA, Fraser Symmans W, et al. High stearoyl-CoA desaturase 1 expression is associated with shorter survival in breast cancer patients. Breast Cancer Res Treat 2013;137:319-27.

23. Ihle NT, Lemos R, Jr., Wipf P, Yacoub A, Mitchell C, Siwak D, et al. Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. Cancer Res

2009;69:143-50.
24. Jia L, Jin H, Zhou J, Chen L, Lu Y, Ming Y, et al. A potential anti-tumor herbal medicine, Corilagin, inhibits ovarian cancer cell growth through blocking the TGF-beta signaling pathways. BMC Complement Altern Med 2013;13:33.
25. Kamel D, Brady B, Tabchy A, Mills GB, Hennessy B. Proteomic classification of breast cancer. Curr Drug Targets 2012;13:1495-509.
26. Kim WY, Chang DJ, Hennessy B, Kang HJ, Yoo J, Han SH, et al. A novel derivative of the natural agent deguelin for cancer chemoprevention and therapy. Cancer Prev Res (Phila) 2008;1:577-87.
27. Kornblau SM, Tibes R, Qiu YH, Chen W, Kantarjian HM, Andreeff M, et al. Functional proteomic profiling of AML predicts response and survival. Blood 2009;113:154-64.
28. Koul D, Shen R, Kim YW, Kondo Y, Lu Y, Bankson J, et al. Cellular and in vivo activity of a novel PI3K inhibitor, PX-866, against human glioblastoma. Neuro Oncol 2010;12:559-69.
29. Liang H, Cheung LW, Li J, Ju Z, Yu S, Stemke-Hale K, et al. Whole-exome sequencing combined with functional genomics reveals novel candidate driver cancer genes in endometrial cancer. Genome Res 2012;22:2120-9.
30. Liu S, Umezu-Goto M, Murph M, Lu Y, Liu W, Zhang F, et al. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. Cancer Cell 2009;15:539-50.
31. Lu Y, Muller M, Smith D, Dutta B, Komurov K, Iadevaia S, et al. Kinome siRNA-phosphoproteomic screen identifies networks regulating AKT signaling. Oncogene 2011;30:4567-77.
32. Luyimbazi D, Akcakanat A, McAuliffe PF, Zhang L, Singh G, Gonzalez-Angulo AM, et al. Rapamycin regulates stearoyl CoA desaturase 1 expression in breast cancer. Mol Cancer Ther 2010;9:2770-84.
33. Mao M, Tian F, Mariadason JM, Tsao CC, Lemos R, Jr., Dayyani F, et al. Resistance to BRAF inhibition in BRAF-mutant colon cancer can be overcome with PI3K inhibition or demethylating agents. Clin Cancer Res 2013;19:657-67.
34. Mazzone M, Selfors LM, Albeck J, Overholtzer M, Sale S, Carroll DL, et al. Dose-dependent induction of distinct phenotypic responses to Notch pathway activation in mammary epithelial cells. Proc Natl Acad Sci U S A 2010;107:5012-7.
35. Meric-Bernstam F, Akcakanat A, Chen H, Do KA, Sangai T, Adkins F, et al. PIK3CA/PTEN mutations and Akt activation as markers of sensitivity to allosteric mTOR inhibitors. Clin Cancer Res 2012;18:1777-89.
36. Meric-Bernstam F, Chen H, Akcakanat A, Do KA, Lluch A, Hennessy BT, et al. Aberrations in translational regulation are associated with poor prognosis in hormone receptor-positive breast cancer. Breast Cancer Res 2012;14:R138.
37. Muranen T, Selfors LM, Worster DT, Iwanicki MP, Song L, Morales FC, et al. Inhibition of PI3K/mTOR leads to adaptive resistance in matrix-attached cancer cells. Cancer Cell 2012;21:227-39.
38. Nieto-Barajas LE, Muller P, Ji Y, Lu Y, Mills GB. A time-series DDP for functional proteomics profiles. Biometrics 2012;68:859-68.
39. Park ES, Rabinovsky R, Carey M, Hennessy BT, Agarwal R, Liu W, et al. Integrative analysis of proteomic signatures, mutations, and drug responsiveness in the NCI 60 cancer cell line set. Mol Cancer Ther 2010;9:257-67.

