FOX CHASE CANCER CENTER
TEMPLE HEALTH

GU-071 *Phase I/II Study of Ixazomib with Pegylated IFN-alpha 2b (pIFN) in Metastatic Renal Cell Carcinoma (mRCC)*

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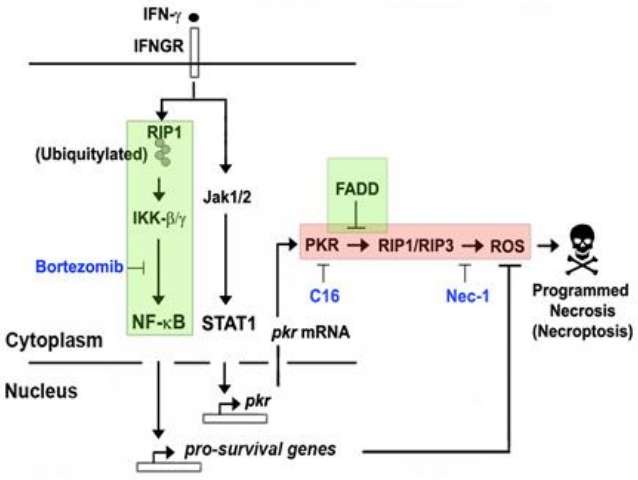
PROTOCOL SUMMARY

Title of Study: Phase I/II Study of Ixazomib with Pegylated IFN-alpha 2b (pIFN) in Metastatic Renal Cell Carcinoma (mRCC)

Investigators: Daniel M. Geynisman, M.D. (PI), Elizabeth Plimack, M.D., M.S., Yu-Ning Wong, M.D., MSCE, Marijo Bilusic, M.D., PhD.

Study Center(s): Fox Chase Cancer Center, Temple University Health System

Concept and Rationale: The Balachandran laboratory at Fox Chase Cancer Center has discovered that interferon (IFN) induces necrotic death in cells lacking either NF-kB pro-survival signaling or the adaptor protein FADD. Although FADD is currently not druggable, NF-kB can be disabled by the proteasome inhibitor bortezomib, and the Balachandran lab has shown that IFN induces the selective necrotic demise of renal cancer cells when NF-kB pro-survival signaling is neutralized by bortezomib. Mechanistically, NF-kB induces the expression of antioxidant enzymes that quench reactive oxygen species (ROS) and prevent accrual of these toxic respiratory byproducts. When NF-kB is disabled, IFN induces ROS accumulation to toxic levels, leading to mitochondrial dysfunction, respiratory failure, and eventual necrotic death. As cancer cells are more reliant on mitochondrial bioenergetics than normal, quiescent cells, it is expected that IFN-induced necrosis in the setting of NF-kB inhibition will be selective for tumor.



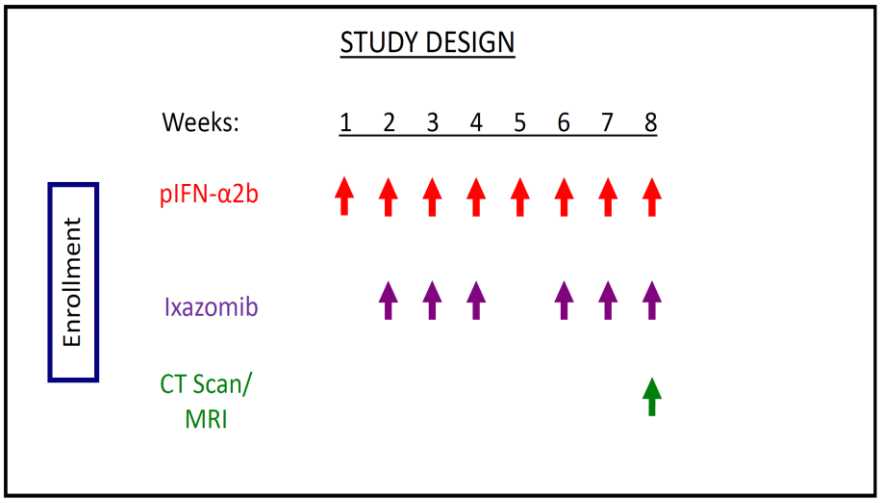
Simultaneous to the above work, Millennium has developed the first orally available proteasome inhibitor, ixazomib. Given the similarities in *in vitro* potency and specificity for proteasome active sites between bortezomib and ixazomib, we propose to test the preclinical findings from the bortezomib/IFN combination in RCC to a phase I/II trial of ixazomib in combination with pegylated IFN-alpha 2b in mRCC patients.

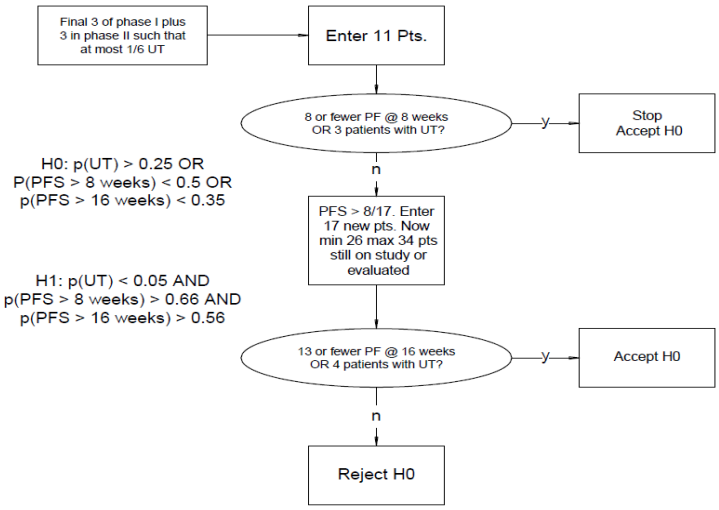
We hypothesize that the combination of ixazomib with pegylated IFN-α will lead to increased necrotic cell death in RCC tumors and consequent clinical benefit to patients.

Phase I Primary Objective: To determine the safety, tolerability and RP2D of the combination of pegylated interferon with ixazomib.

Phase II Primary Objective: To determine progression free survival (PFS)

Phase II Secondary Objectives: To determine the composite rate of unacceptable toxicity at 8 weeks To determine the overall response rate (ORR) using RECIST v1.1
Phase II Exploratory Objectives: **1.** To examine changes in STAT1 phosphorylation and MHC1 up regulation in PBMCs as a pharmacodynamics indicator of IFN activity **2.** To use archived FFPE tumor tissue to correlate activated tissue stroma (i.e., desmoplasia) with serum IL-6 levels and patient outcomes **3.** To use archived FFPE from past biopsies for IHC analysis of nuclear NF-κB p65 and phospho-STAT1 in order to test whether those patients with the most apparent constitutive pathway activation related to either of these two markers are generally more or less responsive to the combination therapy.
Phase I Primary Endpoint: **1.** Presence or absence of dose-limiting toxicity during one cycle of pegylated interferon alpha with ixazomib
Phase II Primary Endpoint: **1.** Progression free survival at 8 and 16 weeks using RECIST v 1.1.
Phase II Secondary Endpoints: **1.** Unacceptable toxicity rate at 8 weeks (any grade 5 toxicity, grade 4 neuropsychiatric toxicity or grade 4 clinically significant non-hematologic toxicity thought to be definitely, probably or possibly related to study drug) **2.** Overall response rate at 8 and 16 weeks using RECIST v1.1.
Exploratory Endpoints for Phase I and II: **1.** To examine changes in STAT1 phosphorylation and MHC1 up regulation in PBMCs as an indicator of IFN activity **2.** To use archived FFPE tumor tissue to correlate activated tissue stroma (i.e., desmoplasia) with IL-6 and patient outcomes **3.** To use archived FFPE from past biopsies for IHC analysis of nuclear NF-κB p65 and STAT1.
Study Design: The study will be conducted at a single center: Fox Chase Cancer Center. It will be open label and non-randomized. It will begin with a '3+3' phase I component to asses initial safety, tolerability and RP2D, followed by a phase II using a two-stage design. The design is meant to incorporate an early stopping rule for futility and toxicity, and to allow for an endpoint of PFS at 8 and 16 weeks rather than overall response. We believe PFS is a more valid endpoint given the scientific rationale of tumor necrosis rather than apoptosis resulting in radiographic disease stabilization rather than shrinkage.
Schema:





Number of Patients:

1. Phase I: Minimum=12; Maximum=18; final 3 patient being part of Phase II
2. Phase II: Minimum=14; Maximum=31
3. Total maximum=49

Main Criteria for Inclusion/Exclusion:

Inclusion: Male or female patients 18 years or older; Patients must have a diagnosis of a metastatic renal cell carcinoma with a ≥50% clear cell component; Eastern Cooperative Oncology Group (ECOG) performance status 0, 1, or 2; Adequate hematologic, hepatic and renal function; Measurable disease by RECIST 1.1.; Receipt of at least two lines of prior therapy for metastatic RCC; Patients with stable brain metastasis are eligible provided they received definitive therapy (EBRT, gamma knife, surgery) no sooner than 14 days prior to registration and are off all steroids.

Exclusion: Previous use of interferon, ixazomib or bortezomib; Untreated central nervous system involvement; ≥ Grade 3 peripheral neuropathy; Grade 2 peripheral neuropathy with pain on clinical examination during the screening period.

Intervention and Mode of Delivery: Pegylated interferon alpha given subcutaneously weekly with weekly oral ixazomib.

Duration of Intervention and Evaluation:
Phase I: Evaluation at day 28 for toxicity, tolerability and dose-limiting toxicity for escalation decision. Treatment can continue indefinitely if beneficial (continual response or stable disease or symptomatic clinical benefit as defined by the investigator) to patient. Evaluation at 8 and 16 weeks for efficacy and these patients may continue indefinitely as well if showing signs of clinical benefit.
Phase II: Evaluation at 8 and 16 weeks. If treatment is beneficial (as per above definition) to the patient, it can continue indefinitely beyond 16 weeks.

Statistical Methods:
Phase I:
The first part of the study will be a phase I dose escalation enrolling at a minimum 12 candidates using a 3+3 stepwise design with doses for escalation and de-escalation and rules for each noted below.

Dose level	Ixazomib mg	Pegylated IFN µg/kg/week
-2	2.3	2
-1	3	2
1 (starting dose level)	3	3
2	3	4.5
3 (expected RP2D)	4	4.5

Phase II:
Definition of primary endpoint: Progression free survival at 8 and 16 weeks using RECIST v 1.1, defined from date of treatment initiation to date of progression according to RECIST or death, or is censored at the date of the last imaging. If patient is not evaluated at 8 and/or 16 weeks then the patient is not considered as alive and progression-free.

Definition of secondary outcomes/endpoints:
1. Composite of unacceptable toxicity at 8 weeks (grade 4 non-hematologic toxicity thought to be definitely, probably or possibly related to study drug)
2. Overall response using RECIST v1.1.

Definition of exploratory objectives:
1. Changes in STAT1 phosphorylation and MHC1 up regulation in PBMCs from pre-treatment to start of cycle 2, 3, 4, 8, 12, 14, 16 and end of study.
2. Degree of desmoplasia in archived FFPE tumor samples and change in serum IL-6
3. Degree of nuclear NF-κB p65 and STAT1 in archived FFPE tumor samples

STUDY OVERVIEW DIAGRAM

Screening with Radiographic Staging
▼
Study Entry
▼
Phase I dose escalation (12-18 patients)
▼
Phase II expansion (14- 31 patients)
▼
Progression free survival follow-up

SCHEDULE OF EVENTS

	Screening		Cycle 1				Cycle 2				Cycle 3				Cycle 4				→ future cycles	
	≤ 28 days of study registration	≤ 7 days of study registration	Wk 1	Wk 2[1]	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 13	Wk 14	Wk 15	Wk 16	Subse-quent Weeks	Off Study
Ixazomib[2]				x	x	x		x	x	x		x	x	x		x	x	x	x →	
pIFN-α 2b[3]			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x →	
Informed consent	x																			
Demographics	x																			
Medical history	x																			
Concomitant Medications		X	x				x				x				x				x[4]	x
Physical exam		X	x	x	x	x	x		x		x				x				x[4]	x
Vital signs[5]		X	x	x	x	x	x		x		x				x				x[4]	x
Adverse event monitoring		X		x	x	x	x		x		x				x				x[4]	x
Height		X																		
Weight		X	x	x	x	x	x		x		x				x				x[4]	x
Performance Status		X	x	x	x	x	x		x		x				x				x[4]	x
CBC with differential		X		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x[4]	x
Serum Chemistry[6]		X		x	x	x	x		x		x				x				x[4]	x
Serum amylase, lipase[7]		X																		
TSH, lipid panel including triglycerides[8]		X													x				x[8]	X
B-HCG (if applicable)		X																		
PT, PTT[9]		X																		
HgbA1C[10]	x																			
Ophthalmic eye evaluation[11]	x																			
Radiologic evaluation with tumor measurement	x									x[12]								x[12]	x[12]	x[12]
ECG	x																			
Blood for correlative analysis			x[13,14]	x	x		x[14]		x		x[14]				x[14]				x[14]	x[14]
Archival Tumor Procurement (optional)[15]		X																		

[1]All patient visits week 2 and thereafter should occur on the same day each week +/- 2 days to allow for logistical issues.
[2]Ixazomib: Weekly dose will depend on whether the patient is in phase I or phase II. Doses will be escalated or de-escalated in phase I and dose de-escalation can occur in phase II based on toxicity. Treatment is weeks 2-4 of each 4 week cycle (week 1 is off).
[3]pIFN: Once weekly dosing continuously. Dose will be determined based on phase of study and detailed below.
[4]At each visit which will occur every 4 weeks after the first 2 cycles.
[5]Vital signs include temperature, pulse, respiratory rate, and blood pressure and will occur at each visit.
[6]Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, lactate dehydrogensase, glucose, potassium, phosphorous, magnesium, total protein, SGOT, SGPT, sodium.
[7]Amylase and lipase will be checked at baseline and re-assessed as clinically indicated.
[8]Triglycerides and TSH have been known to be affected due to pIFN- α. Will be checked every 3 cycles.
[9]PT, PTT, INR may be checked more frequently as clinically indicated.
[10]pIFN can exacerbate diabetes mellitus
[11]If history of diabetic or hypertensive retinopathy
[12]Radiologic evaluation and tumor measurements every 2 cycles while on study (every 8 weeks) for the first 8 months (8 cycles) and then every 3 cycles (every 12 weeks) thereafter. Should be repeated at off study if not done in past 6 weeks.
[13]Blood for correlative analysis should not be performed until patient is confirmed to be eligible and enrolled into the study.
[14]Blood for exploratory analysis will be performed on Day 1, 8, 15 of Cycle 1, Day 1, 15 of Cycle 2, Day 1 of Cycle 3 and 4 (after 3 weeks of ixazomib treatment) and thereafter on Day 1 of every 2 cycles (e.g. C6, C8 …) and at off study. Blood can be collected on each day +/- 3 days.
[15]Archival tumor or metastatic site biopsy tissue will be obtained for correlative analysis. This should not be performed until patient is confirmed to be eligible and enrolled into the study. If none is available, the patient is still eligible to proceed with the trial, but every effort will be made to obtain the tissue.

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LIST OF ABBREVIATIONS AND GLOSSARY OF TERMS

Abbreviation	Term
AE	adverse event
ANC	absolute neutrophil count
ASCO	American Society of Clinical Oncology
AUC	area under the plasma concentration versus time curve
BSA	body surface area
BUN	blood urea nitrogen
CBC	complete blood count
C_{max}	single-dose maximum (peak) concentration
CR	complete response
CT	computed tomography
CYP	cytochrome P_{450}
DLT	dose-limiting toxicity
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EU	European Union
FDA	United States Food and Drug Administration
FFPE	Formalin fixed, paraffin embedded
GCP	Good Clinical Practice
G-CSF	granulocyte colony stimulating factor
GI	Gastrointestinal
GM-CSF	granulocyte macrophage-colony stimulating factor
HIV	human immunodeficiency virus
HNSTD	highest nonseverely toxic dose
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	independent ethics committee
IRB	institutional review board
IV	intravenous; intravenously
KPS	Karnofsky Performance Status
LDH	lactate dehydrogenase

Abbreviation	Term
LFT	liver function test(s)
Millennium	Millennium Pharmaceuticals, Inc., and its affiliates
MTD	maximum tolerated dose
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
OS	Overall survival
PD	progressive disease (disease progression) progressive disease
PF	Progression-free
PFS	progression-free survival
PK	pharmacokinetic(s)
PO	*per os*; by mouth (orally)
PR	partial response
PRO	patient-reported outcome
QD	*quaque die*; each day; once daily
QOL	quality of life
QTc	rate-corrected QT interval (millisec) of electrocardiograph
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	Ready for Phase 2 Dose
RCC	Renal Cell Carcinoma
SAE	serious adverse event
SC	Subcutaneous
SD	stable disease
$t_{1/2}$	terminal disposition half-life
T_{max}	single-dose time to reach maximum (peak) concentration
ULN	upper limit of the normal range
US	United States
UT	Unacceptable toxicity
WBC	white blood cell
WHO	World Health Organization

1. BACKGROUND AND STUDY RATIONALE

Metastatic Renal Cell Carcinoma

Renal cell carcinoma (RCC) is a relatively rare cancer, comprising only 3.9% of all new cancer cases in the United States, with an estimated incidence of new cancers of the kidney and renal pelvis being 65,150 cases in 2013 and leading to 13,680 deaths.[1] World-wide, RCC is diagnosed in approximately 170,000 individuals each year with over 72, 000 deaths.[2] Due to a lack of a proven screening intervention and an often clinically silent disease course, RCC often presents in an advanced or metastatic state and over half of RCC patients will at some point develop metastatic renal cell carcinoma (mRCC).[3] Once metastatic, RCC is resistant to conventional chemotherapy and radiotherapy.[4]

Over the last decade, advances in translational research have led to a rapid paradigm shift in the management of mRCC patients. With the elucidation of the von Hippel-Lindau/hypoxia-inducible factor (VHL-HIF) and mammalian target of rapamycin (mTOR) pathways in mRCC, seven new drugs have been approved since 2005 and many more are in clinical development.[5] Currently FDA approved agents include vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitors (TKIs) sunitinib, pazopanib, axitinib and sorafenib; the mTOR inhibitors temsirolimus and everolimus, and the anti-VEGF monoclonal antibody bevacizumab.[6]

Due to the above development, the median survival of patients with mRCC is now approaching 30 months (up from less than one year in 2000) and is poised to increase even further with the preliminary, but very promising results of novel immunotherapy approaches.[7,8] Many patients are now living with metastatic disease for years as they undergo sequential drug treatments, sometimes surgeries (metastasectomies and/or cytoreductive or palliative nephrectomies) and targeted radiation treatments.[9,10] Unfortunately, although most patients with mRCC will have some benefit from the above mentioned therapy, virtually all will progress and will thus be in need of new interventions.

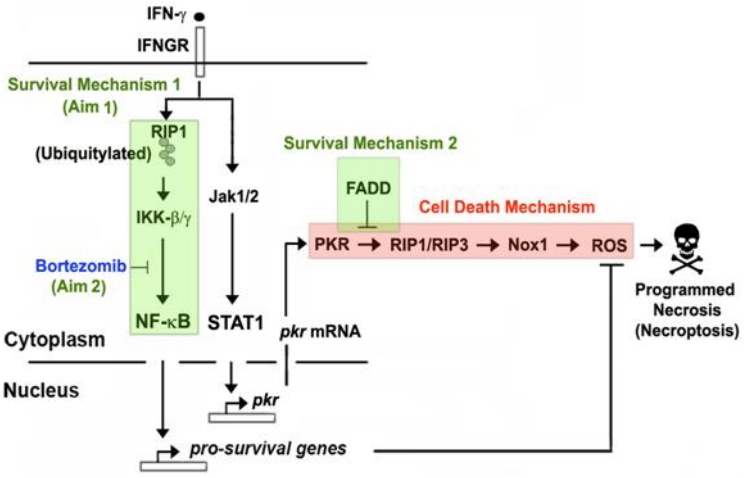
Scientific Rationale for Combination Ixazomib and IFN in RCC

At Fox Chase Cancer Center, the Balachandran laboratory has focused on studying IFN-γ (a cousin of IFN-α) in RCC. They hypothesized that given the ability of IFN-γ to provide lasting remission may stem from its pleiotropic nature: not only does IFN-γ activate a powerful immune response to the tumor, but it is also actively anti-angiogenic, and directly tumoricidal to susceptible RCC cells. Concurrent deployment of this anti-neoplastic triad represents IFN's unique advantage over small-molecule approaches, but these properties cannot be simultaneously exploited unless RCC-specific resistance mechanisms that limit IFN's tumoricidal capacity are disabled and its toxic side effects are reduced or eliminated.

The Balachandran lab showed that interferon (IFN) induces necrotic death in cells lacking either NF-kB pro-survival signaling[11] or the adaptor protein FADD.[12] When either pathway is disabled, IFN-γ activates a novel form of necrotic cell death (also called 'necroptosis') dependent on the kinases PKR, RIP1 and RIP3 (Figure 2, red shading). Although FADD is currently not druggable, NF-kB can be disabled by the proteasome inhibitor bortezomib (Velcade), and the Balachandran lab has shown that IFN induces the selective necrotic demise of renal cancer cells when NF-kB pro-survival signaling is

neutralized by bortezomib.[13] Mechanistically, NF-kB induces the expression of antioxidant enzymes that quench reactive oxygen species (ROS) and prevents accrual of these toxic respiratory byproducts. When NF-kB is disabled, IFN induces ROS accumulation to toxic levels, leading to mitochondrial dysfunction, respiratory failure, and eventual necrotic death (Figure 2). As cancer cells are more reliant on mitochondrial bioenergetics than normal, quiescent cells, it is expected that IFN-induced necrosis in the setting of NF-kB inhibition will be selective for tumor.

Figure 1-1: Novel IFN-γ-activated cell death and survival pathways for exploitation in renal cell carcinoma.



The Balachandran lab tested if Bzb could inhibit NF-κB in RCC cells and sensitize these cells to IFN-γ-induced necrosis. They found that Bzb inhibited both constitutive and inducible NF-κB activity in RCC cells (by 50-90%, depending on the cell line), comparable to that seen with a dedicated IKK inhibitor (IMD-0354).[13] Importantly, Bzb reduced the expression of key NF-κB pro-survival target genes, including the critical target *SOD2* (which encodes the mitochondrial antioxidant enzyme MnSOD). They have previously shown that *SOD2* is both necessary and sufficient for NF-κB-mediated protection against necrosis, suggesting that Bzb co-treament will sensitize RCC cells to IFN-γ-triggered necrosis.[11] Indeed, they have found that Bzb sensitized multiple ATCC- and patient-derived RCC cell lines to IFN-γ-triggered necrotic death at *clinically achievable* doses for both agents. Similar results were obtained when the selective IKK inhibitor IMD-0354 was used, indicating that NF-κB inhibition contributes (at least in part) to Bzb's ability to sensitize RCC cells to IFN-γ. Remarkably, the combination of IFN-γ + Bzb left normal kidney epithelial cells largely unharmed, for reasons we attribute to reduced dependence of constitutive NF-κB signaling (we hypothesize that RCC cells may be 'addicted' to tonic NF-κB signals). In agreement with this hypothesis, patient-derived RCC specimens display nuclear staining of the NF-κB sub-unit RelA and a strikingly-elevated 'NF-κB signature' comprising several pro-survival targets (including *SOD2*), compared to paired normal kidney tissue. Together, these findings strongly suggest that IFN-γ, in the setting of NF-κB blockade, will have clinical benefit in RCC.

Previous clinical experience with IFN in RCC

Immunotherapy with high-dose IL-2 or interferon-alpha was for many years considered the standard treatment for patients with mRCC.[14] Therapy with these agents produced overall-response rates of up to 30% and in up to 10% of the time the responses were durable. With the advent of novel therapies noted above, IFN-α fell out of favor as a frontline therapeutic once small-molecule agents became available for RCC, for two major reasons: (1) poorly-understood resistance pathways prevented IFN-α from directly killing RCC cells, and (2) its side-effect profile.

Extensive work has been done examining IFN in those with metastatic RCC.[15-17] IFN-alpha is approved by the FDA to be used in combination with bevacizumab based on two phase III trials that demonstrated a progression-free survival (PFS), but not an overall survival (OS) benefit.[18,19] In first line setting, an overall response rate (defined as complete response (CR) + PR) of ~7%-15% can be expected with single agent IFN with a 30%-50% SD rate. Furthermore, combinations of IFN with other drugs such as sorafenib,[20,21] sunitinib,[22] IL-2,[23] fluorouracil,[17] and monoclonal antibody therapy[24] has been attempted with a manageable side-effect profiles and at times efficacy signals. Furthermore, pegylated IFN has been studied in RCC with comparable results and toxicity profile to standard IFN, but allowing for once weekly administration.[25-30] Given the rapid rise in VEGF and mTOR directed therapy for mRCC over the last 5-8 years, IFN is infrequently used as a first line therapy today. Nevertheless, none of the recently approved agents for mRCC are curative and the most common response remains stabilization of disease. Thus, further development of a novel IFN based combination based on sound scientific rationale makes sense as it may provide an additional option in the armamentarium for mRCC patients who have failed first line therapy.

Previous clinical experience with proteasome inhibition (bortezomib) in RCC

Two clinical trials of bortezomib in patients with RCC have been reported. After a establishing a dose level in a phase I trial[31], Kondagunata et al treated 37 patients at a dose of 1.3-1.5 mg/m^2 twice weekly, on a two week on, one week off schedule.[32] Eighteen patients were treatment naïve and sixteen received IFN previously. Bortezomib was given i.v. twice weekly. Partial response (PR) rate was 11% with 3 patients experiencing long-term PRs (8-20 + months); stable disease (SD) rate was 38%. Common Grade 2 or higher adverse events (AEs) included constipation, fatigue, neuropathy and dyspnea. A similar study by Davis et al treated 21 patients with only a single objective PR, thus leading to study termination.[33] Though efficacy overall was modest in this study, bortezomab did show antitumor activity as a single agent for some RCC patients. Given the similarity between bortezomib and Ixazomib, we would expect similar findings with Ixazomib.

Previous clinical experience combining IFN with bortezomib

Based on preclinical work by Lesinski et al[34], a phase I trial of fixed dosed IFN-alpha 2b (5 MU/m^2 s.c three times a week) with escalating doses of bortezomib once a week (1.0 mg/m^2, 1.3 mg/m^2 and 1.6 mg/m^2 dose levels) in metastatic melanoma patients was conducted, reported at the ASCO 2013 annual meeting and the manuscript is currently

submitted for review.[35] A total of 16 patients were enrolled with most common Grade 3 toxicity being fatigue, vomiting and diarrhea. Grade 4 toxicity observed was fatigue and lymphopenia. 1 PR, 7 SD and 8 progressive disease (PD) were observed with a median PFS and OS of 2.5 and 10.3 months respectively. Based on this phase I study, a weekly dose of 1.3 mg/m^2 of bortezomib was found to be safe in combination with IFN-alpha 2b. Correlative work showed a decrease in pro-angiongenic factors in the patient with a PR. This work supports the notion that combining IFN with a proteasome inhibitor is feasible and we propose to extend it to ixazomib.

Study Rationale: Based on strong preclinical data showing complementary and novel anti-tumor activity of IFN and bortezomib in RCC noted in the Balachandran laboratory, we propose a phase I/II trial of combination pegylated IFN-alpha 2b with ixazomib in metastatic RCC patients who have failed at least two prior lines of therapy. We hypothesize that by disabling NF-kB via ixazomib, we can promote IFN induced necrotic cell death of RCC. We hypothesize that the combination of ixazomib with IFN will lead to increased necrotic cell death in RCC tumors and consequent clinical benefit to patients.

Ixazomib

Simultaneous to the above work, Millennium has developed the first orally available proteasome inhibitor, ixazomib.[36] Ixazomib and its biologically active form, MLN2238, have a similar mechanism of action to bortezomib, with the most notable difference being a more rapid binding and dissociation from the proteasome.[36] Antitumor activity has been noted in various preclinical xenograft models.[37,38] Several phase I/II trials have examined both intravenous and oral formulations of ixazomib.[39,40] Single-agent oral ixazomib MTD was established at 2.97 mg/m^2 on a weekly dosing schedule (days 1, 8, and 15 every 28 days) which was approximately equal to a fixed dose of 5.5 mg. Adverse events were mostly hematologic or gastrointestinal and were in general manageable. Importantly, peripheral neuropathy appears to be much less frequent with ixazomib compared to bortezomib (only 1 case of grade 3 peripheral neuropathy observed out of 60 patients). In solid tumors, IV formulations of ixazomib have been explored, with most common AE including fatigue, thrombocytopenia, rash and nausea. Thus, there is currently an ongoing randomized phase III trial in multiple myeloma using a weekly oral 4 mg dose of ixazomib in combination with lenalidomide and dexamethasone (clinicaltrials.gov NCT01564537).

Given the similarities in *in vitro* potency and specificity for proteasome active sites between bortezomib and ixazomib, we propose to test the preclinical findings from the bortezomib/IFN combination in RCC to a phase I/II trial of ixazomib in combination with pegylated IFN-alpha 2b in mRCC patients.

Preclinical Experience

Please refer to the current ixazomib Investigator's Brochure (IB) and Safety Management Attachment (SMA).

Clinical Experience

Ixazomib has been evaluated as an oral single agent in phase 1 studies that have included patients with advanced solid tumors, lymphoma, relapse/refractory MM (RRMM), and relapsed or refractory light-chain (AL) amyloidosis and demonstrated early signs of activity. Ongoing studies continue to investigate both single-agent ixazomib and ixazomib in combination with standard treatments. Based on encouraging preliminary data observed in patients with MM requiring systemic treatment, 2 phase 3 trials in newly diagnosed MM (NDMM) (C16014) and RRMM (C16010) patient populations are currently evaluating ixazomib in combination with Revlimid and Dexamethasone (RevDex) versus placebo/RevDex. Both trials are combining ixazomib at a weekly dose of 4.0 mg on Days 1, 8, and 15 in a 28-day cycle to a standard dose of lenalidomide with a weekly dexamethasone dose of 40 mg. In addition, ongoing clinical pharmacology studies include evaluation of drug-drug interactions with ketoconazole and rifampin, effect of food, and oral bioavailability. Studies evaluating the safety and pharmacokinetic (PK) of ixazomib alone (in Japanese patients) and in combination with lenalidomide and dexamethasone in Asian adult patients (including Japanese patients) with a diagnosis of NDMM are ongoing.

As of 27 March 2013, preliminary clinical data is available for a total of 653 patients across 13 studies. The emerging safety profile indicates that ixazomib is generally well tolerated. The adverse events (AEs) are consistent with the class-based effects of proteasome inhibition and are similar to what has been previously reported with VELCADE though the severity of some, for example peripheral neuropathy, is less. While some of these potential toxicities may be severe, they can be managed by clinical monitoring and standard medical intervention, or, as needed, dose modification or discontinuation.

Fatigue was the most common AE reported among 384 patients treated in the oral (PO) studies (47%). Other common AEs reported in the pooled intravenous (IV) and PO safety populations include nausea, thrombocytopenia, diarrhea, and vomiting. Rash is also a commonly reported treatment-emergent event; however, there is some variety in its characterization and causality resulting in different preferred terms to describe it. A high-level term outline of rash events includes rashes, eruptions and exanthems NEC; pruritus NEC; erythemas; papulosquamous conditions; and exfoliative conditions. The dose escalation phases of most trials reported in the IB have now completed enrollment, and gastrointestinal (GI) symptoms were the common dose-limiting toxicities (DLTs) when the use of prophylactic anti-emetics was not permitted per protocol. In the expansion cohorts or phase 2 cohorts (as per each study), the incidence and severity of GI symptoms was mitigated by the use of the lower maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) (as per each study) and standard clinical usage of anti-emetics and/or antidiarrheal medications as deemed appropriate. Prophylactic use of anti-emetics has not been required as with other agents but (as outlined in Section 6.7) has been used according to standard practice and are effective.

The most frequent (at least 20%) treatment-emergent adverse events (TEAEs) reported with the PO formulation pooled from single-agent studies (n = 201) irrespective of causality to ixazomib, include nausea (53%), fatigue (51%), diarrhea (44%), thrombocytopenia (34%), vomiting (38%), decreased appetite (32%), fever (21%), and anemia (21%). The most

frequent (at least 20%) TEAEs reported with the PO formulation pooled from combination trials (irrespective of the combination) (n = 173), irrespective of causality to ixazomib, include diarrhea (47%), fatigue (44%), nausea (38%), peripheral edema (35%), constipation (33%), insomnia (29%), thrombocytopenia (28%), anemia (26%), vomiting (26%), neutropenia (25%), back pain (24%), pyrexia (23%), peripheral edema (21%, each), fever (20%), cough (20%), hypokalemia (20%), neutropenia (20%), and upper respiratory tract infection (20%). Overall rash of all grades is reported in approximately 50% of patients and is more common when ixazomib is given in combination with lenalidomide where rash is an overlapping toxicity.

Additional detailed information regarding the clinical experience of ixazomib may be found in the IB, including information on the IV formulation.

The clinical experience and toxicities associated with oral Ixazomib are summarized below in section 1790898588.

Pharmacokinetics and Drug Metabolism

Clinical IV and PO pharmacokinetic (PK) data show that ixazomib (measured as the biologically active boronic acid form of ixazomib [MLN2238]) has multi-exponential disposition with a rapid initial phase that is largely over by 4 hours. Oral ixazomib is rapidly absorbed with a median time to first maximum plasma concentration (T_{max}) of approximately 0.5 to 2.0 hours and terminal $t_{1/2}$ after multiple dosing of approximately 5 to 7 days.[41] Results of a population PK analysis (N = 137) show that there is no relationship between body surface area (BSA) or body weight and clearance (CL). Also, based on stochastic simulations for fixed dose, exposures are independent of the individual patient's BSA.[42] Based on these data, a recommendation was made for fixed dosing in clinical trials. An absolute bioavailability of 67% was determined for ixazomib using the population PK analysis. Please refer to the current ixazomib IB and Safety Management Attachment (SMA) for information on the PK for IV doses of ixazomib.

Hepatic metabolism appears to be the major route of elimination for ixazomib, with negligible urinary excretion of the parent drug (< 5% of dose). In vitro studies of liver microsomes show that ixazomib is metabolized by multiple cytochrome P450 enzymes (CYPs) and non-CYP enzymes/proteins. The rank order of relative biotransformation activity of the 5 major human CYP isozymes is 3A4 (34.2%) > 1A2 (30.7%) > 2D6 (14.7%) > 2C9 (12.1%) > 2C19 (< 1%). Ixazomib is not an inhibitor of CYPs 1A2, 2C9, 2C19, 2D6, or 3A4, nor is it a time-dependent inhibitor of CYP3A4/5. The potential for ixazomib treatment to produce DDIs via CYP inhibition is inferred to be low; however, there may be a potential for DDIs via CYP inhibition is inferred to be low. However, there may be a potential for DDIs with a concomitant strong CYP3A4 or CYP1A2 inhibitor because of the potential for first-pass metabolism when ixazomib is administered via the PO route and because of the moderate contribution of CYP3A4- and CYP1A2-mediated metabolism of ixazomib in human liver microsomes. Ixazomib may be a weak substrate of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance associated protein (MRP2) efflux pump transporters. Ixazomib is not an inhibitor of P-gp, BCRP, and MRP2. The potential for DDIs with substrates or inhibitors of P-gp, BCRP, and MRP2 is, therefore, inferred to be low. Clinical Study C16009 (Arm 1) with ketoconazole, a strong CYP3A4

inhibitor, showed a 2-fold increase in area under the plasma concentration versus time curve (AUC) in the presence of ketoconazole. This resulted in the continued exclusion of strong CYP3A4 inhibitors in ongoing/planned clinical studies.

Further details on these studies are provided in the IB.

Overall Clinical Trial Experience Using the Oral Formulation of Ixazomib

The emerging safety profile indicates that ixazomib is generally well tolerated with manageable and reversible AEs with both the IV and PO formulations. The types of AEs reported are similar for the 2 formulations, yet the frequency and severity differ. For instance, fatigue was the most common AE reported among the 146 patients treated in the IV studies and among the 384 patients treated in the PO studies, but the incidence was greater with the IV formulation (60% vs 47%, respectively). Other common AEs reported with both formulations include rash (28% vs 25% [all terms pooled]), nausea (40% vs 46%), thrombocytopenia (45% vs 32%), decreased appetite (38% vs 24%), vomiting (40% vs 34%), and diarrhea (34% vs 46%).
The most frequent AEs (those reported in at least 10% of the total safety population) occurring in the pooled safety population, regardless of ixazomib causality, are shown in Table 1-1. The most common TEAEs reported as possibly related to study drug are shown in Table 1-2.

Rash has been reported across studies; however, there is some variety in its characterization resulting in a difference in the use of preferred terms to report it. Therefore, Table 1-3 includes the list of specific rash preferred terms and rates of each for added clarity. The numbers represent reported events and not the number of unique patients or even the number of unique rashes in a given patient; and each patient is counted only once for each preferred term (eg, 2 reported events of rash macular and 1 reported event of pruritus in a single patient is reported as 1 rash macular and 1 pruritus).

Two cases of Stevens-Johnson Syndrome and 3 cases of acute febrile neutrophilic dermatosis have been reported; these events were designated as ≥ Grade 3. Across all studies, rash macula-papular was the most common treatment-emergent rash event reported (65 [12%]).
Rashes, eruptions, exanthems NEC, and exfoliative conditions were fairly evenly distributed between recipients of IV and oral ixazomib.

Table 1-1: Most Frequent Treatment-Emergent Adverse Events (in at Least 10% of Patients), Overall Safety Population

Primary System Organ Class Preferred Term	IV Total n = 146 n (%)	Oral Single Agent (3/4/7/9) n = 201 n (%)	Oral Combo (5/6/8/13) n = 173 n (%)	Oral Total n = 384 n (%)	Overall Total N = 530 n (%)
Subjects with at Least One Adverse Event	145 (99)	197 (98)	163 (94)	370 (96)	515 (97)
Gastrointestinal disorders	115 (79)	160 (80)	139 (80)	306 (80)	421 (79)
Nausea	59 (40)	106 (53)	65 (38)	175 (46)	234 (44)
Diarrhoea	49 (34)	88 (44)	81 (47)	175 (46)	224 (42)
Vomiting	59 (40)	77 (38)	51 (29)	132 (34)	191 (36)
Constipation	36 (25)	46 (23)	57 (33)	105 (27)	141 (27)
Abdominal pain	27 (18)	33 (16)	14 (8)	49 (13)	76 (14)
General disorders and administration site conditions	118 (81)	151 (75)	132 (76)	288 (75)	406 (77)
Fatigue	88 (60)	103 (51)	76 (44)	181 (47)	269 (51)
Pyrexia	45 (31)	51 (25)	39 (23)	93 (24)	138 (26)
Oedema peripheral	30 (21)	27 (13)	61 (35)	89 (23)	119 (22)
Asthenia	10 (7)	31 (15)	20 (12)	51 (13)	61 (12)
Nervous system disorders	86 (59)	92 (46)	115 (66)	210 (55)	296 (56)
Headache	31 (21)	29 (14)	28 (16)	58 (15)	89 (17)
Dizziness	25 (17)	26 (13)	34 (20)	60 (16)	85 (16)
Neuropathy peripheral	16 (11)	21 (10)	45 (26)	66 (17)	82 (15)
Metabolism and nutrition disorders	88 (60)	107 (53)	91 (53)	204 (53)	292 (55)
Decreased appetite	55 (38)	64 (32)	25 (14)	92 (24)	147 (28)
Dehydration	25 (17)	37 (18)	12 (7)	49 (13)	74 (14)
Hypokalaemia	10 (7)	11 (5)	34 (20)	47 (12)	57 (11)
Blood and lymphatic system disorders	88 (60)	98 (49)	88 (51)	195 (51)	283 (53)
Thrombocytopenia	65 (45)	68 (34)	49 (28)	124 (32)	189 (36)
Anaemia	28 (19)	42 (21)	45 (26)	89 (23)	117 (22)

Primary System Organ Class Preferred Term	IV Total n = 146 n (%)	Oral Single Agent (3/4/7/9) n = 201 n (%)	Oral Combo (5/6/8/13) n = 173 n (%)	Oral Total n = 384 n (%)	Overall Total N = 530 n (%)
Neutropenia	16 (11)	29 (14)	43 (25)	79 (21)	95 (18)
Lymphopenia	16 (11)	20 (10)	20 (12)	47 (12)	63 (12)
Skin and subcutaneous tissue disorders	83 (57)	90 (45)	102 (59)	195 (51)	278 (52)
Rash maculo-papular	21 (14)	13 (6)	29 (17)	44 (11)	65 (12)
Musculoskeletal and connective tissue disorders	78 (53)	93 (46)	99 (57)	193 (50)	271 (51)
Back pain	27 (18)	24 (12)	42 (24)	66 (17)	93 (18)
Pain in extremity	21 (14)	18 (9)	31 (18)	49 (13)	70 (13)
Arthralgia	17 (12)	28 (14)	22 (13)	50 (13)	67 (13)
Respiratory, thoracic and mediastinal disorders	87 (60)	78 (39)	80 (46)	161 (42)	248 (47)
Cough	31 (21)	28 (14)	36 (21)	64 (17)	95 (18)
Dyspnoea	30 (21)	30 (15)	26 (15)	56 (15)	86 (16)
Infections and infestations	48 (33)	89 (44)	92 (53)	184 (48)	232 (44)
Upper respiratory tract infection	12 (8)	31 (15)	35 (20)	66 (17)	78 (15)
Psychiatric disorders	32 (22)	35 (17)	73 (42)	113 (29)	145 (27)
Insomnia	14 (10)	12 (6)	50 (29)	67 (17)	81 (15)

Source: \biostatistics\MLNM9708\IB\2013\Tables\T14.1.3-TEAE_Pct10_Pooled 04APR2013 10:48

Treatment emergent is defined as any AE that occurs after administration of the first dose of any study drug through 30 days after the last dose of any study drug, any event that is considered drug-related regardless of the start date of the event, or any event that is present at baseline but worsens in intensity or is subsequently considered by the investigator to be drug-related.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator.