40. Raghav KP, Wang W, Liu S, Chavez-MacGregor M, Meng X, Hortobagyi GN, et al. cMET and phospho-cMET protein levels in breast cancers and survival outcomes. Clin Cancer Res 2012;18:2269- 77.
41. Sangai T, Akcakanat A, Chen H, Tarco E, Wu Y, Do KA, et al. Biomarkers of Response to Akt Inhibitor MK-2206 in Breast Cancer. Clin Cancer Res 2012;18:5816-28.
42. Sohn J, Do KA, Liu S, Chen H, Mills GB, Hortobagyi GN, et al. Functional proteomics characterization of residual triple-negative breast cancer after standard neoadjuvant chemotherapy. Ann Oncol 2013;24:2522-6.
43. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer Res 2008;68:6084-91.
44. Tibes R, Qiu Y, Lu Y, Hennessy B, Andreeff M, Mills GB, et al. Reverse phase protein array: validation of a novel proteomic technology and utility for analysis of primary leukemia specimens and hematopoietic stem cells. Mol Cancer Ther 2006;5:2512-21.
45. Tsavachidou-Fenner D, Tannir N, Tamboli P, Liu W, Petillo D, Teh B, et al. Gene and protein expression markers of response to combined antiangiogenic and epidermal growth factor targeted therapy in renal cell carcinoma. Ann Oncol 2010;21:1599-606.
46. Vasudevan KM, Barbie DA, Davies MA, Rabinovsky R, McNear CJ, Kim JJ, et al. AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. Cancer Cell 2009;16:21-32.
47. Wei X, Guo W, Wu S, Wang L, Lu Y, Xu B, et al. Inhibiting JNK dephosphorylation and induction of apoptosis by novel anticancer agent NSC-741909 in cancer cells. J Biol Chem 2009;284:16948-55.
48. Wong KK, Izaguirre DI, Kwan SY, King ER, Deavers MT, Sood AK, et al. Poor survival with wild- type TP53 ovarian cancer? Gynecol Oncol 2013;130:565-9.
49. Zhang L, Wei Q, Mao L, Liu W, Mills GB, Coombes K. Serial dilution curve: a new method for analysis of reverse phase protein array data. Bioinformatics 2009;25:650-4.
50. Zhang X, Claerhout S, Prat A, Dobrolecki LE, Petrovic I, Lai Q, et al. A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. Cancer Res 2013;73:4885-97.
51. Zwang Y, Sas-Chen A, Drier Y, Shay T, Avraham R, Lauriola M, et al. Two phases of mitogenic signaling unveil roles for p53 and EGR1 in elimination of inconsistent growth signals. Mol Cell 2011;42:524-35.

APPENDIX G COLLECTION AND PROCESSING OF TUMOR BIOPSY AND SAMPLES

MATERIALS & EQUIPMENT REQUIRED

- Liquid nitrogen (LN_2)
- Sterile, disposable tweezers (Fisher #NC9566754)
- Two cryogenic vials per biopsy sample (one cryogenic vial), placed on dry ice in a small insulated bucket (Fisher #72.694.006)
- One cryogenic vial per biopsy sample filled with 2mL 10% formalin
- 50-mL conical tubes (Fisher #14-432-22)
- Computer with specimen processing data sheet
- 3 sample ID labels per biopsy (2 ID labels if collecting at MDACC) with date and time function
- -80°C freezer
- Thermoflask cooler
- Sterile, disposable razor blades

PROCEDURES

1. Print out labels for each biopsy in advance. The labels should include protocol #, patient ID, specimen type, dose level, cycle and time-point of pre-dose or post-dose.
2. The specimen will be divided into 2 portions, one of which will be snap frozen in LN_2 and one of which will be fixed in 10% formalin and sent to the respective MDACC laboratories.
3. Send an email in advance to advice recipients (e.g., laboratory designees, study coordinator, etc.) of scheduled biopsy and the estimated specimen processing time and estimated shipping time.
4. Prepare and bring necessary lab supplies and reagents including a Thermoflask cooler containing approximately 2/3 full LN_2, tweezers, sterile razor blades, cryogenic vials on dry ice, one cryogenic vial filled with 2mL 10% formalin, lab notes and labels.
5. Arrive at the surgery unit about 10 minutes ahead of the scheduled procedure giving time to set up lab supplies, reagents ready for biopsy specimen processing and data recording on time.
6. Complete specimen processing documentation sheet to record time for each processing step as listed on the label sheet and batch record, including:

    a) Local anesthesia administered. Please note: there is a possibility that epinephrine in small quantities may interfere with the assay. Therefore, the local anesthesia used for tumor biopsies should be **only** lidocaine whenever possible and case report forms should specify what agents (i.e., lidocaine alone or lidocaine plus epinephrine) were used for each biopsy procedure.
    b) Skin incision
    c) Guide needle introduced
    d) Guide needle placement confirmed
    e) biopsy needle introduced
    f) portion of biopsy specimen transferred into tube(s) for LN_2

g) portion of biopsy specimen transferred into tube containing 10% formalin

h) Return to the laboratory specimen processing site
i) Cryopreserved biopsies placed at -80°C.

7. For biopsy specimens that are to be snap frozen in LN_2, transfer specimen into a labeled cryogenic vial that is pre-chilled in dry ice, and immediately drop the vial with specimen into LN_2. Dispose of the tweezers into medical waste for sharps.

8. For biopsy specimens that are to be fixed in formalin, transfer specimen into a labeled vial containing 2mL 10% formalin. Dispose of the tweezers into medical waste for sharps.

9. Transport all biopsy specimens with double container from surgical suite to sample processing lab.

10. Transfer all cryopreserved biopsy specimens from LN_2 to -80°C and store until ready to ship or process.

APPENDIX H PROCEDURES FOR ISOLATING PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) AND PLASMA

Isolation of PBMCs:

Equipment

- Two BD Vacutainer CPT Cell Preparation Tube with EDTA
- 50 ml size plastic conical centrifuge tubes with caps
- Pasteur pipettes
- Temperature-controlled centrifuge with swinging bucket rotor and adapters for 16 X 125 mm tube size
- Coulter counter or hemocytometer

Reagents

- Phosphate Buffered Saline (PBS) without Ca^{++} or Mg^{++}
- 0.4% Trypan blue

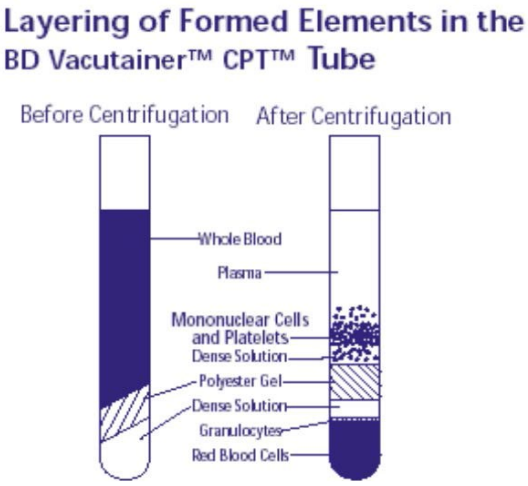
Procedures

1. The BD Vacutainer CPT Tube with EDTA should be used at room temperature (18-25^{o}C) and properly labeled for patient identification and all related information.
2. Collect 8 mL of blood each into two Vacutainer CPTs. Invert tubes approximately 8-10 times to ensure that the whole blood is mixed thoroughly with the anticoagulant.
3. Store tubes upright at room temperature until centrifugation. For best results, centrifuge the tubes within 30 minutes after blood collection.
4. Within 30 minutes of the blood draw, centrifuge samples at **room temperature (18-25 ^{o}C)** for 20 minutes at 1700 RCF (relative centrifugal force.)

   Note: Check to see that the tube is in the proper centrifuge adapter. Excessive centrifuge speed (over 2000 RCF) may cause tube breakage, exposure to blood and possible injury. To calculate the correct centrifuge speed for a given RCF, use the following formula:

$$\text{RPM Speed Setting} = \sqrt{\frac{(\text{RCF}) \times (100{,}000)}{(1.12) \times (r)}}$$

   The variable "r" is the radial **distance in centimeters** from the centrifuge center post to the tube bottom, when the tube is positioned horizontally. RCF is the desired relative centrifugal force, 1700 in this case. (2.5cm = 1 inch).
5. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see below figure).