Data from ongoing blinded pivotal trials (C16010) are not included.

Table 1-2: Most Frequent Treatment-Emergent Treatment-Related Adverse Events (in at Least 10% of Patients) in the Overall Safety Population

Primary System Organ Class Preferred Term	IV Total n = 146 n (%)	Oral Single Agent (3/4/7/9) n = 201 n (%)	Oral Combo Agent (5/6/8/13) n = 173 n (%)	Oral Total n = 384 n (%)	Overall Total N = 530 n (%)
Subjects with at Least One Drug-Related AE	134 (92)	172 (86)	160 (92)	342 (89)	476 (90)
Gastrointestinal disorders	89 (61)	123 (61)	116 (67)	246 (64)	335 (63)
Nausea	49 (34)	88 (44)	55 (32)	147 (38)	196 (37)
Diarrhoea	37 (25)	67 (33)	60 (35)	132 (34)	169 (32)
Vomiting	44 (30)	64 (32)	42 (24)	110 (29)	154 (29)
Constipation	14 (10)	11 (5)	35 (20)	48 (13)	62 (12)
General disorders and administration site conditions	85 (58)	102 (51)	97 (56)	204 (53)	289 (55)
Fatigue	68 (47)	73 (36)	60 (35)	135 (35)	203 (38)
Pyrexia	21 (14)	27 (13)	14 (8)	44 (11)	65 (12)
Blood and lymphatic system disorders	73 (50)	76 (38)	77 (45)	162 (42)	235 (44)
Thrombocytopenia	60 (41)	61 (30)	45 (26)	113 (29)	173 (33)
Neutropenia	14 (10)	26 (13)	37 (21)	70 (18)	84 (16)
Nervous system disorders	55 (38)	56 (28)	100 (58)	158 (41)	213 (40)
Neuropathy peripheral	13 (9)	16 (8)	44 (25)	60 (16)	73 (14)
Skin and subcutaneous tissue disorders	69 (47)	58 (29)	80 (46)	141 (37)	210 (40)
Rash maculopapular[a]	21 (14)	12 (6)	24 (14)	38 (10)	59 (11)
Metabolism and nutrition disorders	62 (42)	57 (28)	56 (32)	117 (30)	179 (34)
Decreased appetite	44 (30)	35 (17)	18 (10)	56 (15)	100 (19)

Source: \biostatistics\MLNM9708\IB\2013\Tables\T14.1.9.3-TEAE_Rel_Pct10_Pooled 03APR2013 12:44.

Treatment emergent is defined as any AE that occurs after administration of the first dose of any study drug through 30 days after the last dose of any study drug, any event that is considered drug-related regardless of the start date of the event, or any event that is present at baseline but worsens in intensity or is subsequently considered by the investigator to be drug-related.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator.

Data from ongoing blinded pivotal trials (C16010) are not included.

a Note that rash maculopapular and rash macular represent the 2 most common terms used to describe rash.

Table 1-3: Treatment-Emergent Rash Events (Overall Safety Population)

MedDRA High-Level Term Preferred Term	IV (C16001/2) n = 146 n (%)	Oral Single Agent (C16003/4/7/9) n = 201 n (%)	Oral Combo Agent (C16005/6/8/13) n = 173 n (%)	Oral Total n = 384 n (%)	Overall Total N = 530 n (%)
Rashes, eruptions and exanthems NEC	41 (28)	40 (20)	54 (31)	96 (25)	137 (26)
Rash maculo-papular	21 (14)	13 (6)	29 (17)	44 (11)	65 (12)
Rash macular	15 (10)	23 (11)	22 (13)	45 (12)	60 (11)
Rash	10 (7)	8 (4)	12 (7)	20 (5)	30 (6)
Rash generalised	0	1 (< 1)	1 (< 1)	2 (< 1)	2 (< 1)
Rash vesicular	0	1 (< 1)	1 (< 1)	2 (< 1)	2 (< 1)
Rash morbilliform	0	1 (< 1)	0	1 (< 1)	1 (< 1)
Pruritus NEC	29 (20)	21 (10)	28 (16)	49 (13)	78 (15)
Rash pruritic	20 (14)	9 (4)	16 (9)	25 (7)	45 (8)
Pruritus	12 (8)	13 (6)	14 (8)	27 (7)	39 (7)
Pruritus generalised	0	0	1 (< 1)	1 (< 1)	1 (< 1)
Erythemas	15 (10)	9 (4)	15 (9)	24 (6)	39 (7)
Rash erythematous	12 (8)	3 (1)	8 (5)	11 (3)	23 (4)
Erythema	3 (2)	6 (3)	7 (4)	13 (3)	16 (3)
Palmar erythema	0	0	1 (< 1)	1 (< 1)	1 (< 1)
Papulosquamous conditions	13 (9)	8 (4)	7 (4)	15 (4)	28 (5)
Rash papular	13 (9)	8 (4)	7 (4)	15 (4)	28 (5)
Exfoliative conditions	4 (3)	8 (4)	4 (2)	12 (3)	16 (3)
Skin exfoliation	2 (1)	5 (2)	2 (1)	7 (2)	9 (2)
Exfoliative rash	2 (1)	2 (< 1)	0	2 (< 1)	4 (< 1)
Dermatitis exfoliative	0	1 (< 1)	2 (1)	3 (< 1)	3 (< 1)
Acute febrile neutrophilic dermatosis	0	2 (< 1)	1 (< 1)	3 (< 1)	3 (< 1)
Dermatitis allergic	0	0	3 (2)	3 (< 1)	3 (< 1)
Dermatitis acneiform	4 (3)	1 (< 1)	3 (2)	4 (1)	7 (1)
Erythema multiforme	0	2 (< 1)	1 (< 1)	3 (< 1)	3 (< 1)

MedDRA High-Level Term Preferred Term	IV (C16001/2) n = 146 n (%)	Oral Single Agent (C16003/4/7/9) n = 201 n (%)	Oral Combo Agent (C16005/6/8/13) n = 173 n (%)	Oral Total n = 384 n (%)	Overall Total N = 530 n (%)
Stevens-Johnson syndrome	0	1 (< 1)	1 (< 1)	2 (< 1)	2 (< 1)
Interstitial granulomatous dermatitis	0	1 (< 1)	0	0	1 (< 1)
Vasculitic rash	0	1 (< 1)	0	1 (< 1)	1 (< 1)

Source: \\biostatistics\MLNM9708\IB\2013\Tables\T14.6.1-TEAE_AESI_Pooled; \\biostatistics\MLNM9708\IB\2013\Tables\T14.1.2-TEAE_All 05APR2013 12:20.

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities Version 15.0; NEC = not elsewhere classified.

The numbers represent reported events and not the number of unique patients or even the number of unique rashes in a given patient; and each patient is counted only once for each preferred term (eg, 2 reported events of rash macular and 1 reported event of pruritus in a single patient is reported as 1 rash macular and 1 pruritus)..

Oral Single-Agent Studies

In the oral ixazomib single-agent studies, 197 (98%) of patients experienced at least 1 TEAE (Table 1-4). The most common TEAEs in these studies included nausea (53%), fatigue (51%), diarrhea (44%), vomiting (38%), thrombocytopenia (34%), decreased appetite (32%), pyrexia (25%), constipation (23%), and anemia (21%). Overall, treatment emergent rash events (skin and subcutaneous tissue disorders) were reported for 45% of patients in the single-agent oral studies.

Table 1-4: Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Single-Agent Studies

Primary System Organ Class Preferred Term	C16003 n = 60 n (%)	C16004 n = 60 n (%)	C16007 n = 27 n (%)	C16009 n = 54 n (%)	Oral Single Agent Total n = 201 n (%)
Subjects with at Least One Adverse Event	60 (100)	59 (98)	26 (96)	52 (96)	197 (98)
Gastrointestinal disorders	45 (75)	46 (77)	24 (89)	45 (83)	160 (80)
Nausea	30 (50)	27 (45)	15 (56)	34 (63)	106 (53)
Diarrhoea	23 (38)	27 (45)	12 (44)	26 (48)	88 (44)
Vomiting	20 (33)	24 (40)	4 (15)	29 (54)	77 (38)
Constipation	11 (18)	10 (17)	7 (26)	18 (33)	46 (23)
Abdominal pain	10 (17)	7 (12)	4 (15)	12 (22)	33 (16)
General disorders and administration site conditions	52 (87)	40 (67)	20 (74)	39 (72)	151 (75)
Fatigue	33 (55)	31 (52)	12 (44)	27 (50)	103 (51)
Pyrexia	20 (33)	11 (18)	6 (22)	14 (26)	51 (25)
Oedema peripheral	5 (8)	6 (10)	6 (22)	10 (19)	27 (13)

Primary System Organ Class Preferred Term	C16003 n = 60 n (%)	C16004 n = 60 n (%)	C16007 n = 27 n (%)	C16009 n = 54 n (%)	Oral Single Agent Total n = 201 n (%)
Asthenia	8 (13)	3 (5)	5 (19)	15 (28)	31 (15)
Nervous system disorders	34 (57)	30 (50)	14 (52)	14 (26)	92 (46)
Headache	10 (17)	12 (20)	4 (15)	3 (6)	29 (14)
Dizziness	11 (18)	7 (12)	3 (11)	5 (9)	26 (13)
Neuropathy peripheral	9 (15)	9 (15)	1 (4)	2 (4)	21 (10)
Metabolism and nutrition disorders	31 (52)	27 (45)	14 (52)	35 (65)	107 (53)
Decreased appetite	17 (28)	19 (32)	7 (26)	21 (39)	64 (32)
Dehydration	10 (17)	8 (13)	3 (11)	16 (30)	37 (18)
Blood and lymphatic system disorders	34 (57)	38 (63)	7 (26)	19 (35)	98 (49)
Thrombocytopenia	27 (45)	29 (48)	4 (15)	8 (15)	68 (34)
Anaemia	12 (20)	15 (25)	3 (11)	12 (22)	42 (21)
Neutropenia	12 (20)	14 (23)	1 (4)	2 (4)	29 (14)
Lymphopenia	4 (7)	15 (25)	1 (4)	0	20 (10)
Skin and subcutaneous tissue disorders	37 (62)	20 (33)	13 (48)	20 (37)	90 (45)
Rash macular[a]	16 (27)	5 (8)	1 (4)	1 (2)	23 (11)
Musculoskeletal and connective tissue disorders	32 (53)	27 (45)	12 (44)	22 (41)	93 (46)
Back pain	8 (13)	7 (12)	3 (11)	6 (11)	24 (12)
Arthralgia	14 (23)	10 (17)	0	4 (7)	28 (14)
Respiratory, thoracic and mediastinal disorders	35 (58)	17 (28)	11 (41)	15 (28)	78 (39)
Cough	18 (30)	4 (7)	2 (7)	4 (7)	28 (14)
Dyspnoea	12 (20)	8 (13)	5 (19)	5 (9)	30 (15)
Infections and infestations	37 (62)	23 (38)	12 (44)	17 (31)	89 (44)
Upper respiratory tract infection	14 (23)	7 (12)	6 (22)	4 (7)	31 (15)

Source: \biostatistics\MLNM9708\IB\2013\Tables\T14.1.3-TEAE_Pct10_Pooled 04APR2013 10:48.

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities, version 15.0.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator.

a Note that rash maculopapular and rash macular represent the 2 most common terms used to describe rash.

Oral Combination Studies

In the oral ixazomib combination studies, 163 (94%) of patients experienced at least 1 TEAE (Table 1-5). The most common TEAEs in these studies included diarrhea (47%), fatigue (44%), nausea (38%), peripheral edema (35%), insomnia (29%), thrombocytopenia (28%), peripheral neuropathy (26%), neutropenia (25%), back pain (24%), and upper respiratory tract infection (20%). Overall, treatment-emergent rash events (skin and subcutaneous tissue disorders) were reported for 59% of patients in the oral combination studies.

Table 1-5: Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Combination Studies

Primary System Organ Class Preferred Term	C16005 n = 65 n (%)	C16006 n = 36 n (%)	C16008 n = 63 n (%)	C16013 n = 9 n (%)	Total Oral Combo Agent (5/6/8/13) n = 173 n (%)
Subjects with at Least One Adverse Event	65 (100)	34 (94)	56 (89)	8 (89)	163 (94)
Gastrointestinal disorders	61 (94)	31 (86)	41 (65)	6 (67)	139 (80)
Nausea	32 (49)	17 (47)	15 (24)	1 (11)	65 (38)
Diarrhoea	39 (60)	18 (50)	19 (30)	5 (56)	81 (47)
Vomiting	25 (38)	18 (50)	6 (10)	2 (22)	51 (29)
Constipation	26 (40)	11 (31)	18 (29)	2 (22)	57 (33)
General disorders and administration site conditions	55 (85)	29 (81)	43 (68)	5 (56)	132 (76)
Fatigue	38 (58)	9 (25)	26 (41)	3 (33)	76 (44)
Pyrexia	17 (26)	12 (33)	9 (14)	1 (11)	39 (23)
Oedema peripheral	25 (38)	11 (31)	24 (38)	1 (11)	61 (35)
Asthenia	5 (8)	12 (33)	3 (5)	0	20 (12)
Nervous system disorders	49 (75)	20 (56)	44 (70)	2 (22)	115 (66)
Headache	11 (17)	4 (11)	13 (21)	0	28 (16)
Dizziness	18 (28)	3 (8)	12 (19)	1 (11)	34 (20)
Neuropathy peripheral	20 (31)	6 (17)	17 (27)	2 (22)	45 (26)
Metabolism and nutrition disorders	40 (62)	17 (47)	31 (49)	3 (33)	91 (53)
Decreased appetite	9 (14)	13 (36)	3 (5)	0	25 (14)
Hypokalaemia	14 (22)	3 (8)	14 (22)	3 (33)	34 (20)
Blood and lymphatic system disorders	39 (60)	29 (81)	17 (27)	3 (33)	88 (51)
Thrombocytopenia	21 (32)	23 (64)	4 (6)	1 (11)	49 (28)
Anaemia	16 (25)	16 (44)	11 (17)	2 (22)	45 (26)
Neutropenia	18 (28)	22 (61)	2 (3)	1 (11)	43 (25)
Lymphopenia	8 (12)	10 (28)	2 (3)	0	20 (12)
Skin and subcutaneous tissue disorders	45 (69)	17 (47)	37 (59)	3 (33)	102 (59)
Rash maculopapular[a]	6 (9)	7 (19)	16 (25)	0	29 (17)
Rash macular[a]	10 (15)	8 (22)	4 (6)	0	22 (13)
Musculoskeletal and connective tissue disorders	45 (69)	18 (50)	35 (56)	1 (11)	99 (57)

Primary System Organ Class Preferred Term	C16005 n = 65 n (%)	C16006 n = 36 n (%)	C16008 n = 63 n (%)	C16013 n = 9 n (%)	Total Oral Combo Agent (5/6/8/13) n = 173 n (%)
Back pain	22 (34)	6 (17)	13 (21)	1 (11)	42 (24)
Pain in extremity	16 (25)	7 (19)	8 (13)	0	31 (18)
Arthralgia	14 (22)	1 (3)	7 (11)	0	22 (13)
Respiratory, thoracic and mediastinal disorders	37 (57)	13 (36)	26 (41)	4 (44)	80 (46)
Cough	19 (29)	8 (22)	6 (10)	3 (33)	36 (21)
Dyspnoea	13 (20)	4 (11)	9 (14)	0	26 (15)
Infections and infestations	42 (65)	19 (53)	28 (44)	3 (33)	92 (53)
Upper respiratory tract infection	22 (34)	4 (11)	8 (13)	1 (11)	35 (20)
Psychiatric disorders	29 (45)	12 (33)	30 (48)	2 (22)	73 (42)
Insomnia	20 (31)	8 (22)	20 (32)	2 (22)	50 (29)

Source: \biostatistics\MLNM9708\IB\2013\Tables\T14.1.3-TEAE_Pct10_Pooled 04APR2013 10:48.

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities, version 15.0.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator.

Data from ongoing blinded pivotal trials (C16010) are not included.

a Note that rash maculopapular and rash macular represent the 2 most common terms used to describe rash..

Most Common ≥ Grade 3 Treatment-Related Adverse Events in Overall Oral Population

Across all studies, 278 (52%) patients experienced at least one Grade 3 or greater TEAE that was considered at least possibly related to study treatment. These events are presented in (Table 1-6) according to route of administration and study type. Details for IV studies and the oral single-agent and combination studies are provided in the sections that follow. Overall, 52% of patients across all studies experienced a Grade 3 or greater TEAE considered at least possibly related to treatment. Across all oral administration studies, transient, reversible thrombocytopenia (19%) was the most common Grade 3 or greater drug-related TEAE. The most common drug-related Grade 4 TEAE was also thrombocytopenia (9%). In the oral single-agent studies, 48% of patients experienced at least one Grade 3 or greater TEAE considered possibly related to treatment. The most common Grade 3 or greater drug related TEAE was transient, reversible thrombocytopenia (19%). Grade 4 TEAEs considered at least possibly related to treatment were reported for 9% of patients (Table 1-9); thrombocytopenia (11%) was the most common drug-related TEAE.

In the oral combination studies, 55% of patients experienced at least 1 Grade 3 or greater
TEAE considered possibly related to treatment. The most common Grade 3 TEAE considered possibly related to treatment was transient, reversible thrombocytopenia (14%).

The most common Grade 4 TEAE considered at least possibly related to treatment was thrombocytopenia (6%).

Table 1-6: Most Common Grade ≥ 3 Drug-Related TEAEs (Pooled Safety Population)

Primary System Organ Class Preferred Term	IV Total n = 146 n (%)	Oral Single Agent (3/4/7/9) n = 201 n (%)	Oral Combo Agent (5/6/8/13) n = 173 n (%)	Oral Total n = 384 n (%)	Overall Total n = 530 n (%)
Subjects with at least one Drug-Related Grade 3 or Higher AE	80 (55)	96 (48)	95 (55)	198 (52)	278 (52)
Blood and lymphatic system disorders	41 (28)	59 (29)	48 (28)	113 (29)	154 (29)
Thrombocytopenia	31 (21)	47 (23)	24 (14)	74 (19)	105 (20)
Neutropenia	8 (5)	22 (11)	23 (13)	49 (13)	57 (11)
Lymphopenia	8 (5)	8 (4)	9 (5)	23 (6)	31 (6)
Anaemia	3 (2)	6 (3)	6 (3)	13 (3)	16 (3)
Leukopenia	3 (2)	6 (3)	5 (3)	13 (3)	16 (3)

Primary System Organ Class Preferred Term	IV Total n = 146 n (%)	Oral Single Agent (3/4/7/9) n = 201 n (%)	Oral Combo Agent (5/6/8/13) n = 173 n (%)	Oral Total n = 384 n (%)	Overall Total n = 530 n (%)
Febrile neutropenia	0	1 (< 1)	1 (< 1)	2 (< 1)	2 (< 1)
Pancytopenia	1 (< 1)	1 (< 1)	0	1 (< 1)	2 (< 1)
Skin and subcutaneous tissue disorders	20 (14)	13 (6)	23 (13)	36 (9)	56 (11)
Rash maculo-papular	8 (5)	4 (2)	7 (4)	11 (3)	19 (4)
Rash pruritic	5 (3)	0	4 (2)	4 (1)	9 (2)
Rash macular	3 (2)	3 (1)	1 (< 1)	4 (1)	7 (1)
Rash papular	1 (< 1)	2 (< 1)	3 (2)	5 (1)	6 (1)
Rash erythematous	2 (1)	0	3 (2)	3 (< 1)	5 (< 1)
Erythema multiforme	0	1 (< 1)	1 (< 1)	2 (< 1)	2 (< 1)

Source: \biostatistics\MLNM9708\IB\2013\Tables\T14.5.1-TEAE_RelGr3High_Pooled 03APR2013 12:45.

Treatment emergent is defined as any AE that occurs after administration of the first dose of any study drug through 30 days after the last dose of any study drug, any event that is considered drug-related regardless of the start date of the event, or any event that is present at baseline but worsens in intensity or is subsequently considered by the investigator to be drug-related.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator.

Data from ongoing blinded pivotal trials (C16010) are not included.

Most Common ≥ Grade 3 Treatment-Related Adverse Events in Oral Single-Agent Studies

In the oral single-agent studies, Grade 3 or greater AEs related to study treatment were reported for 96 patients (48%) (Table 1-7). Thrombocytopenia was the most common TEAE regardless of study population or drug schedule being investigated. Rash maculopapular and diarrhea were equally common with thrombocytopenia in Study C16007 in patients with relapsed or refractory AL amyloidosis.