6. IMMEDIATELY after centrifugation, collect the plasma layer and aliquot into labeled screw cap cryovials at 1mL per vial and freeze at -80°C. Plasma samples will be used in ELISAs as outlined in section 9.3.

   Collect PBMC according to the following procedures:

   - Gently invert each vacutainer tube 3 times to resuspend leftover layer (which contains the PBMCs). Under sterile conditions, decant the liquid from the Vacutainer CPT tubes into a 50 ml conical tube with cap. Discard the vacutainer containing red blood cells.
   - Centrifuge the conical tube at 300 RCF for 10 minutes at **4°C**.
   - Aspirate as much supernatant as possible without disturbing the cell pellets. This should be achieved by progressively tilting the tube while having the pipette tip touch the side of the tube. Do not lower pipette tip close to the cell pellet.

PBMC Counting

7. Add cold PBS to bring the volume to 10 ml. Re-suspend cell pellets by tapping the tube with fingers, then mix by pipetting gently to get an equal cell distribution.
8. Use either an electronic coulter counter or hemocytometer to determine the number of cells/mL..

PBMC Aliquoting:

9. Centrifuge the original cell suspension from Step 7 at 300 RCF for 10 min at 4°C.
10. Immediately after centrifugation, carefully remove and discard supernatant without disturbing the cell pellet.
11. Based on the cell count, add appropriate amount of PBS to adjust the cell concentration to 1 x 10^6/mL. Re-suspend the cell pellets by tapping the tube with your finger until no clumps are visible.
12. Label the appropriate number of microcentrifuge tubes according to the number of cells available.

13. Aliquot the cells to microcentrifuge tubes:

- Aliquot cells at 1 mL (1 x 10^6 cells) per tube. If there are not enough cells for two tube, aliquot 1 mL (1 x 10^6 cells) to one tube, and the rest to the other tube.
- Centrifuge the tubes at 2862 RCF for 5 minutes at 4^{o}C.
- Carefully remove all remaining supernatant from the microcentrifuge tube, again being careful not to disturb the pellet.
- Freeze the PBMC pellets immediately at -80^{o}C.

Preparation of Platelet Rich Plasma

1. Collect whole blood in labeled 4.5 mL CTAD blue hemogard-top vacutainer tube (BD Medical #367947) tubes and mix thoroughly by inversion.

2. Centrifuge the blood tube at 180 x g (RCF) for 12 minutes at room temperature.

***NOTE:** The RCF varies according to the centrifuge rotor radius. The formula for computing RCF from rotation speed and centrifuge radius is RCF = 11.2r (RPM/1000)2, where r is rotor radius, in cm, and RPM is the rotations per minute setting of the centrifuge.

3. Without disturbing the bottom red blood cell layer, slowly and carefully collect most of the platelet rich plasma (PRP) from the top layer of the tube (within 5mm of meniscus) using a P- 1000 pipettor**.** Transfer the PRP into a pre-labeled 15 mL polypropylene tube. Discard the remaining red blood cell layer appropriately.

4. Centrifuge the 15ml tube containing the PRP at 1800 x g (RCF) for 10 minutes at room temperature. A white pellet should be visible at the bottom of the centrifuge tube.

5. Gently remove and discard all of the plasma supernatant using a P-1000 pipettor without disrupting the white platelet pellet.

6. Add 1mL of sterile PBS to the pellet and gently re-suspend the pellet by pipetting up and down. Transfer the entire suspended pellet to the previously labeled 1.5ml microfuge tube OR cryovial.

7. Centrifuge the cryovial OR microcentrifuge tube at **450 x g for 5 minutes** at room temperature. Discard the supernatant.

8. Record the date and time of collection and the subject‟s initials on the label in permanent ink. Secure the label by wrapping with clear tape.

9. Quick freeze the pellet by embedding the cryovial (or microfuge tube) in dry ice for 10 minutes and then transfer the frozen cryovial into a secure, monitored -70°C freezer.

Samples will be kept in -80◦C at the following address: Meric-Bernstam lab,
M.D. Anderson Cancer Center
1515 Holcombe Blvd. Room T5.3849
Houston, TX 77030

APPENDIX I IMMUNOHISTOCHEMICAL (IHC) MARKER TEMPLATE FOR INTEGRAL MARKERS IN CLINICAL TRIALS

Immunohistochemical (IHC) Marker Template For Integrated Markers in Clinical Trials

This is a template to describe the analytical and clinical performance of an assay that is essential for performance of a trial. It will be used to assess whether assays are ready for use in a trial by Disease Steering Committees and CTEP. The FDA may also use it to evaluate integral assays and diagnostics for their pre-IDE evaluation. Not all parameters may be known a priori. Please enter as much information as you can and N/A for not available or applicable where appropriate.

This template requires detailed information that may be known only by laboratorians, scientists who work in clinical laboratories, and should be collaborating closely with clinical trialists. Please be sure to collect the appropriate responses before filling out this form. The template has the following sections with information needed from trialists and laboratorians:

1. **Assay, Patient and Specimen Information** –Trialists and Laboratorians
2. **Primary Antibody Characteristics** – Laboratorians
3. **Design of Immunohistochemical Assay** - Laboratorians
4. **Assay Performance** – Laboratorians
5. **Laboratory Information** – Trialists and Laboratorians

Section 1. Assay, Patient and Specimen Information

A. Name of marker (Please use *HUGO Gene Nomenclature Committee (HGNC)* gene or protein name for molecular marker or the *Atlas of Genetics and Cytogenetics in Oncology and Haematology* for cytogenetic or FISH markers)
HGNC Site: http://www.genenames.org/
Atlas Site: http://atlasgeneticsoncology.org/index.html

Marker name: pAkt (Ser473), pS6(235/236), pS6(240/244), VEGFR, IGF-1R, p4EBP1(Thr70)

B. How will assay and its marker be used in clinical trial?
__ Integral Marker __ Integrated Marker _X_ Research Marker

- Integral markers are required for the trial to proceed (e.g., patient eligibility, assignment to treatment, stratification, risk classifier or medical decision-making -often requires performance in a CLIA laboratory).
- Integrated markers are performed on all or a statistical subset of patients but are not used for medical decision-making.
- Research markers are all other assays and commonly referred to as correlative research.
- For other definitions, please see References at end of form.

B1. Assay Purpose
_X_ Treatment Assignment __ Eligibility Criterion
__ Stratification Factor __ Other (Specify) _____

C. Assay type
_X_ IHC __ ISH __ FISH __ ELISA __ Microarray __ RT-PCR
__ Other (Specify) _____

D. Will the assay be performed in a central reference CLIA lab, multiple CLIA-certified labs, or in research labs?
__ Central Reference CLIA Lab __ Multiple CLIA Labs _X_ Research Labs

E. Anatomic source of specimens (organ site) _All Comers

E1. Type of Specimen __ N/A
__ ascites __ bone marrow __ cell __ normal __ plasma __ serum
__ blood __ buccal mucosa __ CSF _X_ tumor __ skin __ pleural fluid __ urine __ Other (Specify) _____

E2. Tissue collection

__ mandatory (must be performed on trial)
_X_ mandatory on consent (must be performed when consent obtained)
__ voluntary
__ not specified

F. Patient conditions or co-morbidities that may affect assay and must be noted: NA

G. Preanalytic Specimen Requirements

G1. Maximum Warm ischemia time (= time from cutting blood supply to removal from body) allowed in minutes if known __ Known _X_ Unknown

Please specify if known ____ Minutes

G2. Maximum Cold ischemia time (= time from removal from body to being frozen or put into preservative) allowed in minutes if known __ Known _X_ Unknown

Please specify if known _____ Minutes

G3. Type of stabilization of Specimen: _X_ Fixed __ Frozen __ Both

G3a. If fixed, what fixation buffer? __ Bouin's _X_ 10% Neutral Buffered formalin
__ Other (Specify) _____

G3a1. What is the shortest fixation time allowed (Hours or fractions thereof)?
6 hrs
G3a2. What is the longest fixation time allowed (Hours or fractions thereof)?
24 hrs

G3b. If frozen, how will the specimen be frozen?
_X_ Flash Frozen (to -80°C)
__ Embedded in OCT and then frozen
__ Cryopreserved with controlled rate freezing