Table 1-7: Most Common Grade ≥ 3 Treatment-Related TEAEs: Oral Single- Agent Studies

Primary System Organ Class Preferred Term	C16003 n = 60 n (%)	C16004 n = 60 n (%)	C16007 n = 27 n (%)	C16009 n = 54 n (%)	Oral Single Agent (3/4/7/9) n = 201 n (%)
Patients with at least one Drug-Related Grade 3 or Higher AE	37 (62)	32 (53)	12 (44)	15 (28)	96 (48)
Blood and lymphatic system disorders	27 (45)	26 (43)	3 (11)	3 (6)	59 (29)
Thrombocytopenia	22 (37)	20 (33)	3 (11)	2 (4)	47 (23)
Neutropenia	10 (17)	11 (18)	0	1 (2)	22 (11)
Lymphopenia	3 (5)	5 (8)	0	0	8 (4)
Anaemia	1 (2)	4 (7)	1 (4)	0	6 (3)
Leukopenia	2 (3)	3 (5)	0	1 (2)	6 (3)
Febrile neutropenia	1 (2)	0	0	0	1 (< 1)
Pancytopenia	0	0	0	1 (2)	1 (< 1)
Skin and subcutaneous tissue disorders	5 (8)	2 (3)	3 (11)	3 (6)	13 (6)
Rash maculo-papular	0	0	3 (11)	1 (2)	4 (2)
Rash macular	3 (5)	0	0	0	3 (1)
Rash papular	1 (2)	1 (2)	0	0	2 (< 1)
Erythema multiforme	0	1 (2)	0	0	1 (< 1)
Rash	0	0	0	1 (2)	1 (< 1)
Acute febrile neutrophilic dermatosis	0	0	0	1 (2)	1 (< 1)
Pruritus	0	0	1 (4)	0	1 (< 1)
Rash generalised	1 (2)	0	0	0	1 (< 1)
Swelling face	0	0	0	1 (2)	1 (< 1)
Gastrointestinal disorders	3 (5)	10 (17)	6 (22)	5 (9)	24 (12)
Diarrhoea	1 (2)	10 (17)	3 (11)	2 (4)	16 (8)
Vomiting	1 (2)	3 (5)	1 (4)	2 (4)	7 (3)
Nausea	1 (2)	4 (7)	1 (4)	3 (6)	9 (4)

Primary System Organ Class Preferred Term	C16003 n = 60 n (%)	C16004 n = 60 n (%)	C16007 n = 27 n (%)	C16009 n = 54 n (%)	Oral Single Agent (3/4/7/9) n = 201 n (%)
Abdominal pain	2 (3)	0	1 (4)	0	3 (1)
Metabolism and nutrition disorders	5 (8)	7 (12)	3 (11)	1 (2)	16 (8)
Dehydration	1 (2)	2 (3)	2 (7)	1 (2)	6 (3)
Hypokalaemia	1 (2)	0	1 (4)	0	2 (< 1)
Hyponatraemia	0	1 (2)	0	0	1 (< 1)
Decreased appetite	0	4 (7)	0	0	4 (2)
Hypophosphataemia	2 (3)	0	1 (4)	0	3 (1)
Hyperuricaemia	1 (2)	1 (2)	0	0	2 (< 1)
Electrolyte imbalance	0	0	1 (4)	0	1 (< 1)
Hypocalcaemia	0	1 (2)	0	0	1 (< 1)
General disorders and administration site conditions	5 (8)	5 (8)	2 (7)	7 (13)	19 (9)
Fatigue	4 (7)	5 (8)	1 (4)	7 (13)	17 (8)
Asthenia	0	0	1 (4)	0	1 (< 1)
Non-cardiac chest pain	1 (2)	0	0	0	1 (< 1)
Investigations	4 (7)	3 (5)	2 (7)	0	9 (4)
Platelet count decreased	1 (2)	1 (2)	2 (7)	0	4 (2)
White blood cell count decreased	2 (3)	1 (2)	0	0	3 (1)
Neutrophil count decreased	1 (2)	0	0	0	1 (< 1)
Nervous system disorders	0	2 (3)	0	1 (2)	3 (1)
Neuropathy peripheral	0	1 (2)	0	0	1 (< 1)
Dizziness	0	1 (2)	0	0	1 (< 1)
Posterior reversible encephalopathy syndrome	0	0	0	1 (2)	1 (< 1)
Infections and infestations	2 (3)	1 (2)	0	0	3 (1)
Pneumonia	1 (2)	1 (2)	0	0	2 (< 1)
Oral candidiasis	1 (2)	0	0	0	1 (< 1)
Vascular disorders	2 (3)	1 (2)	1 (4)	1 (2)	5 (2)
Hypertension	0	0	1 (4)	0	1 (< 1)
Orthostatic hypotension	2 (3)	1 (2)	0	1 (2)	4 (2)
Renal and urinary disorders	1 (2)	1 (2)	1 (4)	0	3 (1)
Renal failure acute	0	1 (2)	1 (4)	0	2 (< 1)
Renal failure	1 (2)	0	0	0	1 (< 1)
Cardiac disorders	0	1 (2)	2 (7)	0	3 (1)
Atrial fibrillation	0	0	1 (4)	0	1 (<1)
Cardiac arrest	0	0	1 (4)	0	1 (< 1)

Primary System Organ Class Preferred Term	C16003 n = 60 n (%)	C16004 n = 60 n (%)	C16007 n = 27 n (%)	C16009 n = 54 n (%)	Oral Single Agent (3/4/7/9) n = 201 n (%)
Cardiac failure congestive	0	1 (2)	0	0	1 (< 1)
Respiratory, thoracic and mediastinal disorders	1 (2)	0	0	0	1 (< 1)
Hypoxia	1 (2)	0	0	0	1 (< 1)
Pulmonary hypertension	1 (2)	0	0	0	1 (< 1)
Musculoskeletal and connective tissue disorders	0	0	0	1 (2)	1 (< 1)
Muscular weakness	0	0	0	1 (2)	1 (< 1)
Hepatobiliary disorders	0	0	0	1 (2)	1 (< 1)
Hyperbilirubinaemia	0	0	0	1 (2)	1 (< 1)
Injury, poisoning and procedural complications	1 (2)	0	0	0	1 (< 1)
Fall	1 (2)	0	0	0	1 (< 1)

Source: \biostatistics\MLNM9708\IB\2013\Tables\T14.5.1-TEAE_RelGr3_Pooled 03APR2013 12:46.

Treatment emergent is defined as any AE that occurs after administration of the first dose of any study drug through 30 days after the last dose of any study drug, any event that is considered drug-related regardless of the start date of the event, or any event that is present at baseline but worsens in intensity or is subsequently considered by the investigator to be drug-related.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator.

Data from ongoing blinded pivotal trials (C16010) are not included.

Most Common ≥ Grade 3 Treatment-Related Adverse Events in Oral Combination Studies

In the oral combination studies, at least 1 Grade 3 or greater AE deemed related to study treatment was reported for 95 patients (55%) (Table 1-8). Thrombocytopenia is less common when given in combination with lenalidomide and dexamethasone as compared to the frequency reported with single-agent ixazomib. Rash events are more common in 2 of the Len/Dex combination dose escalation studies (Studies C16005 and C16008), with anemia being most commonly reported in the phase 1 study in Asian patients (C16013). Ixazomib in combination with melphalan/ prednisone (Study C16006) demonstrates a higher frequency of hematologic toxicity, specifically thrombocytopenia and neutropenia, than reported in other combination studies.

Table 1-8: Most Common Grade ≥ 3 Treatment-Related Adverse Events: Oral Combination Studies

Primary System Organ Class Preferred Term	C16005 n = 65 n (%)	C16006 n = 36 n (%)	C16008 n = 63 n (%)	C16013 n = 9 n (%)	Oral Coml Agent (5/6/8/13) n = 173 n (%)
Patients with at least one Drug-Related Grade 3 or Higher AE	41 (63)	24 (67)	27 (43)	3 (33)	95 (55)
Blood and lymphatic system disorders	19 (29)	22 (61)	4 (6)	3 (33)	48 (28)
Thrombocytopenia	6 (9)	14 (39)	3 (5)	1 (11)	24 (14)
Neutropenia	10 (15)	12 (33)	1 (2)	0	23 (13)
Lymphopenia	3 (5)	5 (14)	1 (2)	0	9 (5)
Anaemia	3 (5)	1 (3)	0	2 (22)	6 (3)
Leukopenia	2 (3)	3 (8)	0	0	5 (3)
Febrile neutropenia	0	0	0	1 (11)	1 (< 1)
Pancytopenia	0	0	0	0	0
Skin and subcutaneous tissue disorders	11 (17)	3 (8)	9 (14)	0	23 (13)
Rash maculo-papular	2 (3)	2 (6)	3 (5)	0	7 (4)
Rash pruritic	3 (5)	1 (3)	0	0	4 (2)
Rash macular	0	0	1 (2)	0	1 (< 1)
Rash papular	1 (2)	0	2 (3)	0	3 (2)
Rash erythematous	3 (5)	0	0	0	3 (2)
Erythema multiforme	0	0	1 (2)	0	1 (< 1)
Rash	0	0	0	0	0
Urticaria	1 (2)	0	1 (2)	0	2 (1)
Acute febrile neutrophilic dermatosis	0	0	0	0	0
Angioedema	1 (2)	0	0	0	1 (< 1)
Dermatitis exfoliative	0	0	1 (2)	0	1 (< 1)
Erythema nodosum	0	0	0	0	0
Palmar-plantar erythrodysaesthesia syndrome	1 (2)	0	0	0	1 (< 1)
Pruritus	0	0	0	0	0
Rash generalised	0	0	0	0	0
Skin ulcer	0	0	0	0	0
Stevens-Johnson syndrome	0	0	1 (2)	0	1 (< 1)
Swelling face	0	0	0	0	0
Gastrointestinal disorders	9 (14)	6 (17)	1 (2)	0	16 (9)
Diarrhoea	2 (3)	3 (8)	0	0	5 (3)
Vomiting	4 (6)	2 (6)	0	0	6 (3)
Nausea	3 (5)	0	0	0	3 (2)
Abdominal pain	0	0	0	0	0

Primary System Organ Class Preferred Term	C16005 n = 65 n (%)	C16006 n = 36 n (%)	C16008 n = 63 n (%)	C16013 n = 9 n (%)	Oral Combo Agent (5/6/8/13) n = 173 n (%)
Constipation	0	1 (3)	1 (2)	0	2 (1)
Ileus	0	1 (3)	0	0	1 (< 1)
Abdominal distension	1 (2)	0	0	0	1 (< 1)
Colitis	0	1 (3)	0	0	1 (< 1)
Gastrooesophageal reflux disease	1 (2)	0	0	0	1 (< 1)
Haematemesis	0	1 (3)	0	0	1 (< 1)
Intestinal perforation	1 (2)	0	0	0	1 (< 1)
Oesophageal ulcer haemorrhage	0	1 (3)	0	0	1 (< 1)
Small intestinal obstruction	0	0	0	0	0
Metabolism and nutrition disorders	10 (15)	0	9 (14)	1 (11)	20 (12)
Dehydration	2 (3)	0	0	0	2 (1)
Hypokalaemia	4 (6)	0	1 (2)	1 (11)	6 (3)
Hyponatraemia	1 (2)	0	2 (3)	0	3 (2)
Decreased appetite	1 (2)	0	0	0	1 (< 1)
Hypophosphataemia	3 (5)	0	0	0	3 (2)
Hyperglycaemia	0	0	5 (8)	0	5 (3)
Hyperuricaemia	1 (2)	0	0	0	1 (< 1)
Electrolyte imbalance	0	0	0	0	0
Hypocalcaemia	0	0	0	0	0
Hypomagnesaemia	0	0	1 (2)	0	1 (< 1)
General disorders and administration site conditions	8 (12)	1 (3)	1 (2)	0	10 (6)
Fatigue	6 (9)	1 (3)	0	0	7 (4)
Asthenia	0	0	0	0	0
Non-cardiac chest pain	1 (2)	0	0	0	1 (< 1)
Oedema peripheral	1 (2)	0	1 (2)	0	2 (1)
Investigations	5 (8)	0	4 (6)	0	9 (5)
Platelet count decreased	1 (2)	0	2 (3)	0	3 (2)
White blood cell count decreased	1 (2)	0	0	0	1 (< 1)
Alanine aminotransferase increased	1 (2)	0	0	0	1 (< 1)
Blood creatinine increased	1 (2)	0	0	0	1 (< 1)
Aspartate aminotransferase increased	1 (2)	0	0	0	1 (< 1)
Neutrophil count decreased	1 (2)	0	0	0	1 (< 1)
Transaminases increased	0	0	2 (3)	0	2 (1)
Nervous system disorders	5 (8)	3 (8)	7 (11)	0	15 (9)
Neuropathy peripheral	1 (2)	0	2 (3)	0	3 (2)

Primary System Organ Class Preferred Term	C16005 n = 65 n (%)	C16006 n = 36 n (%)	C16008 n = 63 n (%)	C16013 n = 9 n (%)	Oral Combo Agent (5/6/8/13) n = 173 n (%)
Dizziness	1 (2)	1 (3)	2 (3)	0	4 (2)
Peripheral sensory neuropathy	2 (3)	1 (3)	1 (2)	0	4 (2)
Syncope	2 (3)	0	1 (2)	0	3 (2)
Neuralgia	0	1 (3)	0	0	1 (< 1)
Cognitive disorder	0	0	1 (2)	0	1 (< 1)
Polyneuropathy	0	1 (3)	0	0	1 (< 1)
Tremor	0	0	1 (2)	0	1 (< 1)
Infections and infestations	5 (8)	1 (3)	4 (6)	2 (22)	12 (7)
Pneumonia	2 (3)	1 (3)	2 (3)	1 (11)	6 (3)
Brain abscess	1 (2)	0	0	0	1 (< 1)
Cellulitis	0	0	1 (2)	0	1 (< 1)
Gastroenteritis	0	0	0	1 (11)	1 (< 1)
Influenza	0	0	1 (2)	0	1 (< 1)
Pneumonia respiratory syncytial viral	1 (2)	0	0	0	1 (< 1)
Rash pustular	1 (2)	0	0	0	1 (< 1)
Subcutaneous abscess	0	0	1 (2)	0	1 (< 1)
Vascular disorders	5 (8)	0	2 (3)	0	7 (4)
Hypertension	4 (6)	0	0	0	4 (2)
Orthostatic hypotension	0	0	1 (2)	0	1 (< 1)
Deep vein thrombosis	0	0	1 (2)	0	1 (< 1)
Embolism	1 (2)	0	0	0	1 (< 1)
Renal and urinary disorders	0	1 (3)	0	0	1 (< 1)
Neurogenic bladder	0	1 (3)	0	0	1 (< 1)
Cardiac disorders	1 (2)	0	2 (3)	0	3 (2)
Atrial fibrillation	1 (2)	0	1 (2)	0	2 (1)
Atrial flutter	0	0	1 (2)	0	1 (< 1)
Musculoskeletal and connective tissue disorders	1 (2)	1 (3)	0	0	2 (1)
Muscular weakness	1 (2)	0	0	0	1 (< 1)
Myalgia	0	1 (3)	0	0	1 (< 1)
Psychiatric disorders	2 (3)	0	2 (3)	0	4 (2)
Agitation	1 (2)	0	0	0	1 (< 1)
Anxiety	0	0	1 (2)	0	1 (< 1)
Insomnia	0	0	1 (2)	0	1 (< 1)
Mood altered	1 (2)	0	0	0	1 (< 1)
NOT CODED	2 (3)	0	0	0	2 (1)

Primary System Organ Class Preferred Term	C16005 n = 65 n (%)	C16006 n = 36 n (%)	C16008 n = 63 n (%)	C16013 n = 9 n (%)	Oral Combo Agent (5/6/8/13) n = 173 n (%)

Source: biostatistics\MLNM9708\IB\2013\Tables\T14.5.1-TEAE_RelGr3_Pooled 03APR2013 12:44.

Treatment emergent is defined as any AE that occurs after administration of the first dose of any study drug through 30 days after the last dose of any study drug, any event that is considered drug-related regardless of the start date of the event, or any event that is present at baseline but worsens in intensity or is subsequently considered by the investigator to be drug-related.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator. Data from ongoing blinded pivotal trials (C16010) are not included.

NOT CODED = preferred terms were not coded at time of clinical cutoff.

Table 1-9: Grade 3, Grade 3 or Greater, and Grade 4 Drug-Related Adverse Events in Oral Studies

MedDRA High-Level Term Preferred Term	Grade 3 or greater n = 384 n (%)	Grade 3 n = 384 n (%)	Grade 4 n = 384 n (%)
Subjects with at Least 1 Drug-Related Grade 3 or 4 AE	198 (52)	194 (51)	47 (12)
Blood and lymphatic system disorders	113 (29)	102 (27)	42 (11)
Thrombocytopenia	74 (19)	62 (16)	36 (9)
Neutropenia	49 (13)	46 (12)	6 (2)
Lymphopenia	23 (6)	21 (5)	5 (1)
Anaemia	13 (3)	13 (3)	0
Leukopenia	13 (3)	13 (3)	1 (< 1)
Febrile neutropenia	2 (< 1)	2 (< 1)	0
Pancytopenia	1 (< 1)	1 (< 1)	0
Skin and subcutaneous tissue disorders	36 (9)	36 (9)	0
Rash maculo-papular	11 (3)	11 (3)	0
Rash pruritic	4 (1)	4 (1)	0
Rash macular	4 (1)	4 (1)	0
Rash papular	5 (1)	5 (1)	0
Rash erythematous	3 (< 1)	3 (< 1)	0
Erythema multiforme	2 (< 1)	2 (< 1)	0
Rash	1 (< 1)	1 (< 1)	0
Urticaria	2 (< 1)	2 (< 1)	0
Acute febrile neutrophilic dermatosis	1 (< 1)	1 (< 1)	0
Angioedema	1 (< 1)	1 (< 1)	0
Dermatitis exfoliative	1 (< 1)	1 (< 1)	0

MedDRA High-Level Term Preferred Term	Grade 3 or greater n = 384 n (%)	Grade 3 n = 384 n (%)	Grade 4 n = 384 n (%)
Palmar-plantar erythrodysaesthesia syndrome	1 (< 1)	1 (< 1)	0
Pruritus	1 (< 1)	1 (< 1)	0
Rash generalised	1 (< 1)	1 (< 1)	0
Stevens-Johnson syndrome	1 (< 1)	1 (< 1)	0
Swelling face	1 (< 1)	1 (< 1)	0
Gastrointestinal disorders	42 (11)	42 (11)	1 (< 1)
Diarrhoea	23 (6)	23 (6)	0
Vomiting	14 (4)	14 (4)	0
Nausea	13 (3)	13 (3)	0
Abdominal pain	3 (< 1)	3 (< 1)	0
Constipation	2 (< 1)	2 (< 1)	0
Ileus	1 (< 1)	1 (< 1)	0
Abdominal distension	1 (< 1)	1 (< 1)	0
Colitis	1 (< 1)	1 (< 1)	0
Gastrooesophageal reflux disease	1 (< 1)	1 (< 1)	0
Haematemesis	1 (< 1)	1 (< 1)	0
Intestinal perforation	1 (< 1)	1 (< 1)	0
Oesophageal ulcer haemorrhage	1 (< 1)	0	1 (< 1)
Metabolism and nutrition disorders	37 (10)	36 (9)	3 (< 1)
Dehydration	8 (2)	8 (2)	0
Hypokalaemia	9 (2)	9 (2)	1 (< 1)
Hyponatraemia	5 (1)	5 (1)	0
Decreased appetite	5 (1)	5 (1)	0
Hypophosphataemia	6 (2)	6 (2)	0
Hyperglycaemia	5 (1)	5 (1)	0
Hyperuricaemia	3 (< 1)	1 (< 1)	2 (< 1)
Electrolyte imbalance	1 (< 1)	0	1 (< 1)
Hypocalcaemia	1 (< 1)	1 (< 1)	0
Hypomagnesaemia	1 (< 1)	1 (< 1)	0
General disorders and administration site conditions	31 (8)	31 (8)	0
Fatigue	26 (7)	26 (7)	0
Asthenia	1 (< 1)	1 (< 1)	0
Non-cardiac chest pain	2 (< 1)	2 (< 1)	0
Oedema peripheral	2 (< 1)	2 (< 1)	0
Investigations	18 (5)	18 (5)	0
Platelet count decreased	7 (2)	7 (2)	0

MedDRA High-Level Term Preferred Term	Grade 3 or greater n = 384 n (%)	Grade 3 n = 384 n (%)	Grade 4 n = 384 n (%)
White blood cell count decreased	4 (1)	4 (1)	0
Alanine aminotransferase increased	1 (< 1)	1 (< 1)	0
Blood creatinine increased	2 (< 1)	2 (< 1)	0
Aspartate aminotransferase increased	1 (< 1)	1 (< 1)	0
Neutrophil count decreased	2 (< 1)	2 (< 1)	0
Transaminases increased	2 (< 1)	2 (< 1)	0
Nervous system disorders	18 (5)	18 (5)	0
Neuropathy peripheral	4 (1)	4 (1)	0
Dizziness	5 (1)	5 (1)	0
Peripheral sensory neuropathy	4 (1)	4 (1)	0
Syncope	3 (< 1)	3 (< 1)	0
Neuralgia	1 (< 1)	1 (< 1)	0
Cognitive disorder	1 (< 1)	1 (< 1)	0
Polyneuropathy	1 (< 1)	1 (< 1)	0
Posterior reversible encephalopathy syndrome	1 (< 1)	1 (< 1)	0
Tremor	1 (< 1)	1 (< 1)	0
Infections and infestations	15 (4)	15 (4)	0
Pneumonia	8 (2)	8 (2)	0
Oral candidiasis	1 (< 1)	1 (< 1)	0
Brain abscess	1 (< 1)	1 (< 1)	0
Cellulitis	1 (< 1)	1 (< 1)	0
Gastroenteritis	1 (< 1)	1 (< 1)	0
Influenza	1 (< 1)	1 (< 1)	0
Pneumonia respiratory syncytial viral	1 (< 1)	1 (< 1)	0
Rash pustular	1 (< 1)	1 (< 1)	0
Subcutaneous abscess	1 (< 1)	1 (< 1)	0
Vascular disorders	13 (3)	13 (3)	0
Hypertension	6 (2)	6 (2)	0
Orthostatic hypotension	5 (1)	5 (1)	0
Deep vein thrombosis	1 (< 1)	1 (< 1)	0
Embolism	1 (< 1)	1 (< 1)	0
Renal and urinary disorders	4 (1)	4 (1)	0
Renal failure acute	2 (< 1)	2 (< 1)	0
Renal failure	1 (< 1)	1 (< 1)	0
Neurogenic bladder	1 (< 1)	1 (< 1)	0
Cardiac disorders	6 (2)	4 (1)	2 (< 1)
Atrial fibrillation	3 (< 1)	3 (< 1)	0
Atrial flutter	1 (< 1)	1 (< 1)	0
Cardiac arrest	1 (< 1)	0	1 (< 1)
Cardiac failure congestive	1 (< 1)	0	1 (< 1)

MedDRA High-Level Term Preferred Term	Grade 3 or greater n = 384 n (%)	Grade 3 n = 384 n (%)	Grade 4 n = 384 n (%)
Respiratory, thoracic and mediastinal disorders	1 (< 1)	1 (< 1)	0
Hypoxia	1 (< 1)	1 (< 1)	0
Pulmonary hypertension	1 (< 1)	1 (< 1)	0
Musculoskeletal and connective tissue disorders	3 (< 1)	3 (< 1)	0
Muscular weakness	2 (< 1)	2 (< 1)	0
Myalgia	1 (< 1)	1 (< 1)	0
Psychiatric disorders	4 (1)	4 (1)	0
Agitation	1 (< 1)	1 (< 1)	0
Anxiety	1 (< 1)	1 (< 1)	0
Insomnia	1 (< 1)	1 (< 1)	0
Mood altered	1 (< 1)	1 (< 1)	0
NOT CODED	2 (< 1)	2 (< 1)	0
NOT CODED[a]	2 (< 1)	2 (< 1)	0
Hepatobiliary disorders	1 (< 1)	1 (< 1)	0
Hyperbilirubinaemia	1 (< 1)	1 (< 1)	0
Injury, poisoning and procedural complications	1 (< 1)	1 (< 1)	0
Fall	1 (< 1)	1 (< 1)	0

Source: \biostatistics\MLNM9708\IB\2013\Tables\T14.5.1.1-TEAE_RelGr3_Pooled 03APR2013 12:46;
\biostatistics\MLNM9708\IB\2013\Tables\T14.5.1.2-TEAE_RelGr4_Pooled 03APR2013 12:47;
\biostatistics\MLNM9708\IB\2013\Tables\T14.5.1-TEAE_RelGr3High_Pooled 03APR2013 12:45.

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities, version 15.0.

Subject Incidence: A subject counts once for each preferred term and system organ class. Percentages use the number of treated subjects as the denominator.

Data from ongoing pivotal trial (C16010) are not included.

a Preferred term was fatigue.

Clinical Safety Summary

Ixazomib has been evaluated as a single agent in phase 1 studies that have included patients with advanced solid tumors (Study C16001), lymphoma (Study C16002), RRMM (Studies C16003 and C16004), and AL amyloidosis (Study C16007). Ongoing clinical pharmacology studies include Study C16009, a clinical trial evaluating drug-drug interactions with ketoconazole and rifampin, effect of food, and oral bioavailability. Study C16013 is investigating the safety and PK of ixazomib in combination with lenalidomide and dexamethasone in Asian adult patients with a diagnosis of RRMM. Study TB-MC010034 (Japan) is a phase 1/1b PK and tolerability study of ixazomib in Japanese patients with RRMM. Ixazomib continues to be evaluated in combination with lenalidomide and low-dose dexamethasone in patients with newly diagnosed MM requiring systemic treatment (Study C16005, weekly in a 28-day cycle and Study C16008, twice weekly in a 21-day cycle).

Another combination being evaluated is ixazomib in combination with melphalan and prednisone in patients with newly diagnosed MM not eligible for stem cell transplant (Study C16006).

Late stage development is focused on development of the oral formulation. A phase 3 trial is ongoing to evaluate oral ixazomib in combination with LenDex in RRMM (Study C16010) with a similar trial planned at the time of data cut for patients with NDMM. A phase 3 study in relapsed or refractory AL amyloidosis (Study C16011) is currently evaluating ixazomib in combination with dexamethasone versus physician's choice of a Dex-based regimen.

As of 27 March 2013, preliminary clinical data is available for a total of 653 patients across 10 studies. (The overall safety population comprises 530 patients, but blinded aggregate data for SAEs and deaths is provided for an additional 123 patients enrolled in Study C16010. Therefore, for SAE and death data, the total number of patients is 653.) The emerging safety profile indicates that ixazomib is generally well tolerated with AEs generally consistent with the class-based effects of proteasome inhibition. While some of these potential toxicities may be severe, they can be managed by clinical monitoring and standard medical intervention.

Fatigue was the most common AE reported among the 146 patients treated in the IV studies and 384 patients treated in the PO studies (60% and 47%, respectively). Other common AEs reported in the pooled IV and PO safety populations include nausea, thrombocytopenia, diarrhea, vomiting, and rash. Rash is a commonly reported TEAE; however, there is some variation in its characterization and causality resulting in different preferred terms to describe it. The dose escalation phase of most trials reported are now complete. GI symptoms were the most common DLTs when the use of prophylactic anti-emetics was not permitted per protocol. In the expansion cohorts or phase 2 cohorts (as per each study), the incidence and severity of GI symptoms will be mitigated by the use of the lower MTD/ RP2D (as per each study) and standard clinical usage of anti-emetics and/or anti-diarrheal medications as deemed appropriate.

As of 27 March 2013, at least 1 TEAE regardless of causality has been reported in 515 of 530 patients (97%) across all ongoing/enrolling open label studies. The MTD has been determined in 8 clinical trials. The most frequent (at least 20%) TEAEs reported with the IV formulation pooled from Studies C16001 and C16002 (n = 146), regardless of causality to Ixazomib, include fatigue (60%), thrombocytopenia (45%), nausea and vomiting (40% each), decreased appetite (38%), diarrhea (34%), pyrexia (31%), constipation (25%), and cough, anemia, peripheral edema, dyspnea, and headache (21% each).

The most frequent (at least 20%) TEAEs reported with the PO formulation pooled from single-agent Studies C16003, C16004, C16007, and C16009 (n = 201) regardless of causality with Ixazomib, include nausea (53%), fatigue (51%), diarrhea (44%), thrombocytopenia (34%), vomiting (38%), decreased appetite (32%), fever (21%), and anemia (21%). In the single-agent group, the most frequent (at least 20%) TEAEs reported with the PO formulation pooled from combination trials (irrespective of the combination) in Studies C16005, C16006, C16008, and C16013 (n = 173), irrespective of causality to Ixazomib, include diarrhea (47%), fatigue (44%), nausea (38%), peripheral edema (35%), constipation (33%), insomnia (29%), thrombocytopenia (28%), anemia (26%), vomiting (26%), neutropenia (25%), back pain (24%), pyrexia (23%), peripheral edema (21% each), fever, cough, hypokalemia, neutropenia, and upper respiratory tract infection (20% each).

The frequency of rash with single-agent Ixazomib is higher with the twice-weekly schedule than with the weekly schedule regardless of the formulation; however, the frequency with the weekly schedule approaches the twice-weekly rate when Ixazomib is

given in combination with an agent where rash is an overlapping toxicity, such as lenalidomide. One approach to managing the frequency of rash was the careful consideration of the RP2D for the combination trials where all toxicities over multiple cycles were taken into consideration. Additional approaches include the use of standard medical interventions such as the use of oral or topical antihistamines as medically appropriate or oral corticosteroids in more severe cases.
Additionally, a rare risk is Stevens-Johnson Syndrome, a severe, life threatening, or deadly rash with skin peeling and mouth sores.

As of 27 March 2013, at least 1 SAE has been reported for 241 patients (37%) across all ongoing Millennium-sponsored clinical studies with Ixazomib (including blinded data reported for patients enrolled in Study C16010). Regardless of causality, the most common SAEs include pneumonia (27 patients), pyrexia (21 patients), dehydration (20 patients), thrombocytopenia (17 patients), and vomiting (14 patients).

As of 27 March 2013, a total of 83 patients experienced AEs that resulted in study drug discontinuation: 18 patients in Study C16001, 3 patients in Study C16002, 9 patients in Study C16003, 7 patients in Study C16004, 11 patients in Study C16005, 4 patients in Study C16006, 7 patients in Study C16007, 8 patients in Study C16008, 11 patients in Study C16009, 4 patients in Study C16010, and 1 patient in Study TB-MC010034. Fatigue and GI events were common reasons for discontinuation.

As of 27 March 2013, 8 overdoses have been reported in Ixazomib clinical studies. One patient in Study C16001 received 2 doses of Ixazomib that were approximately 46% higher than was intended per the protocol. The patient experienced Grade 4 thrombocytopenia (nadir 22,000/□L) and some liver function elevations, but no other issues including no elevation in creatinine. All laboratory abnormalities resolved 8 days after the overdose; the patient died due to disease progression 38 days after last dose of Ixazomib. In Study C16008, 1 patient was hospitalized for observation after taking Ixazomib on Days 1 and 2 instead of Days 1 and 4; no AEs were reported. In Study C16005, there were 2 cases determined to be not serious. In Study C16001, 1 patient had a dose calculated incorrectly due to an error in height measurement; the patient received a 3% overdose, but did not experience any related SAEs. In Study C16008, a nonserious overdose was reported for a patient who mistakenly took the correct dose of Ixazomib on the wrong day (Day 2 vs Day 4). In Study C16010, a patient mistakenly took 3 capsules of study drug instead of 1 capsule despite receiving proper instructions from study site personnel; the patient was withdrawn from the study for compliance reasons. Also in Study C16010, a patient took 40 mg of dexamethasone every day for 7 days instead of Day 1 only; the drug schedule was again provided to the patient following the overdose.
As of 27 March 2013, a total of 32 on-study deaths were reported in the clinical studies. One of the 32 events was deemed by the investigator to be related to treatment with Ixazomib (pneumonia RSV in Study C16005). The most common cause of death was disease progression.

Potential Benefits

The clinical benefit of ixazomib continues to be studied in a comprehensive and global development plan that involves studies sponsored by Millennium. Ixazomib appears to show early signs of anti-tumor activity as evidenced by at least 50% reduction in disease burden in

some patients, including patients that have been heavily pretreated as well as those with newly diagnosed MM, and prolongs stabilization of the underlying disease in other patients across all ongoing trials. The preliminary findings are favorable when considering historical and currently available therapies for the patient populations evaluated. Though additional data are needed to characterize the clinical benefit of this drug, the emerging data supports expanded development of Ixazomib for the treatment of patients with advanced malignancy.

IFN-α-2b: Description, PK, PD, and safety data

Pegylated IFN-α-2b is a covalent conjugate of recombinant alfa interferon with monomethoxy polyethylene glycol (PEG). The biological activity of pIFN is derived from its interferon α-2b moiety. Interferons exert their cellular activities by binding to specific membrane receptors on the cell surface and initiate a complex sequence of intracellular events. These include the induction of certain enzymes, suppression of cell proliferation, immunomodulating activities such as enhancement of the phagocytic activity of macrophages and augmentation of the specific cytotoxicity of lymphocytes for target cells, and inhibition of virus replication in virus-infected cells. Interferon alfa upregulates the Th1 T-helper cell subset in in vitro studies. Please see Section 1790898588 for more background from the Balachandran lab.

Pegylated IFN has a single PEG-moiety and a molecular weight of 12 kDa and is a conjugate of interferon with polyethylene glycol (PEG). As a single agent therapy it is well tolerated up to 6.0 mcg/kg per week, which achieves an exposure comparable to 180 MIU nonpegylated interferon per week.[26,43] The conjugate decreases the clearance, thus prolonging the plasma half time (t½) to approximately 40 hours (in patients with chronic hepatitis) from otherwise 3-7 hours for not conjugated interferon alfa-2b.[44,45] The prolonged plasma half-life of Peg-interferon achieves a more constant exposure which leads to an increased area under the concentration curve (AUC). This may more effectively inhibit the angiogenesis as repeated injections of low-dose interferon.[27] Thereby, the efficacy of the drug may be increased as it has an antiangiogenic effect apart from the immunological effect.

The pharmacokinetics of pIFN allows for administration subcutaneously once weekly and this regimen has shown acceptable safety.[44] pIFN has been used in different clinical settings such as hepatitis C,[46] chronic myelogenous leukaemia (CML)[47], malignant melanoma,[26] and mRCC.[25,26,29,48] Pegylated IFN raises concentrations of effector proteins such as serum neopterin and 2'5' oligoadenylate synthetase, raises body temperature, and causes reversible decreases in leukocyte and platelet counts. The correlation between the *in vitro* and *in vivo* pharmacologic and pharmacodynamic and clinical effects is unknown.

In Hepatitis C studies with pIFN, following a single subcutaneous (SC) dose of PEG-Intron, the mean absorption half-life (t ½ k_a) was 4.6 hours. Maximal serum concentrations (C_{max}) occur between 15-44 hours post-dose, and are sustained for up to 48-72 hours. The C_{max} and AUC measurements of PEG-Intron increase in a dose-related manner. After multiple dosing, there is an increase in bioavailability of PEG-Intron. Week 48 mean trough concentrations (320 pg/mL; range 0, 2960) are approximately 3-fold higher than Week 4 mean trough concentrations (94 pg/mL; range 0, 416). The mean PEG-Intron elimination half-life is approximately 40 hours (range 22 to 60 hours) in patients with HCV infection.

The apparent clearance of PEG-Intron is estimated to be approximately 22.0 mL/hr·kg. Renal elimination accounts for 30% of the clearance. Single dose peginterferon alfa-2b pharmacokinetics following a subcutaneous 1.0 µg/kg dose suggest the clearance of peginterferon alfa-2b is reduced by approximately half in patients with impaired renal function (creatinine clearance <50 mL/minute).

The pharmacokinetics of geriatric patients (> 65 years of age) treated with a single subcutaneous dose of 1.0 µg/kg of PEG-Intron were similar in C_{max}, AUC, clearance, or elimination half-life as compared to younger subjects (28 to 44 years in age).

During the 48 week treatment period with PEG-Intron no differences in the pharmacokinetic profiles were observed between male and female patients with chronic hepatitis C infection.

In cancer patients, Bukowski et al conducted a phase I/II study using pIFN-α-2b to determine the MTD. 70 patients were treated out whom 35 had mRCC. The MTD was 6.0 mcg/kg/week with one year of treatment well tolerated with dose modifications. The PK data demonstrated that pIFN was readily absorbed and the AUC at weeks 1 and 4 demonstrated a dose-related but not dose-proportional increase over the 0.75 to 7.5 mcg/kg dose range.

The toxicity profile of pIFN is summarized in the below four tables (Figures 1-2 to 1-5) showing AE from three separate trials using pIFN in mRCC patients. Some of the more common and dose-dependent AEs include fatigue, fevers, diarrhea, nausea, myalgias, asthenia and flu-like symptoms.

Figure 1-2: Most Frequently Reported Treatment-Emergent Adverse Events (all grades) by Pegylated IFNα-2b Dose Cohort Over 12 Weeks (core phase)[26]

	Dose Cohort (µg/kg/wk)											
	0.75 (n = 3)		1.5 (n = 3)		3.0 (n = 4)		4.5 (n = 6)		6.0 (n = 29)		7.5 (n = 25)	
Adverse Event	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Nausea	3	100	1	33	2	50	4	67	23	79	20	80
Anorexia	3	100	3	100	2	50	4	67	20	69	19	76
Fatigue	3	100	2	67	4	100	1	17	17	59	21	84
Rigors	1	33	2	67	4	100	2	33	18	62	21	84
Headache	2	67	2	67	3	75	0		17	59	17	68
Vomiting	2	67	2	67	0		3	50	14	48	15	60
Diarrhea	1	33	2	67	0		2	33	12	41	17	68
Injection-site reaction	2	67	3	100	3	75	1	17	16	55	7	28
Myalgia	0		1	33	1	25	2	33	9	31	18	72
Dyspnea	2	67	2	67	0		2	33	12	41	8	32
Fever	1	33	2	67	3	75	2	33	19	66	17	68
Asthenia	2	67	1	33	0		0		9	31	13	52
Pain	1	33	1	33	2	50	1	17	10	34	8	32
Back pain	2	67	1	33	2	50	2	33	9	31	7	28
Dizziness	2	67	0		2	50	0		10	34	8	32
Dry mouth	1	33	2	67	0		1	17	10	34	6	24

Figure 1-3: Most Frequently Reported Treatment-Emergent Adverse Events by Severity (n=29) Over 1 Year (extension phase) using Pegylated IFNα-2b[26]

Adverse Event	All Grades		Grade 3 or 4	
	No. of Patients	%	No. of Patients	%
Fatigue	11	38	0	
Anorexia	10	34	0	
Pain	10	34	1	3
Headache	9	31	1	3
Injection-site reaction	8	28	0	
Myalgia	8	28	0	
Weight loss	8	28	0	
Nausea	7	24	0	
Alopecia	6	21	0	
Coughing	6	21	0	
Dyspnea	6	21	0	
Fever	6	21	1	3
Rigors	6	21	1	3
Laboratory abnormalities				
Thrombocytopenia	22	76	1	3
Leukopenia	20	69	2	7
Neutropenia	15	52	3	10

Figure 1-4: Toxicity. Grade 1-3, reported at least once during treatment with Pegylated IFNα-2b in mRCC[48]

Toxicity. Grade 1-3, reported at least once during treatment, n =28 (%)

Side effect	≤2 months	>2 months
Fatigue	23 (82)	28 (100)
Nausea	18 (64)	16 (57)
Fever	16 (57)	11 (39)
Rigors/Chills	10 (36)	8 (29)
Myalgia	9 (32)	8 (29)
Diarrhoea	7 (26)	3 (11)
Other Neurological Events	8 (29)	10 (36)
Artralgia	7 (26)	4 (14)
Mood	6 (21)	4 (14)
Anorexia	6 (21)	7 (26)
Skin-Rash	5 (18)	8 (29)
Headache	5 (18)	6 (21)
Diaphoresis	5 (18)	3 (11)
Vomiting	4 (14)	3 (11)
Alopecia	3 (11)	3 (11)
Weight loss	1 (4)	7 (26)
Cardiac-Dysrythmia	2 (7)	1 (4)

Figure 1-5: Patient Toxicities withi Pegylated IFNα-2b in mRCC (no grade 4 toxicities were observed)[25]

Toxicity	Grade 2, n (%)	Grade 3, n (%)
Laboratory		
Neutropenia	7 (22)	4 (13)
Lymphopenia	0	4 (13)
Anemia	5 (16)	2 (6)
Thrombocytopenia	2 (6)	1 (3)
Hyperkalemia	8 (25)	3 (9)
Transaminitis	6 (19)	2 (6)
Hyponatremia	0	3 (9)
Symptoms		
Fatigue	8 (25)	4 (13)
Rash	1 (3)	2 (6)
Depression	0	2 (6)
Diarrhea	0	1 (3)

Of note, there are some notable rare toxicities that are known to occur with pIFN. These include:

1. Neuropsychiatric events: Life-threatening or fatal neuropsychiatric events, including suicide, suicidal and homicidal ideation, depression, relapse of drug addiction/overdose, and aggressive behavior have occurred in patients with and without a previous psychiatric disorder during PEG-Intron treatment and follow-up.
2. Bone marrow toxicity: Severe cytopenias may occur. Very rarely alpha interferons may be associated with aplastic anemia.
3. Endocrine disorders: pIFN can cause or aggravates hypothyroidism and hyperthyroidism. Hyperglycemia has been observed in patients treated with PEG-Intron. Diabetes mellitus has been observed in patients treated with alpha interferons.

Patients with these conditions who cannot be effectively treated by medication should not begin PEG-Intron therapy. Patients who develop these conditions during treatment and cannot be controlled with medication should not continue PEG-Intron therapy.