H. How will the specimens be stored?

__ -20°C
__ -80°C
__ -100°C to -130°C
__ Vapor Phase Liquid Nitrogen
__ 4°C
_X_ Room Temperature

I. Specimen size to be stored __Inches __ Centimeters

Length _____
Width _____
Height _____

J. Tissue section thickness on slide in microns

_4_

K. Antigen retrieval solution/procedures

_Slides will be subjected to HEIR (heat induced epitope retrieval) in 10 mmol/L citrate buffer (pH 6.0) for 20 min. Endegenous peroxidase activity will be blocked for 10 minutes in 0.3% hydrogen peroxide. Slides will be blocked with 1% goat serum for 1 hour at room temperature and subsequently primary antibodies will be dispensed on tissue

Section 2. Primary Antibody Characteristics

A. Source of primary antibody (purchased from xxx as lot # xxx, or generated in house, etc.)

purchased from Cell Signal

B. What was the immunogen

__ Protein __ Peptide __ Oligosaccharide __ Phosphorylated Protein
__ Other (describe) _____

B1. Species of immunogen (e.g., human or mouse gene product)

X Human __ Mouse __ Recombinant __ Other (Specify) _____

B2. Are there specific isoform(s) of the immunogen that are recognized (e.g., one or all isoforms or unknown)?

__ One Isoform __ All Isoforms X Unknown

B3. Preparation of immunogen

__ Purified Protein __ Recombinant __ Synthetic Peptide __ Oligosaccharide

C. Other attributes of the primary antibody

C1. X Monoclonal __ Polyclonal

C2. __ Human
__ Mouse
X Rabbit
__ Goat
__ Horse
__ Chicken
__ Other (specify) _____

D. How was the antibody specificity demonstrated?
__ IHC X Western Blot __ Immunoprecipitation __ Immunocompetition
__ Other (specify) _____

D1. Are there band(s) at the expected mass(es) on a Western blot?
X Yes __ No __Unknown

If no, please explain _____

D2. Is immunostaining abolished in knock out/knock-down cells or with epitope-absorbed antibody?
__ Yes __ No X Unknown

D3. Is immunostaining abolished when antibody absorbed or blocked with epitope?
__ Yes __ No X Unknown

E. What is the targeted organ/tissue/cell (e.g., normal melanocytes, breast ductal carcinoma)?
all tissue types_

E1. What non-targeted organ/tissue/cell is also stained?
_positive and negative controls_____

F. Have any cross-reactive proteins or peptides been identified that may confound interpretation of IHC?
__ Yes X No __ Unknown

If yes and known, what are they? _____

K. Is the antigen stable when the period between tissue sectioning and staining is
__ <7 days __ 7-30 days __ >30 X Not Known

Section 3. Design of Immunohistochemical Assay

A. Assay Design (Complete assay details are needed if multiple labs will perform the assay).

A1. Describe the platform of the assay, e.g. instrument (manufacturer, model, UDI number if known)

A1a. Platform _____

A1b. Manufacturer _ Thermo Scitific Autostainer

A1c. Model Number _____

A1d. UDI Number (Universal Device Number) AS1014A0905

A1e. Is the platform cleared or approved by the FDA
__ Yes __ No X Unknown

A2. Is there an SOP?
__ Yes __ No X Unknown

A2a. Is the SOP attached as an Appendix?
__ Yes X No __ Unknown

B. Type of Immunoassay

B1. Is the assay qualitative, semi-quantitative or quantitative?
X Qualitative __ Semi-quantitative __ Quantitative

B1a. If an image analyzer is used, what manufacturer and model was used?
_____

B1b. Is it cleared or approved by the FDA?
__ Yes __ No X Unknown

B2. Nature of the reporter signal
_____

B3. Assay method (e.g. direct, indirect, 3-step immunoperoxidase assay)
__ direct X indirect __ 3-step Immunoperoxidase
__ other (specify) _____

B3a. What secondary reagent(s) is used for the indirect or 3-step assay
_polymer detection kit_____

C. Are there positive and negative controls for the assay?
X Yes __ No __ Unknown

C1. If there are controls, what are they?
468 DMSO treated cell lines and ZR75-1 MK2206 treated cell lines

D. What is the smallest specimen that can be analyzed by the assay? <1.0__ cm

D1. Is the minimum specimen size determined by a particular characteristic of the tissue?
__Yes __ No _X Unknown

D1a. If so, is it
__ Number of cell nuclei __ Nuclear area
__ Cytoplasmic area _X_ Other (specify) _at minimum must have 20% tumor____

Section 4. Assay Performance

A. Details regarding how the analysis is measured

A1. What statistical test(s) were used to validate the assay results
_ no statistical test was used. Pls see section B for reproducibility of assay for validation purposes _____

A2. How was a clinically relevant threshold selected?
__ Literature __ Pilot Clinical Study __ Medical Practice Guidelines
__ Non-clinical data (e.g., *in vitro* or *in vivo* animal) _X_ Other (specify) _____

A3. Were results obtained on retrospective or prospective data sets?
__ Retrospective _X_ Prospective Sample Size __30_____

A3a. Training sets or other validation method
X Separate Training & Validation Sets __ Other Method (specify) _____

A4. What is the cut-off? _____

A5. How well was the cut-off validated before using it in these trials? _____

A6. Were assay conditions standardized to minimize variance, (e.g., automated tissue processors and/or strainers)?
X Yes __ No __ Unknown
If yes, what tissue processor/stainer was used? __Thermo Scientific Autostainer___

A7. Were calibrators/controls used? _X Yes __ No __ Unknown

A7a. Were the controls stained as separate slides with slides?
X Yes __ No __ Unknown

A7b. Were the controls included in each slide and stained as internal controls?
__ Yes __ No __ Unknown

A7c. Were the controls not stained in each staining run?
__ Yes __ No __ Unknown

B. Reproducibility of assay

B1. Was reproducibility assessed?
X Yes __ No __ Unknown

If yes, please describe the specimen type(s) used
_used cell lines of known protein expression confirmed by Western Blot to make positive and negative controls._____

If no, please explain
_____

B2. How many replicates were done? _Antibody was titered prior to running 5 sets of 10 known positive and negative controls._____

B3. What is the intra-lab reproducibility (%CV)? __95%___

B4. What is the inter-lab reproducibility (same specimens, different lab, and number of different technicians)? __95%___

B4a. How many on the same specimens?
_50 total of each positive and negative_____

B4b. How many different labs?
_one_____

B4c. How many different technicians?
_____

B4d. What types of specimens (e.g., tissue sections, TMA)?
___cell pellet____

B4e. Over how many different days?
___several weeks____

B4f. How many readers?
_one____

B5. What is the agreement between readers? _____

B5a. How are differences resolved?
X Different runs of the same assay
__ Different runs of another assay of the same technology
__ Different runs of another assay of a different technology
__ Different reading by the same reader or instrument
__ Different reading by a different reader or instrument
__ Panel or arbitration
__ Other (please specify) _____

C. Image Measurement

C1. What strategy was used to select the fields to be analyzed?
_____

C2. How was a threshold to distinguish positive from negative determined?
_____

C3. How were the cells of interest distinguished from other cells?
_____

C4. Was reference material used to generate a standard curve?
__ Yes __ No __ Unknown

C4a. What was the reference material?
_____

C4b. Has it been cleared by the FDA?
__ Yes __ No __ Unknown

D. Assay Discrimination

D1. What is the accuracy of the assay for detecting the analyte? _____

D2. How are staining and tissue artifacts identified and handled (especially if image analysis is used)? _____

Section 5. Laboratory Information

A. Will the assay be performed in a research or clinical lab?
X Research __ Clinical

B. Does the lab meet GLP standards
_X Yes __ No __ Unknown

C. What is the training and experience of the Technician/Operators? _Senior Histotechnologist has 20+ years experience in processing IHC assays_____

References

(Section, Ref #, Citation)

1. 20215558 Dancey JE, Dobbin KK, Groshen S, Jessup JM, Hruszkewycz AH, Koehler M, Parchment R, Ratain MJ, Shankar LK, Stadler WM, True LD, Gravell A, Grever MR; Biomarkers Task Force of the NCI Investigational Drug Steering Committee. Guidelines for the development and incorporation of biomarker studies in early clinical trials of novel agents. Clin Cancer Res. 2010 Mar 15;16(6):1745-55.