4. Cardiovascular events: Cardiovascular events, which include hypotension, arrhythmia, tachycardia, cardiomyopathy, angina pectoris, and myocardial infarction, have been observed in patients treated with PEG-Intron. PEG-Intron should be used cautiously in patients with cardiovascular disease.
5. Pulmonary disorders: Dyspnea, pulmonary infiltrates, pneumonia, bronchiolitis obliterans, interstitial pneumonitis and sarcoidosis some resulting in respiratory failure and/or patient deaths, may be induced or aggravated by PEG-Intron or alpha interferon therapy. Recurrence of respiratory failure has been observed with interferon rechallenge. PEG-Intron combination treatment should be suspended in patients who develop pulmonary infiltrates or pulmonary function impairment. Patients who resume interferon treatment should be closely monitored.
6. Colitis: Fatal and nonfatal ulcerative or hemorrhagic/ischemic colitis have been observed within 12 weeks of the start of alpha interferon treatment. Abdominal pain, bloody diarrhea, and fever are the typical manifestations. PEG-Intron treatment should be discontinued immediately in patients who develop these symptoms and signs. The colitis usually resolves within 1-3 weeks of discontinuation of alpha interferons.
7. Pancreatitis: Fatal and nonfatal pancreatitis has been observed in patients treated with alpha interferon. PEG-Intron therapy should be suspended in patients with signs and symptoms suggestive of pancreatitis and discontinued in patients diagnosed with pancreatitis.
8. Autoimmune disorders: Development or exacerbation of autoimmune disorders (e.g. thyroiditis, thrombocytopenia, rheumatoid arthritis, interstitial nephritis, systemic lupus erythematosus, psoriasis) have been observed in patients receiving PEG-Intron. PEG-Intron should be used with caution in patients with autoimmune disorders.
9. Ophthalmologic disorders: Decrease or loss of vision, retinal artery or vein thrombosis, retinal hemorrhages and cotton wool spots, optic neuritis, and papilledema are induced or aggravated by treatment with PEG-Intron or other alpha interferons. All patients should receive an eye examination at baseline. Patients with preexisting ophthalmologic disorders (e.g. diabetic or hypertensive retinopathy) should receive periodic ophthalmologic exams during interferon alpha treatment. Any patient who develops ocular symptoms should receive a prompt and complete eye examination. PEG-interferon treatment should be discontinued in patients who develop new or worsening ophthalmologic disorders.
10. Hypersensitivity: Serious, acute hypersensitivity reactions (e.g., urticaria, angioedema, bronchoconstriction, anaphylaxis) have been rarely observed during alpha interferon therapy. If such a reaction develops during treatment with PEG-Intron, discontinue treatment and institute appropriate medical therapy immediately.

2. STUDY OBJECTIVES

Phase I Primary Objective

1. To determine the safety, tolerability and RP2D of the combination of pegylated interferon with ixazomib.

Phase II Primary Objective

1. To determine progression free survival (PFS)

Phase II Secondary Objectives

1. To determine the composite rate of unacceptable toxicity at 8 weeks
2. To determine the overall response rate (ORR) using RECIST v1.1

Phase II Exploratory Objectives

1. To examine changes in STAT1 phosphorylation and MHC1 up regulation in PBMCs as an indicator of IFN activity
2. To use archived FFPE tumor tissue to correlate activated tissue stroma (i.e., desmoplasia) with serum IL-6 levels and patient outcomes
3. To use archived FFPE from past biopsies for IHC analysis of nuclear NF-κB p65 and phospho-STAT1 in order to test whether those patients with the most apparent constitutive pathway activation related to either of these two markers are generally more or less responsive to the combination therapy.

3. STUDY ENDPOINTS

Phase I Primary Endpoint

1. Presence or absence of dose-limiting toxicity during one cycle of pegylated interferon alpha with ixazomib.

Phase II Primary Endpoint

1. Progression free survival at 8 and 16 weeks using RECIST v 1.1.

Phase II Secondary Endpoints

1. Unacceptable toxicity rate at 8 weeks (any grade 5 toxicity, grade 4 neuropsychiatric toxicity or grade 4 clinically significant non-hematologic toxicity thought to be definitely, probably or possibly related to study drug)

2. Overall response rate at 8 and 16 weeks using RECIST v1.1.

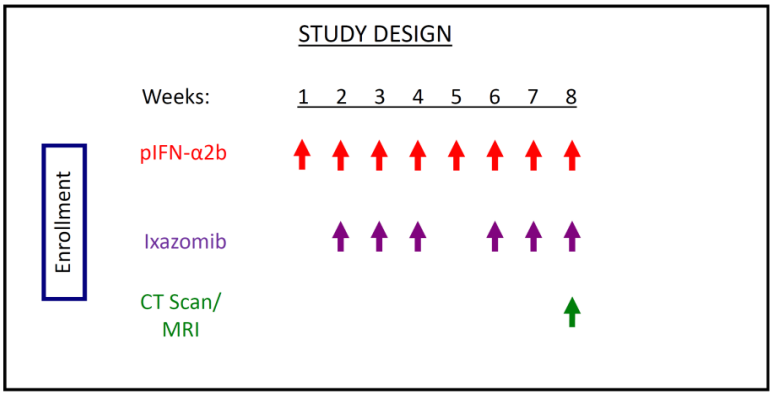
Exploratory Endpoints for Phase I and II

1. To examine changes in STAT1 phosphorylation and MHC1 up regulation in PBMCs as an indicator of IFN activity
2. To use archived FFPE tumor tissue to correlate activated tissue stroma (i.e., desmoplasia) with IL-6 and patient outcomes
3. To use archived FFPE from past biopsies for IHC analysis of nuclear NF-κB p65 and STAT1.

4. STUDY DESIGN

The study will be conducted at a single center: Fox Chase Cancer Center. It will be open label and non-randomized. It will begin with a '3+3' phase I component to asses initial safety, tolerability and RP2D, followed by a phase II using a two-stage design previously described.[49] The design is meant to incorporate an early stopping rule for futility and toxicity, and to allow for an endpoint of PFS at 8 and 16 weeks rather than overall response. We believe PFS is a more valid endpoint given the scientific rationale of tumor necrosis rather than apoptosis resulting in radiographic disease stabilization rather than shrinkage. The overall treatment plan can be seen in Schedule of Events (page 6) and Figure 4-1.

Figure 4-1: Study Schema



4.1 Phase I: Dose Escalation

The first part of the study will be a phase I dose escalation enrolling at a minimum 12 candidates using a 3+3 stepwise design with doses for escalation and de-escalation and rules for each noted below in Table 4-1 and 4-2.

Evaluation will occur at C2D1) for toxicity, tolerability and dose-limiting toxicity during the first cyclefor escalation decision. Treatment can continue indefinitely if beneficial (continual response or stable disease or symptomatic clinical benefit as defined by the

investigator) to patient. Evaluation at 8 and 16 weeks for efficacy and theses patients may continue indefinitely as well if showing signs of clinical benefit.

Table 4-1: Dose escalation and de-escalation for phase I

Dose level	Ixazomib mg	Pegylated IFN µg/kg/week
-2	2.3	2
-1	3	2
1 (starting dose level)	3	3
2	3	4.5
3 (expected RP2D)	4	4.5

Table 4-2. Escalation and De-Escalation Decision Rules

Number of Patients with DLT	Escalation/De-escalation Decision Rules
0 out of 3	**If dose level 1**: Open dose levels 2. **If dose level 2**: Open dose level 3. **If dose level 3**: Declare this dose level the maximally administered dose (MAD). Move forward at this dose level to the expansion phase II. **If dose level -1 or -2:** Dose level -1 or -2 will become the MTD and be used to move to phase II.
1 out of 3	**For all dose levels**: Enter 3 more patients at this dose level. If 0 of these 3 patients experience DLT, consider this dose acceptable and escalate to the next dose(s) as described above. If another patient experiences DLT at this dose level (2 DLTs total), consider this dose unacceptable and the MAD. Declare the next lowest dose level(s) to be the MTD. If the MAD is dose level -2 and there are at least 2/6 DLTs the study will be halted for re-evaluation of lower dose levels.
≥2	**For dose level 3**: Consider this dose unacceptable. Dose level 2 will become the MTD and be used to move to phase II. **For dose level 2**: Consider this dose unacceptable. Dose level 1 will become the MTD and be used to move to phase II. **For dose level 1**: Consider this dose unacceptable. De-escalate to dose level -1 and enroll additional 3 patients at dose level -1 followed by assessment as per above. **For dose level -1**: Consider this dose unacceptable. De-escalate to dose level -2 and enroll additional 3 patients at dose level -2 followed by

	assessment as per above. If there are at least 2/6 DLTs the study will be halted for re-evaluation of lower dose levels.

The table below indicates the probability of dose escalation given various true DLT rates.

Probability of dose escalation.

	True Probability of DLT at Current Dose						
	0.10	0.20	0.30	0.40	0.50	0.60	0.70
Probability that dose is escalated	0.91	0.71	0.49	0.31	0.17	0.08	0.03

Only those patients that have received at least one dose of ixazomib will be counted for phase I analysis. If a patient stops the trial for reasons other than DLT in the first 28 days, they will be replaced for phase I analysis.

4.1.1 Definition of Dose Limiting Toxicity

Dose-limiting toxicity (DLT) is any drug related toxicity occurring during the first 28 days on study (cycle 1) that meets the parameters defined in Table 4-3.

Table 4-3. Dose Limiting Toxicity

Any grade 3 or 4 treatment related non-hematologic toxicity regardless of duration, except: • Grade 3 nausea or Grade 3 vomiting ≤ 72 hours that recovers to grade 0-2 with maximal antiemetic therapy. • Grade 3 diarrhea that resolves to grade 0-2 with loperamide or diphenoxylate/atropine within 48 hours. • Grade 3 hypercholesterolemia, hypertriglyceridemia, hyperglycemia or hypophosphatemia that resolves to grade 0-2 with medical management. • Transient electrolyte abnormalities lasting ≤ 1 week.
Grade 3 or 4 thrombocytopenia.
Grade 4 neutropenia associated with fever or hospitalization for infection.
Grade 4 neutropenia lasting longer than 5 days.
Any toxicity felt to be possibly or probably related to study medication that causes the patient to miss more than 1 dose of either Ixazomib or pIFN in the first 28 days.
Any unacceptable toxicity (UT) as defined for phase II below (Section 4.5)

4.2 Phase II: Dose Expansion

Once the RP2D is reached in phase I in at least 3 patients, 3 additional patients will be enrolled that together will make up 6 patients that will count toward the toxicity and feasibility assessment for the phase I part, but also for the first step of phase II. See Figure 7-1 for full flow diagram of study enrollment.

The treatment plan for both phases I and II of the study is summarized in Figure 4-1. A cycle is defined as 28 days. IFN will be dosed once a week continuously with no scheduled interruptions. Ixazomib will be administered once a week, in weeks 2-4, with a break during week 1.

The schedule of administration and target dose (level 3) of IFN and ixazomib is based on a) trials of pegylated IFN-alpha 2b in RCC which demonstrated comparable safety and efficacy of pegylated IFN to standard IFN,[25,26,28] b) the study of IFN and bortezomib combination in melanoma,[35] and c) the current dosing schedule for oral ixazomib. The use of pegylated IFN will improve both feasibility and theoretically provide more sustained levels (half-life ~50 hours) of IFN compared to short-acting IFN. Once weekly dosing of ixazomib will also be conducive to patient adherence and ease of administration.

Radiologic evaluation will occur after every 2 cycles for the first 8 cycles (at 8 weeks, 16 weeks, 24 weeks and 32 weeks) and then every 3 cycles thereafter. Treatment will continue indefinitely or until PD, DLT or patient withdrawal. Given the i) historic PFS of ~5 months for first line use of IFN[18,19], ii) PFS of <2 months for single agent bortezomib[32] in RCC, iii) a PFS of 4.0 months for FDA-approved second line everolimus in RCC[50] and iv) the inclusion of patients after an unlimited number of treatment regimens, we will consider the regimen not worthy of pursuit if ≥66% of patients are progression-free at 8 weeks. If ≤56% of patients are progression free at 16 weeks, this regimen would be worthy of further investigation (see below for abbreviated statistical design). This will approximate the 4 month PFS of second line everolimus. Patients who develop a CR, PR or SD at study completion, may continue on treatment post study end.

4.3 Number of Patients

For the phase I portion of the study we will enroll at a minimum 12 and at a maximum 18 (please see Section 4.1 and 7 for more on the statistical design). For the phase II portion of the study we will enroll up to 31 patients. For phase II portion, a patient is considered enrolled at cycle 1 day 1 when at least one of the drugs has been administered. All patients will be included in the toxicity and PFS and RR analysis.

4.4 Duration of Study

From a study perspective, the anticipated duration of study being open for enrollment will be 2 years. Amongst Fox Chase Cancer Center and its community partners (FCCCP) there were 450 analytic (new to the system without ever having had any treatment for their RCC) cases of RCC in 2012 alone and FCCCP saw approximately 224 of those cases. Out of these cases at FCCCP ~35/year were de novo metastatic and ~30/year were locally advanced and thus at high risk for short-term metastatic progression. In addition, ~30 non-analytic (primary treatment conducted elsewhere) cases per year were seen at FCCCP sites.

According to the FCCC RCC database 256 nephrectomies were performed in 2010, 234 in 2011, 227 in 2012 and 204 in 2013. Over 75 new patients with RCC were seen by GU medical oncology in 2013 and over 43 patients with RCC were enrolled on therapeutic clinical trials. Given this volume of patients we believe we can accrue 1-2 patients a month to this trial.

Once an eligible patient is identified and consented, screening will occur within 28 days of study initiation (first day of drug administration). The patient will stay on study unless he or she has PD or unacceptable toxicity or chooses to withdraw consent. Once a patient is no longer on active treatment, follow up for progression and survival will occur for a maximum of 2 years.

4.5 Definition of Unacceptable Toxicity

For this study we will have a category defined as unacceptable toxicity (UT). This will be used in the statistical design of the phase II part of the study and incorporated into an early stopping rule (see Figure 7-1). If a patient develops UT at any point in the study, he or she will be withdrawn from the study. If more than 1 UT occurs during the final 6 patients of Phase I of the study, the study will be halted for assessment and possible amendment (Figure 7-1).

UT is defined as any grade 5 toxicity, grade 4 neuropsychiatric toxicity or grade 4 clinically significant non-hematologic toxicity thought to be definitely, probably or possibly related to study drug.

4.6 Exploratory endpoints and Protocol for Exploratory Analysis

See Lab Manual for details of specimen collection and storage.

1. To examine changes in STAT1 phosphorylation and MHC1 up regulation in PBMCs as an indicator of IFN activity

a. STAT1 phosphorylation by immunoblot analysis. PBMCs will be isolated from patient blood by Ficoll-Paque Plus-based centrifugal separation (GE Healthcare Bio-Sciences, Uppsala, Sweden) and crypreserved. The phosphorylated (p) form of STAT1 (Tyr701) as well as total STAT1 in cryopreserved PBMC pellets will be measured by immunoblot analysis and/or by intracellular flow cytometry performed on fresh PBMC. For immunoblot analysis, Whole-cell extracts from PBMCs (5×10^5) will be prepared in TL buffer (1% [v/v] Triton-X100, 150 mM NaCl, 20 mM HEPES [pH 7.3], 5 mM EDTA, 5 mM NaF, 0.2 mM $NaVO_3$ [*ortho*], and Complete Protease Inhibitor cocktail [Roche]) and clarified by centrifugation (15000×g, 10 minutes at 4°C). Samples will be denatured by boiling in Laemmli buffer (0.1% [v/v] 2-mercaptoethanol, 0.0005% [w/v] bromophenol blue, 10% [v/v] glycerol, 2% [w/v] SDS, 63 mM Tris-HCl [pH 6.8]) for 2 minutes and separated by 12% SDS-PAGE. Protein from gels will be transferred onto PVDF membranes (Millipore) and blocked in blocking buffer (0.1% [v/v] Tween20, 5% [w/v] non-fat dry milk in PBS) at room temperature for 15 minutes. Blots will be incubated for at least 18 h at 4°C with primary antibody (p-STAT1, 1:500 (Upstate); total STAT1, 1:2000 (BD

Pharmingen) diluted in blocking buffer, followed by 3 washes for 10 minutes each in washing buffer (0.1% [v/v] Tween 20 in PBS) at room temperature. Next, blots will be incubated with secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit/mouse IgG (H+L), 1:5000, Jackson ImmunoResearch) for at least 18 h at 4°C, followed by 3 washes of 10 minutes each in washing buffer at room temperature. Blots will then be incubated in enhanced chemiluminescence substrate (Pierce ECL Plus Western Blotting Substrate, Pierce) for one minute, and proteins will be detected by chemiluminescence using X-ray film (Denville Scientific Inc.).

b. STAT1 phosphorylation by intracellular flow cytometry. Fresh PBMCs (5×10^5 cells) obtained from patients will be suspended in 100 µL of RPMI-1640 medium supplemented with 10% FBS, fixed by incubating in 100 µL of Fix & Perm Reagent A (Caltag Laboratories, Burlingame, CA) for 2–3 minutes at room temperature, and incubated for 10 minutes in 3 mL of cold methanol. Cells will then be washed in flow buffer (PBS supplemented with 5% FBS) and permeabilized with 100 µL of Fix & Perm Reagent B (Caltag Laboratories. Cells will then be incubated for a total of 30 minutes at room temperature in Fix & Perm Reagent B containing 1 µg of a mouse anti-human FITC-conjugated anti-pSTAT1 antibody (BD Pharmingen) or an appropriate conjugated isotype control antibody. Fluorescence staining data will be acquired on a BD FACSAria II, which is calibrated daily for clinical use. Data will be processed with FlowJo software.

c. MHC1 upregulation by flow cytometry. Fresh PBMCs Cells will then be stained using fluorophore-conjugated pan-MHC-I specific antibody (clone W6/32, DAKO) or an appropriate isotype control. Fluorescence staining data will be acquired on a BD FACSAria II, which is calibrated daily for clinical use. Data will be processed with FlowJo software.

2. To use archived FFPE tumor tissue to correlate activated tissue stroma (i.e., desmoplasia) with IL-6 and patient outcomes

The Cukierman lab has observed that *in vivo* stromal activation (measured by a specific stromal isoform of the actin binding protein palladin) corresponds to worse outcomes regardless of grade or stage of the tumor in RCC.[51] Also, using stromal (patient derived tumors) ECMs they have observed increased tumoral IL-6 and NF-kB signaling (Cukierman unpublished results). We will thus examine the correlation between activated stroma (by assessing palladin IHC levels, as published by Dr. Cukierman and briefly described below, in the primary renal tumor, a metastasectomy sample or a biopsy) and plasma levels of IL-6 (using ELISA based assays) with patient outcome and response to treatment a la previous work. We want to answer whether activated stroma could predispose for increased IL-6 levels and whether both together will present a better response to the proposed combination treatment. The rationale behind this is that patients with high desmoplasia (represented by plasma IL-6 levels) will not only be at increased risk of worse outcome and recurrence but the same patients may also present better responses to

the combinational treatment approach.

Immunohistochemistry of paraffin-embedded samples will be performed using rabbit anti-palladin (1:100) antibody. A hybridoma via an MTA agreement is also in place and thus unlimited access to monoclonal antibody is available. An avidin biotin-peroxide kit (Vectastain Elite; Vector Laboratories, Burlingame, CA), together with chromogen 3',3'-diaminobenzidine, was used following manufacturer's instructions. Negative controls will consist of treated samples incubated using iso-matched non-specific primary antibodies and normal rabbit or mouse pre-immune sera. All sections will be counterstained with hematoxylin and mounted for inspection. The score for the intensity of staining in the immunohistochemistry will be performed by a 'blinded' pathologist using semiquantitative measurements. The assigned intensities consist of four categories; 0, 0.5, 1, and 2. Absence of staining will be assigned with the lowest value (0) and the strongest set of intensities will be assigned the highest (2). The correlation between immunohistological staining and clinical parameters and outcomes will be analyzed.

Serum levels of IL-6 will be assessed using ELISA. Sera will be procured via centrifugation of peripheral blood samples of study patients and stored at -80° until analysis. Sera samples will be thawed at room temperature and then assayed in duplicate wells using the premixed Bio-Plex® Pro Human Cytokine Array (Bio-Rad) according to manufacturer's instructions.

3. To use archived FFPE from past biopsies for IHC analysis of nuclear NF-κB p65 and STAT1

FFPE sections will be cut to a thickness of 4 microns, deparaffinized by xylene, and rehydrated in decreasing concentration of ethanol. Antigen retrieval will be achieved by boiling sections in 10mM citrate buffer for 20 minutes. After blocking of endogenous peroxidase with 3% hydrogen peroxidase in methanol, sections will be incubated with Background Sniper (Biocare Medical) at room temperature for 30 minutes. The sections will next be incubated with primary antibodies, NF-κB p65 (Cell Signaling) at a dilution of 1:500, and STAT1 (BD Biosciences) at a dilution of 1:100, at 4°C overnight. After washing in PBS, sections will be incubated with Labeled Polymer-HRP anti-rabbit and anti-mouse (DAKO) secondary antibody at RT for 1h, exposed to diaminobenzidine tetrahydrochloride solution, and counterstained with hematoxylin. After dehydrating in increasing concentrations of ethanol and clearing in xylene, sections will mounted in Permount. Images will be taken on a Nikon Eclipse E600 microscope with NIS Elements D3.0 software and scored across multiple fields for percentage of cells with nuclear NF-kB or STAT1. Only nuclear staining for p65 and STAT1 are demonstrative of activity and thus only nuclear staining will be scored.

5. STUDY POPULATION

5.1 Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be enrolled in the study:

1. Male or female patients 18 years or older.

2. Voluntary written consent must be given before performance of any study related procedure not part of standard medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.

3. Female patients must be:

   - Postmenopausal for at least 1 year before the screening visit, OR

   - Surgically sterile, OR

   - If they are of childbearing potential, agree to practice 2 effective methods of contraception, at the same time, from the time of signing the informed consent form through 90 days after the last dose of study drug, OR

   - Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception.)