1.A 18327244 Deutsch EW, Ball CA, Berman JJ, Bova GS, Brazma A, Bumgarner RE, Campbell D, Causton HC, Christiansen JH, Daian F, Dauga D, Davidson DR, Gimenez G, Goo YA, Grimmond S, Henrich T, Herrmann BG, Johnson MH, Korb M, Mills JC, Oudes AJ, Parkinson HE, Pascal LE, Pollet N, Quackenbush J, Ramialison M, Ringwald M, Salgado D, Sansone SA, Sherlock G, Stoeckert CJ Jr, Swedlow J, Taylor RC, Walashek L, Warford A, Wilkinson DG, Zhou Y, Zon LI, Liu AY, True LD. Minimum information specification for in situ hybridization and immunohistochemistry experiments (MISFISHIE). Nat Biotechnol. 2008 Mar;26(3):305-12.

1.B OBBR https://brd.nci.nih.gov/BRN/brnHome.seam?conversationId=12427

1.F1 12414521 Dash A, Maine IP, Varambally S, Shen R, Chinnaiyan AM, Rubin MA. Changes in differential gene expression because of warm ischemia time of radical prostatectomy specimens. Am J Pathol. 2002 Nov;161(5):1743-8.

1.F2 19734848 Khoury T, Sait S, Hwang H, Chandrasekhar R, Wilding G, Tan D, Kulkarni S. Delay to formalin fixation effect on breast biomarkers. Mod Pathol. 2009 Nov;22(11):1457-67.

1.F1-2. 19415952 Middleton LP, Price KM, Puig P, Heydon LJ, Tarco E, Sneige N, Barr K, Deavers MT. Implementation of American Society of Clinical Oncology/College of American Pathologists HER2 Guideline Recommendations in a tertiary care facility increases HER2 immunohistochemistry and fluorescence in situ hybridization concordance and decreases the number of inconclusive cases. Arch Pathol Lab Med. 2009 May;133(5):775-80.

2.E 20093391 Brevet M, Arcila M, Ladanyi M. Assessment of EGFR mutation status in lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR. J Mol Diagn. 2010 Mar;12(2):169-76.

20359301 Bordeaux J, Welsh A, Agarwal S, Killiam E, Baquero M, Hanna J, Anagnostou V, Rimm D. Antibody validation. Biotechniques. 2010 Mar;48(3):197-209.

18796405 Pradidarcheep W, Labruyère WT, Dabhoiwala NF, Lamers WH. Lack of specificity of commercially available antisera: better specifications needed. J Histochem Cytochem. 2008 Dec;56(12):1099-111.

2.E7 NCBI Blast Search http://blast.ncbi.nlm.nih.gov/Blast.cgi

4. 20524870 Fitzgibbons PL, Murphy DA, Hammond ME, Allred DC, Valenstein PN. Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays. Arch Pathol Lab Med. 2010 Jun;134(6):930-5.

ISBN978-0-387-72805-6 Discrete Multivariate Analysis: Theory and Practice. Authors: Paul W. Holland (Author)Stephen E. Fienberg (Author)Yvonne M. Bishop (Author)F. Mosteller (Contributor)R.J. Light (Contributor). Springer. 30 July 2007. English. Paperback, 558 pages

18829475 Chau CH, Rixe O, McLeod H, Figg WD. Validation of analytic methods for biomarkers used in drug development. Clin Cancer Res. 2008 Oct 1;14(19):5967-76.

Appendix to CLSI document IL-28a

Appendix to CLSI document IL-28a

4.B5 2189948 Cicchetti DV, Feinstein AR. High agreement but low kappa:

II. Resolving the paradoxes. J Clin Epidemiol. 1990;43(6):551-8.

4.B 11459866 Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001 Jul 18;93(14):1054-61.

4.B 20586616 Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, Hicks DG, Lester S, Love R, Mangu PB, McShane L, Miller K, Osborne CK, Paik S, Perlmutter J, Rhodes A, Sasano H, Schwartz JN, Sweep FC, Taube S, Torlakovic EE, Valenstein P, Viale G, Visscher D, Wheeler T, Williams RB, Wittliff JL, Wolff AC; American Society of Clinical Oncology; College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med. 2010 Jul;134(7):e48-72.

4.8 2578140 Gross DS, Rothfeld JM. Quantitative immunocytochemistry of hypothalamic and pituitary hormones: validation of an automated, computerized image analysis system. J Histochem Cytochem. 1985 Jan;33(1):11-20.