4. Male patients, even if surgically sterilized (ie, status post-vasectomy), must agree to practice effective barrier contraception during the entire study treatment period and through 90 days after the last dose of study drug, or agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception.)

5. Patients must have a diagnosis of a metastatic renal cell carcinoma with a ≥50% clear cell component.
6. Eastern Cooperative Oncology Group (ECOG) performance status 0, 1, or 2.
7. Patients must meet the following clinical laboratory criteria:

   - Absolute neutrophil count (ANC) ≥ 1,000/mm^3 and platelet count ≥ 100,000/mm^3 and hemoglobin ≥ 9 g/dL. Platelet or red cell transfusions to help patients meet eligibility criteria are not allowed within 3 days before study enrollment.

   - Total bilirubin ≤ 1.5 × the upper limit of the normal range (ULN).

- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 3 × ULN.

- Calculated by Cockcroft-Gault or measured by 24 hour urine creatinine clearance ≥ 50 mL/min (see Section 12.2).

8. Measurable disease by RECIST 1.1.
9. Receipt of at least two line of prior therapy for metastatic RCC.
10. Patients with stable brain metastasis are eligible provided they received definitive therapy (EBRT, gamma knife, surgery) no sooner than 14 days prior to registration and are off all steroids.

5.2 Exclusion Criteria

Patients meeting any of the following exclusion criteria are not to be enrolled in the study:

1. Female patients who are lactating or have a positive serum pregnancy test during the screening period.

2. Failure to have fully recovered (ie, ≤ Grade 1 toxicity) from the reversible effects of prior chemotherapy, radiation therapy or targeted therapy

3. Previous use of interferon, ixazomib or bortezomib.

4. Washout periods for prior therapy are as follows

    - Bevacizumab – last dose must be ≥ 6 weeks prior to day 1 of study treatment.

    - Targeted therapy – last dose must be ≥ 5 half-lives prior to initiation of day 1 of study treatment.

    - Other chemotherapy, immunotherapy, or radiotherapy – Last dose must be ≤ 3 weeks prior to day 1 of study treatment

5. Major surgery within 14 days before enrollment.

6. Radiotherapy within 14 days before enrollment. If the involved field is small, 7 days will be considered a sufficient interval between treatment and administration of the Ixazomib.

7. Untreated central nervous system involvement.

8. Uncontrolled thyroid disease.

9. Infection requiring systemic antibiotic therapy or other serious infection within 14 days before study enrollment.

10. Evidence of current uncontrolled cardiovascular conditions, including uncontrolled hypertension, uncontrolled cardiac arrhythmias, symptomatic congestive heart failure, unstable angina, or myocardial infarction within the past 6 months.

11. Systemic treatment, within 14 days before the first dose of Ixazomib, with strong inhibitors of CYP1A2 (fluvoxamine, enoxacin, ciprofloxacin), strong inhibitors of CYP3A (clarithromycin, telithromycin, itraconazole, voriconazole, ketoconazole, nefazodone, posaconazole) or strong CYP3A inducers (rifampin, rifapentine, rifabutin, carbamazepine, phenytoin, phenobarbital), or use of Ginkgo biloba or St. John's wort.

12. Known ongoing or active systemic infection, active hepatitis B or C virus infection, or known human immunodeficiency virus (HIV) positive.

13. Decompensated liver disease (Child-Pugh score >6) or active or past auto-immune hepatitis.

14. Any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of treatment according to this protocol. In particular, a history of a serous psychiatric illness that might be exacerbated by IFN-α-2b; a history of significant or unstable cardiovascular, hepatic or gastrointestinal disease; a history of autoimmune disease of any kind.

15. Known allergy to any of the study medications, their analogues, or excipients in the various formulations of any agent.

16. Known GI disease or GI procedure that could interfere with the oral absorption or tolerance of ixazomib including difficulty swallowing.

17. Evidence of another clinically or radiographically active invasive malignancy OR Diagnosed or treated for another malignancy within 2 years before study enrollment or previously diagnosed with another malignancy and have any evidence of residual disease. Patients with nonmelanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection.

18. Patient has ≥ Grade 3 peripheral neuropathy, or Grade 2 peripheral neuropathy with pain on clinical examination during the screening period.

19. Having received an investigational agent with 21 days of receiving the first dose of study drug on this trial.

5.3 Participant Registration

5.3.1 Patient Registration

Eligible patients will be entered on study centrally by the Fox Chase Cancer Center QA Coordinator or their designee. Following registration, patients should begin protocol

treatment within 7 days of registration. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be cancelled and the subject will be replaced. The QA Coordinator should be notified of cancellations as soon as possible.

Subjects may be registered from 9:00 am to 5:00 pm excluding holidays by calling the QA Coordinator at 215-728-4770. The site's investigator or designee will then fax the completed registration form, consent and HIPAA signature pages and eligibility checklist to 215-728-2687. The QA Coordinator or designee will notify the site by phone and fax when registration is confirmed and the sequence number has been assigned. Subjects must be registered and have received a sequence number assigned by the QA Coordinator prior to the initiation of treatment. The following forms must be completed at the time of registration:

- Signed and dated informed consent form
- Signed and dated HIPAA consent form
- Registration form
- Signed eligibility checklist

6 STUDY DRUG

6.1 Description of Investigational Agents

Ixazomib Capsules

The ixazomib drug product is provided in strengths of 4.0-, 3.0-, and 2.3-mg and 2.0-, 0.5-, and 0.2 mg capsules as the active boronic acid. The different dose strengths are differentiated by both capsule size and color as described below:

Dose Strength	Capsule Size	Capsule Color
4.0 mg	Size 4	Ivory
3.0 mg	Size 3	Light gray
2.3 mg	Size 2	Light pink
2.0 mg	Size 2	Swedish orange
0.5 mg	Size 3	Dark green
0.2 mg	Size 4	White opaque

For additional details, please see the Ixazomib IB.

Pegylated Interferon alpha 2b

The pIFN will be obtained commercially for each patient.

6.2 Study Drug Administration

Ixazomib Administration

All protocol-specific criteria for administration of study drug must be met and documented before drug administration. Study drug will be administered or dispensed only to eligible patients under the supervision of the investigator or identified subinvestigator(s). Patients should be monitored for toxicity, as necessary, and doses of ixazomib should be modified as needed to accommodate patient tolerance to treatment; this may include symptomatic treatment, dose interruptions, and adjustments of ixazomib dose (see Section 6.3).

Capsules of ixazomib will also be referred to as study drug. Study drug will be supplied by Millennium as capsules of 2.0 mg, 2.3 mg, 3.0, and 4.0 mg ixazomib. If during the dose escalation phase I we find that the lowest dose (ixazomib 2.3 mg and pIFN 3 µg/kg/week) is not tolerable and have to dose de-escalate, we will use 0.5 mg capsules and the protocol will be amended. If the MTD in Phase I is found to be 2.3 mg, dose levels for phase II will be 2.3 mg, 2.0 mg and 1.5 mg.

The prescribed administration of ixazomib doses in this study is 1.5-4.0 mg ixazomib weekly for 3 out of 4 weeks in each cycle (1 cycle=28 days).

Patients should be instructed to swallow ixazomib capsules whole, with water, and not to break, chew, or open the capsules. Study drug should be taken on an empty stomach (no food or drink) at least 1 hour before or 2 hours after a meal. Each capsule should be swallowed separately with a sip of water. A total of approximately 8 ounces (240 mL) of water should be taken with the capsules.

Missed doses can be taken as soon as the patient remembers if the next scheduled dose is 72 hours or more away. A double dose should not be taken to make up for a missed dose. If the patient vomits after taking a dose, the patient should not repeat the dose but should resume dosing at the time of the next scheduled dose.

The patient will take the pill in clinic during the first 2 cycles and either in clinic or at home during all subsequent cycles depending on whether they are in clinic for a visit on a particular week or not. They will write down in a patient diary the time of administration and dose when taking at home.

Ixazomib Destruction

Investigational ixazomib (expired or end of study) should be destroyed on site according to the institution's standard operating procedure. The investigators will document removal and destruction on drug accountability logs.

Pegylated IFN-α-2b Administration

Pegylated IFN-α-2b (PegIntron): Each patient will be treated with Pegylated IFN-α-2b by subcutaneous injection in clinic by a registered nurse or at home by self-injection after proper training. They will write down in a patient diary the time of administration and dose

if they are injecting at home. The dose will be based on phase of study (see section 4-1). The drug will be obtained commercially in various vial strengths depending on patient's weight in kg (see below). The injection site will be rotated between thigh, outer surface of upper arm, and abdomen. Injections will not be given near navel or waistline; patients who are thin will only have thigh or upper arm used. No injection will be given into a bruised, infected, irritated, red, or scarred skin.

All patients will be pre-medicated with acetaminophen (500-1000 mg orally) 30 minutes prior to the first dose and as needed for subsequent doses thereafter.

Dose in mL=Weight (kg) x Dose (μg/kg)/Reconstituted Vial Strength (μg/mL)

For patients whose CrCl was acceptable for study eligibility/entry per a 24 hour urine creatinine clearance, but not Cockcroft-Gault, if serum creatinine increases by 20% or more, the dose of pegylated IFN will be reduced by 25% for the particular dose level the patient is at and not re-escalated.

If a patient's CrCl was acceptable per Cockcroft-Gault for study eligibility/entry, if CrCl drops to between 30-50 ml/min while on treatment, the dose of pegylated IFN will be reduced by 25% for the particular dose level the patient is at and not re-escalated.

6.3 Dose-Modification Guidelines

Treatment with Ixazomib and Pegylated IFN-α-2b will use a cycle length of 28 days. Evaluations will occur as listed in the Study Calendar. For a new treatment cycle to begin, the patient must meet the following criteria:

- ANC must be $\geq 1{,}000/mm^3$.
- Platelet count must be $\geq 75{,}000/mm^3$.
- Hemoglobin must be ≥ 8.0 g/dl.
- All other non-hematologic toxicity (except for alopecia) must have resolved to $\leq$ Grade 1 or to the patient's baseline condition

If the patient fails to meet the above-cited criteria for initiation of the next treatment, dosing should be delayed for 1 week for both drugs. If the patient continues to fail to meet the above-cited criteria, delay therapy and continue to re-evaluate. The maximum delay before treatment should be discontinued will be 4 weeks or at the discretion of the Principal Investigator.

For dosing recommendations upon recovery, refer to Table 6-1, Table 6-2 and Table 6-3. If starting doses will be different than below, the protocol will be amended prior to moving on to Phase II.

6.4 Management of Clinical Events

Adverse drug reactions such as thrombocytopenia, diarrhea, fatigue, nausea, vomiting, and rash have been associated with ixazomib treatment. Management guidelines regarding these events are outlined below. Further details of management of ixazomib AEs are described in Section 6 of the Ixazomib IB.

Prophylaxis Against Risk of Infection

If lymphopenia is noted, patients may be at an increased risk of infection. In particular, lymphopenia can be associated with reactivation of herpes zoster and herpes simplex viruses. Antiviral therapy such as acyclovir or valacyclovir may be initiated as clinically indicated. Other antivirals are also acceptable.

Nausea and/or Vomiting

Standard anti-emetics, including 5-HT_3 antagonists, are recommended for emesis occurring upon treatment initiation; prophylactic anti-emetics may also be considered. Dexamethasone should not be administered as an anti-emetic. Fluid deficits should be corrected before initiation of study drug and during treatment.

Diarrhea

Diarrhea should be managed according to clinical practice, including the administration of antidiarrheals such as loperamide and diphenoxylate/atropine according to the package inserts. If there is clinical suspicion for c. difficile infection, send stool sample for toxin assay. Avoid loperamide and diphenoxylate/atropine if there is blood or mucous in the stool, or if diarrhea is accompanied by fever. Fluid intake should be maintained to avoid dehydration. Fluid deficits should be corrected before initiation of treatment and during treatment.

Erythematous Rash With or Without Pruritus

As with bortezomib, rash with or without pruritus has been reported with ixazomib, primarily at the higher doses tested and when given with agents where rash is an overlapping toxicity. The rash may range from limited erythematous areas, macular and/or small papular bumps that may or may not be pruritic over a few areas of the body, to a more generalized eruption that is predominately on the trunk or extremities. Rash has been most commonly characterized as maculopapular or macular. To date, when it does occur, rash is most commonly reported within the first 3 cycles of therapy. The rash is often transient, self-limiting, and is typically Grade 1 to 2 in severity.

Symptomatic measures such as antihistamines or corticosteroids (oral or topical) have been successfully used to manage rash and have been used prophylactically in subsequent cycles. The use of a topical, IV, or oral steroid (eg, prednisone $\leq$ 10 mg per day or equivalent) is permitted. Management of a Grade 3 rash may require intravenous antihistamines or corticosteroids. Administration of ixazomib (and/or other causative agent if given in

combination) should be modified per protocol and re-initiated at a reduced level from where rash was noted (also, per protocol).

In line with clinical practice, dermatology consult and biopsy of Grade 3 or higher rash or any SAE involving rash is recommended. Prophylactic measures should also be considered if a patient has previously developed a rash (eg, using a thick, alcohol-free emollient cream on dry areas of the body or oral or topical antihistamines). A rare risk is Stevens-Johnson Syndrome, a severe and potentially life-threatening rash with skin peeling and mouth sores, which should be managed symptomatically according to standard medical practice. Punch biopsies for histopathological analysis are encouraged at the discretion of the investigator.

Thrombocytopenia

Thrombocytopenia has been reported to date primarily at the higher doses tested. Blood counts should be monitored regularly as outlined in the protocol with additional testing obtained according to standard clinical practice. Thrombocytopenia may be severe but has been manageable with platelet transfusions according to standard clinical practice. Thrombocytopenia nadirs commonly recover without intervention by the beginning of the next scheduled cycle. Ixazomib administration should be modified as noted as per dose modification recommendations in Table 6-2 when thrombocytopenia occurs. Therapy can be reinitiated at a reduced level upon recovery of platelet counts. A rare risk is thrombotic thrombocytopenic purpura (TTP), a rare blood disorder where blood clots form in small blood vessels throughout the body characterized by thrombocytopenia, petechiae, fever, or possibly more serious signs and symptoms. TTP should be managed symptomatically according to standard medical practice.

Neutropenia

Neutropenia has been reported with ixazomib. Blood counts should be monitored regularly as outlined in the protocol with additional testing obtained according to standard clinical practice. Neutropenia may be severe but has been manageable with G-CSF according to standard clinical practice. Neutropenic nadirs commonly recover without intervention by the beginning of the next scheduled cycle or with a short delay in treatment. Ixazomib administration should be modified when neutropenia occurs, as noted in the dose modification recommendations in Table 6-2. Therapy can be reinitiated at a reduced level upon recovery of absolute neutrophil counts.

Fluid Deficits

Dehydration should be avoided because ixazomib may cause vomiting, diarrhea, and dehydration. Acute renal failure has been reported with ixazomib. Fluid deficits should be corrected before initiation of study drug and during treatment and as needed during therapy. Until further information is available, intake of NSAIDs while on this protocol should be avoided.

Hypotension

Symptomatic hypotension and orthostatic hypotension with or without syncope have been reported with ixazomib. Blood pressure should be closely monitored while the patient is on study treatment and fluid deficit should be corrected as needed, especially in the setting of concomitant symptoms such as nausea, vomiting, diarrhea, or anorexia. Patients taking medications and/or diuretics to manage their blood pressure (for either hypo- or hypertension) should be managed according to standard clinical practice, including considerations for dose adjustments of their concomitant medications during the course of the trial.

Posterior Reversible Encephalopathy Syndrome

One case of posterior reversible encephalopathy syndrome (PRES) has been reported with ixazomib. While this case ultimately resolved, PRES has also been reported rarely with another proteasome inhibitor, VELCADE. PRES is characterized by headache, seizures and visual loss, as well as abrupt increase in blood pressure. Prompt diagnosis and initiation of antihypertensive and anticonvulsant therapy are important to prevent irreversible end-organ damage.

Transverse Myelitis

Transverse myelitis has also been reported with ixazomib. It is not known if ixazomib causes transverse myelitis; however, because it happened to a patient receiving ixazomib, the possibility that ixazomib may have contributed to transverse myelitis cannot be excluded.

Table 6-1 MLN908 and Pegylated IFN-α-2b Dose Adjustments during Phase II: Note levels for dose modification do not correspond with levels for dose escalation. Dose modifications will depend on the RP2D. Final dose modification schedule will be decided upon once the RP2D is determined. As an example, if the RP2D is, as expected, 4.5 mcg/kg/week of pIFN and 4.0 mg per week of Ixazomib, then the dose modification schedule will be as below.

Dose Mod Level	pIFN Dose (mg)	Ixazomib Dose (mg)
1	4.5 mcg/kg/week	4.0 mg
-1	3.0 mcg/kg/week	3.0 mg
-2	1.0 mcg/kg/week	2.3 mg
-3	Discontinue	Discontinue

Table 6-2 Ixazomib and Pegylated IFN-α-2b Dose Adjustments for Hematologic Toxicities	
Within-Cycle Dose Modifications	
• If platelet count ≤ 75×10^9/L or ANC ≤ 0.50×10^9/L on a Ixazomib dosing day (other than Day 1 (if Day 1, see section 6.3))	• Ixazomib dose should be withheld. • Complete blood count (CBC) with differential should be repeated at least every other day until the ANC and/or platelet counts have exceeded the prespecified values (see Section 6.3.1) on at least 2 occasions. • Upon recovery, Ixazomib may be reinitiated with 1 dose level reduction.
• If ANC ≤ 0.50×10^9/L and the patient is febrile (febrile neutropenia)	• All treatment suspended until ANC count has exceeded the prespecified value (see Section 6.3.1) on at least 2 occasions. • Upon recovery, Ixazomib and pegylated IFN may be reinitiated with 1 dose level reduction.
Dose Modifications for Subsequent Treatment Cycles	
• Delay of > 2 weeks in the start of a subsequent cycle due to lack of toxicity recovery as defined in Section 6.3**Error! Reference source not found.** • ANC < 1.0×10^9/L, platelet count < 75×10^9/L, or other non-hematologic toxicities > Grade 2 or not to the patient's baseline condition	• Hold Ixazomib until resolution as per criteria Section 6.3. • Upon recovery, reduce Ixazomib 1 dose level. • The maximum delay before treatment should be discontinued will be 3 weeks or at the discretion of the PI.
Dose Modifications for Subsequent Treatment Cycles	
• All hematologic toxicities	For hematologic toxicity that occurs during a cycle but recovers in time for the start of the next cycle: o If dose was reduced within the cycle, start the next cycle at that same dose. o If due to toxicity timing, ie, during the off week for Ixazomib, a dose

Table 6-2 Ixazomib and Pegylated IFN-α-2b Dose Adjustments for Hematologic Toxicities	
	reduction was not required at that point in the cycle, reduce Ixazomib by 1 dose level at the start of the following cycle. o Do not reduce the dose both within a cycle and at the start of the cycle for the same most severe toxicity.

Table 6-3 Ixazomib and Pegylated IFN-α-2b Dose Adjustments (Delays, Reductions, and Discontinuations) Due to Adverse Events (Non-Hematologic Toxicities)

Grade 1 peripheral neuropathy	No action	
New or worsening Grade 1 peripheral neuropathy with pain or Grade 2 neuropathy without pain	• Hold Ixazomib ONLY until resolution to Grade ≤ 1 or baseline	
New or worsening Grade 2 peripheral neuropathy with pain (not grade Grade 2 peripheral neuropathy without pain, see above for that modification) or Grade 3 neuropathy	• Hold Ixazomib ONLY until resolution to Grade ≤ 1 or baseline • Reduce Ixazomib ONLY to next lower dose upon recovery	
New or worsening Grade 4 peripheral neuropathy	• Discontinuation of study drug	
Grade 2 Rash Grade 3 Rash	• Symptomatic recommendations as per Section 6.4 with no dose modification • Hold Ixazomib ONLY until resolution to Grade ≤ 1 or baseline. Initiate symptomatic recommendations as per	The investigator may discuss considerations for dose modifications and symptom management with the PI

Grade 4 Rash	Section 6.4. • Reduce Ixaxomib ONLY to next lower dose upon recovery. • Hold both study drugs and discontinue study treatment for the patient.	
Grade 2 diarrhea	• Continue drugs, but initiate symptomatic treatment as per Section 6.4	
Grade 3 diarrhea	• Hold Ixazomib AND Interferon until resolution to Grade ≤ 1 or baseline. Initiate symptomatic recommendations as per Section 6.4. • Reduce both drugs to next lower dose upon recovery.	
Grade 4 diarrhea	• Hold Ixazomib AND Interferon until resolution to Grade ≤ 1 or baseline. Initiate symptomatic treatment. Reduce Ixazomib by 2 dose levels and pegylated IFN by 1 dose level. If the current dose level is at a point where the above adjustment is not possible (i.e., dose adjustments have already been done), patient will be withdrawn from the study.	
Grade 2 nausea/vomiting	• Continue drugs, but initiate symptomatic treatment as per Section 6.4	

Grade 3 nausea/vomiting Grade 4 nausea/vomiting	• Hold Ixazomib AND Interferon until resolution to Grade ≤ 1 or baseline. Initiate symptomatic recommendations as per Section 6.4. • Reduce both drugs to next lower dose upon recovery. • Hold Ixazomib AND Interferon until resolution to Grade ≤ 1 or baseline. Initiate symptomatic treatment. Reduce Ixazomib by 2 dose levels and pegylated IFN by 1 dose level. If the current dose level is at a point where the above adjustment is not possible (i.e., dose adjustments have already been done), patient will be withdrawn from the study.	
Grade 3 non-hematologic toxicity judged to be related to **both study drugs**	• Hold BOTH drugs until resolution to Grade ≤ 1 or baseline	Symptomatic recommendations noted in Section 6.4
If not recovered to ≤ Grade 1 or baseline within 4 weeks	• Reduce BOTH drugs to next lower dose upon return to ≤ Grade 1 or baseline	
Subsequent recurrence Grade 3 that does not recover to ≤ Grade 1 or baseline within 4 weeks	• Hold BOTH drugs until resolution to Grade ≤ 1 or baseline • Reduce BOTH drugs to next lower dose	Monitor closely, take appropriate medical precautions, and provide appropriate symptomatic care

Grade 3 non-hematologic toxicity judged to be related to **IFN only.**	• Hold IFN ONLY until resolution to Grade ≤ 1 or baseline	Monitor closely, take appropriate medical precautions, and provide appropriate symptomatic care
If not recovered to ≤ Grade 1 or baseline within 4 weeks	• Reduce IFN to next lower dose upon return to ≤ Grade 1 or baseline	Monitor closely, take appropriate medical precautions, and provide appropriate symptomatic care
Subsequent recurrence Grade 3 that does not recover to ≤ Grade 1 or baseline within 4 weeks	• Hold IFN until resolution to Grade ≤ 1 or baseline • Reduce IFN to next lower dose	Monitor closely, take appropriate medical precautions, and provide appropriate symptomatic care
Grade 4 non-hematologic toxicities judged to be related to study drug	Consider permanently discontinuing study drug	Exceptions may be made in cases in which the investigator determines the patient is obtaining a clinical benefit

Renal Impairment Dose Adjustment:

For patients whose CrCl was acceptable for study eligibility/entry per a 24 hour urine creatinine clearance, but not Cockcroft-Gault, if serum creatinine increases by 20% or more, the dose of pegylated IFN will be reduced by 25% for the particular dose level the patient is at and not re-escalated.

If a patient's CrCl was acceptable per Cockcroft-Gault for study eligibility/entry, then if CrCl drops to between 30-50 ml/min while on treatment, the dose of pegylated IFN will be reduced by 25% for the particular dose level the patient is at and not re-escalated.

Once Ixazomib or pIFN is reduced for any toxicity, the dose may not be re-escalated.

6.5 Excluded Concomitant Medications and Procedures

The following medications and procedures are prohibited during the study:

Systemic treatment with any of the following metabolizing enzyme inhibitors is not permitted during this study. A DDI with a strong inhibitor would increase ixazomib exposure.

Strong inhibitors of CYP1A2: fluvoxamine, enoxacin, ciprofloxacin

Strong inhibitors of CYP3A: clarithromycin, telithromycin, itraconazole, voriconazole, ketoconazole, nefazodone, and posaconazole

Systemic treatment with any of the following metabolizing enzyme inducers should be avoided unless there is no appropriate alternative medication for the patient to use (Rationale: Unlike with inhibitors, if there were to be a DDI with an inducer, ixazomib exposure would be less; therefore, there would be a reduced chance of an AE. However, there may be less chance for an antitumor effect, but that is not an absolute reason to be taken off Ixazomib):

Strong CYP3A inducers: rifampin, rifapentine, rifabutin, carbamazepine, phenytoin, and phenobarbital.

The dietary supplements St John's wort and Ginkgo biloba are not permitted.

The following procedures are prohibited during the study:

Any antineoplastic treatment with activity against RCC except for drugs in this treatment regimen.

Radiation therapy (the requirement for local radiation therapy generally indicates disease progression).

The following drugs are not permitted during the study: zidoudine, theophylline derivatives, telbivudine, ribavirin, pegloticase, methadone, fluoxetine, aldesleukin, clozapine.

Monitor closely any CYP2D6 or CYP2C9 substrate drugs as Pegylated IFN may decrease serum concentration of CYP2D6 substrates.

6.6 Permitted Concomitant Medications and Procedures

The following medications and procedures are permitted during the study:

- Antiemetics, including 5-HT3 serotonin receptor antagonists, may be used at the discretion of the investigator.

- Loperamide or other antidiarrheal should be used for symptomatic diarrhea at discretion of the investigator. The dose and regimen will be according to institutional guidelines. Intravenous fluids should be given as needed to prevent volume depletion.

- Growth factors (eg, granulocyte colony stimulating factor [G-CSF], granulocyte macrophage-colony stimulating factor [GM-CSF], recombinant erythropoietin) are

permitted. Their use should follow published guidelines and/or institutional practice; however, alternative usage may be reviewed with the PI. Erythropoietin will be allowed in this study. Its use should follow published guidelines and/or institutional practice.

- Patients should be transfused with red cells and platelets as clinically indicated and according to institutional guidelines.

- Antiviral therapy such as acyclovir may be administered if medically appropriate.

- Concomitant treatment with bisphosphonates and RANKL inhibitor denosumab will be permitted, as appropriate.

- Patients who experience worsening neuropathy from baseline may be observed for recovery and have dose reductions/delays as indicated in the protocol, and any supportive therapy or intervention may be initiated as appropriate at the discretion of the investigator.

- Supportive measures consistent with optimal patient care may be given throughout the study.

6.7 Precautions and Restrictions

Pregnancy

It is not known what effects ixazomib has on human pregnancy or development of the embryo or fetus. Therefore, female patients participating in this study should avoid becoming pregnant, and male patients should avoid impregnating a female partner. Nonsterilized female patients of reproductive age group and male patients should use effective methods of contraception through defined periods during and after study treatment as specified below.

Female patients must meet 1 of the following criteria:

Postmenopausal for at least 1 year before the screening visit, OR

Surgically sterile, OR

If they are of childbearing potential, agree to practice 2 effective methods of contraception, at the same time, from the time of signing the informed consent form through 90 days after the last dose of study drug, OR

Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception.)

Male patients, even if surgically sterilized (ie, status postvasectomy), must agree to 1 of the following:

- Agree to practice effective barrier contraception during the entire study treatment period and through 90 days after the last dose of study drug, OR
- Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception.)
- Pegylated IFN is a category C pregnancy risk and the above should be followed.

6.8 Preparation, Reconstitution, and Dispensing

Ixazomib

As with any anticancer drug caution should be exercised when handling ixazomib capsules.

Pegylated Interferon

Two B-D Safety LokTM syringes are provided in the package; one syringe is for the reconstitution steps and one for the patient injection. There is a plastic safety sleeve to be pulled over the needle after use. The syringe locks with an audible click when the green stripe on the safety sleeve covers the red stripe on the needle. Brief instructions for the preparation and administration of PEG-Intron Powder for Injection are provided below. Please refer to the Medication Guide for detailed, step-by-step instructions.

Reconstitute the PEG-Intron lyophilized product with only 0.7 mL of supplied diluent (Sterile Water for Injection, USP). The diluent vial is for single use only. The remaining diluent should be discarded. No other medications should be added to solutions containing PEG-Intron, and PEG-Intron should not be reconstituted with other diluents. Swirl gently to hasten complete dissolution of the powder. The reconstituted solution should be clear and colorless. Visually inspect the solution for particulate matter and discoloration prior to administration. The solution should not be used if discolored or cloudy.

The reconstituted solution should be used immediately and cannot be stored for more than 24 hours at 2-80 C. The appropriate PEG-Intron dose should be withdrawn and injected subcutaneously. The PEG-Intron vial is a single use vial and does not contain a preservative. DO NOT REENTER VIAL. DISCARD UNUSED PORTION. Once the dose from a single dose vial has been withdrawn, the sterility of any remaining product can no longer be guaranteed. Pooling of unused portions of some medications has been linked to bacterial contamination and morbidity.

After preparation and administration of the PEG-Intron injection, it is essential to follow the procedure for proper disposal of syringes and needles. A puncture-resistant container should

be used for disposal of syringes. Patients should be instructed in the technique and importance of proper syringe disposal and be cautioned against reuse of these items (See MEDICATION GUIDE for detailed instructions.)

PEG-Intron is a white to off-white lyophilized powder supplied in 2-mL vials. The PEG-Intron Powder for Injection should be reconstituted with 0.7 mL of the supplied Diluent (Sterile Water for Injection, USP) prior to use. Below are the various vial sizes available and will be ordered based on a patient's weight in kg.

Each PEG-Intron Package Contains	
A box containing one 50 µg per 0.5 ml vial of PEG-Intron Powder for Injection and one 5 mL vial of Diluent (Sterile Water for Injection, USP), 2 B-D Safety Lok™ syringes with a safety sleeve and 2 alcohol swabs.	(NDC 0085-1368-01)
A box containing one 80 µg per 0.5 mL vial of PEG-Intron Powder for Injection and one 5 mL vial of Diluent (Sterile Water for Injection, USP), 2 B-D Safety Lok™ syringes with a safety sleeve and 2 alcohol swabs.	(NDC 0085-1291-01)
A box containing one 120 µg per 0.5 mL vial of PEG-Intron Powder for Injection and one 5 mL vial of Diluent (Sterile Water for Injection, USP), 2 B-D Safety Lok™ syringes with a safety sleeve and 2 alcohol swabs.	(NDC 0085-1304-01)
A box containing one 150 µg per 0.5 mL vial of PEG-Intron Powder for Injection and one 5 mL vial of Diluent (Sterile Water for Injection, USP), 2 B-D Safety Lok™ syringes with a safety sleeve and 2 alcohol swabs.	(NDC 0085-1279-01)

6.9 Packaging and Labeling

The study drug ixazomib capsules will be provided by Millennium. The study drug will be labeled and handled as open-label material, and packaging labels will fulfill all requirements specified by governing regulations.

Ixazomib capsules should be stored unopened at 2°C to 8°C (36°F-46°F). The capsules are individually packaged in cold form foil-foil blisters in a child-resistant package. The 0.2-, 0.5-, and 2.0 mg capsules are in 1 × 4 blister strips that are individually perforated. The strips (1 × 4) are placed in cartons containing 6 strips (24 total capsules) of the same

strength. The 2.3-, 3.0-, and 4.0 mg capsules are supplied as a 1 x 3 blister card in a child-resistant cardboard wallet.

PEG-Intron, should be stored at 25°C (77°F): excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature]. After reconstitution with supplied Diluent the solution should be used immediately, but may be stored up to 24 hours at 2° to 8°C (36° to 46°F). The reconstituted solution contains no preservative, is clear and colorless. Do not freeze.

6.10 Storage, Handling, and Accountability

Upon receipt at the investigative site, ixazomib should remain in the blister and carton provided until use or until drug is dispensed. The container should be stored at the investigative site refrigerated (36°F to 46°F, 2°C to 8°C). Ensure that the drug is used before the retest expiry date provided by Millennium. Expiry extensions will be communicated accordingly with updated documentation to support the extended shelf life.

In countries where local regulations permit, ixazomib capsules dispensed to the patient for take-home dosing should remain in the blister packaging and refrigerated as noted above until the point of use. The investigative site is responsible for providing the medication to the patient in the correct daily dose configurations. Comprehensive instructions should be provided to the patient in order to ensure compliance with dosing procedures. Patients who are receiving take-home medication should be given only 1 cycle of medication at a time. Patients should be instructed to store the medication refrigerated (36°F to 46°F, 2°C to 8°C) for the duration of each cycle. Patients should be instructed to return their empty blister packs to the investigative site, rather than discarding them. Reconciliation will occur accordingly when the patient returns for their next cycle of take-home medication. Any extreme in temperature should be reported as an excursion and should be dealt with on a case-by-case basis.

Because ixazomib is an investigational agent, it should be handled with due care. Patients should be instructed not to chew, break, or open capsules. In case of contact with broken capsules, raising dust should be avoided during the clean-up operation. The product may be harmful by inhalation, ingestion, or skin absorption. Gloves and protective clothing should be worn during cleanup and return of broken capsules and powder to minimize skin contact.

The area should be ventilated and the site washed with soap and water after material pick-up is complete. The material should be disposed of as hazardous medical waste in compliance with federal, state, and local regulations.

In case of contact with the powder (eg, from a broken capsule), skin should be washed immediately with soap and copious amounts of water for at least 15 minutes. In case of contact with the eyes, copious amounts of water should be used to flush the eyes for at least 15 minutes. Medical personnel should be notified. Patients are to be instructed on proper storage, accountability, and administration of ixazomib, including that ixazomib is to be taken as intact capsules.

See above for handing, storage and administration of IFN.

6.11 Study Compliance

Study drug will be administered or dispensed only to eligible patients under the supervision of the investigator or identified sub-investigator(s). The appropriate study personnel will maintain records of study drug receipt and dispensing.

6.12 Treatment Assignment

All patients will be assigned combination treatment. In phase I this will be based on the cohort and in phase II it will be a set dose based on phase I. Dose modifications are permitted as described in section 6.2.

6.13 Termination of Treatment and/or Study Participation

In the absence of treatment delays due to adverse events, treatment may continue for unlimited cycles or until one of the following criteria applies:

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

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care.

Patients who are withdrawn from the study will not be replaced.

Treatment delay of ≥ 21 days for any hematologic or non-hematologic toxicity will lead to permanent treatment discontinuation.

At the time of withdrawal, all study procedures outlined for the End of Study visit should be completed. The primary reason for patient's withdrawal from the study should be recorded in the source documents and CRF.

6.14 Long Term Follow Up

In regard to long-term follow-up, unless there is progression or withdrawal of consent, study follow-up will continue until 5 years maximum. Upon progression, patient will continue to be followed up for three years maximum with phone calls from the study coordinator every 3 months.

7 ADVERSE EVENTS

7.1 Definitions

7.1.1 Adverse Event Definition

Adverse event (AE) means any untoward medical occurrence in a patient or subject administered a pharmaceutical product; the untoward medical occurrence does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not it is related to the medicinal product. This includes any newly occurring event, or a previous condition that has increased in severity or frequency since the administration of study drug.

An abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered by the investigator to be a clinically significant change from baseline.

7.1.2 Serious Adverse Event Definition

Serious AE (SAE) means any untoward medical occurrence that at any dose:

- Results in **death.**
- Is **life-threatening** (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient **hospitalization or prolongation of an existing hospitalization** (see clarification in the paragraph below on planned hospitalizations).
- Results in **persistent or significant disability or incapacity**. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- Is a **congenital anomaly/birth defect**.
- Is a **medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent 1 of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (eg, prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term *severe* is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as *serious*, which is based on patient/event outcome or action criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm^3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

7.1.3 Severity Rating

The investigator will evaluate the severity of each adverse event. NCI Common Terminology Criteria for Adverse Events (CTCAE v.4.0) or study specific toxicity tables provided in the protocol define severity. If not included in CTCAE v.4.0, severity is expressed in numerical grade using the following definitions:

Grade 1: Mild-asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate-minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL.

Grade 3: Severe-severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE

Attribution/Relationship to study drug:

Definite – clearly related

Probable – likely related

Possible – may be related

Unlikely – doubtfully related

Unrelated – clearly not related

7.1.4 Expectedness

An Expected Adverse Event is one where the specificity or severity is consistent with the current information available from the resources.

An Unexpected Adverse Event is one where the nature, severity, or frequency of the event is related to participation in the research is not consistent with either:

1. The known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts: or

2. The expected natural progression of any underlying disease, disorder, or condition of the subject (s) experiencing the adverse event and the subjects(s) predisposing risk factor profile for the adverse event.

(OHRP Guidance on reviewing unanticipated problems 2007)

7.2 Procedures for Reporting Serious Adverse Events

AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. For serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration. For serious pretreatment events, the investigator must determine both the intensity of the event and the relationship of the event to study procedures.

AEs which are serious must be reported to Millennium Pharmacovigilance (or designee) from the date the participant signs Informed Consent through 30 days after administration of the last dost of Ixazomib. Any SAE that occurs at any time after completion of Ixazomib treatment or after the designated follow-up period that the sponsor-investigator and/or sub-investigator considers to be related to any study drug must be reported to Millennium Pharmacovigilance (or designee). In addition, new primary malignancies that occur during the follow-up periods must be reported, regardless of causality to study regimen, for a minimum of three years after the last dose of the investigational product, starting from the first dose of study drug. All new cases of primary malignancy must be reported to Millennium Pharmacovigilance (or designee).

Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

7.2.1 Site reporting responsibilities:

Since this is an investigator-initiated study, the principal investigator Daniel M. Geynisman, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor- investigator's EC or IRB.

Regardless of expectedness or causality, all SAEs (including serious pretreatment events) must also be reported to Millennium Pharmacovigilance:

Fatal and Life Threatening SAEs within 24 hours of the sponsor-investigator's observation or awareness of the event

All other serious (non-fatal/non life threatening) events within 4 calendar days of the sponsor-investigator's observation or awareness of the event

See below for contact information for the reporting of SAEs to Millennium Pharmacovigilance.

The sponsor-investigator must fax the SAE Form per the timelines above.

The SAE report must include at minimum:

- **Event term(s)**
- **Serious criteria**
- **Intensity of the event(s):** Sponsor-investigator's or sub-investigator's determination. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version specified in the protocol, as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

- **Causality of the event(s):** Sponsor-investigator's or sub-investigator's determination of the relationship of the event(s) to study drug administration.

Follow-up information on the SAE may be requested by Millennium.

Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version used at your institution, as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

In the event that this is a multisite study, the sponsor-investigator is responsible to ensure that the SAE reports are sent to Millennium Pharmacovigilance (or designee) from all sites participating in the study. Sub-investigators must report all SAEs to the sponsor-investigator so that the sponsor-investigator can meet his/her foregoing reporting obligations to the required regulatory agencies and to Millennium Pharmacovigilance, unless otherwise agreed between the sponsor-investigator and sub-investigator(s).

Relationship to all study drugs for each SAE will be determined by the investigator or sub-investigator by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the study drug(s)?

Sponsor-investigator must also provide Millennium Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to the study or study drug(s), including, but not limited to, telephone conversation logs within 24 hours but no later than 4 calendar days of such communication.

SAE and Pregnancy Reporting Contact Information

SAE and Pregnancy Reporting Contact Information

Fax Number: 1-800-963-6290
Email: TakedaOncoCases@cognizant.com

Reporting Form:

- US FDA MedWatch 3500A: http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

1. The investigator/ site is responsible to report all SAEs to the Study Monitor within 24 hours of becoming aware of the event. A written report must follow within 48 hours.

2. Each investigator is responsible to report all AEs/SAEs to their local IRB following guidelines set by that IRB. The FCCC ERP reserves the right to request an event be reported to the IRB at their discretion. Copies of events reviewed by the IRB must be sent via fax to the ERP Regulatory Coordinator at (215) 728-2914

3. Any investigator who is in doubt of whether a particular AE needs to be reported is directed to call the Study Monitor for confirmation with the Principal Investigator

4. If the results of an investigator or ERP investigation show an adverse event not initially determined to be reportable is so reportable, the investigator will report the event following the above guidelines based on the date the determination is made.

5. Copies of all related correspondence and reporting documents must be submitted to the ERP Regulatory Coordinator and will be maintained in a regulatory file.

The participating site should report events to:

Study Monitor
Fox Chase Cancer Center
Clinical Trials Operations
333 Cottman Avenue
Philadelphia, PA 19111
Telephone 215-214-3704
Fax 215-214-1511

7.2.2 ERP Reporting Responsibilities:

1. Adverse events which meet all of the following criteria must be reported to all participating institutions for IRB submission within 2 weeks of notification of the event.

i. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

ii. Possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

iii. Serious (refer to above definition) or otherwise one that suggests that the research places subjects or others at a greater risk of physical or psychological harm than was previously known or recognized.

2. If the adverse event requires modification of the study protocol and informed consent, these changes will be provided to all participating institutions in the form of an amendment from the ERP for each site's IRB of record along with the report of the adverse event.

3. Copies of all related correspondence and reporting documents will be maintained in a centralized regulatory file for this study by the ERP.

4. SAEs that are related, unexpected, fatal, or life-threatening are reportable through the Food and Drug Administration (FDA) MedWatch program by telephone or fax no later than 7 calendar days after initial receipt of the information. Further information on the timing of submissions is as directed by FDA guidelines (www.fda.gov/medwatch/index.html). Serious, unexpected events that suggest significant clinical risk will be submitted to within 15 calendar days after initial receipt of this information.

Food and Drug Administration:
Telephone 1-800-FDA-1088
Fax 1-800-FDA-0178
http://www.fda.gov/medwatch/report.htm

Mandatory Drug Reporting:
Central Document Room
Center for Drug Evaluation and Research
Food and Drug Administration
12229 Wilkins Avenue
Rockville, MD 20852
Office of Post-Marketing Drug Risk Assessment (HFD 730)
Center for Drug Evaluation and Research
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857
(301) 827-3169 for any further questions regarding where to send drug mandatory reporting forms

7.3 Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study or within 90 days after the last dose, she must inform the investigator immediately and permanently discontinue study drug. The sponsor-investigator must immediately fax a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee. The pregnancy must be followed for the final pregnancy outcome.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately fax a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

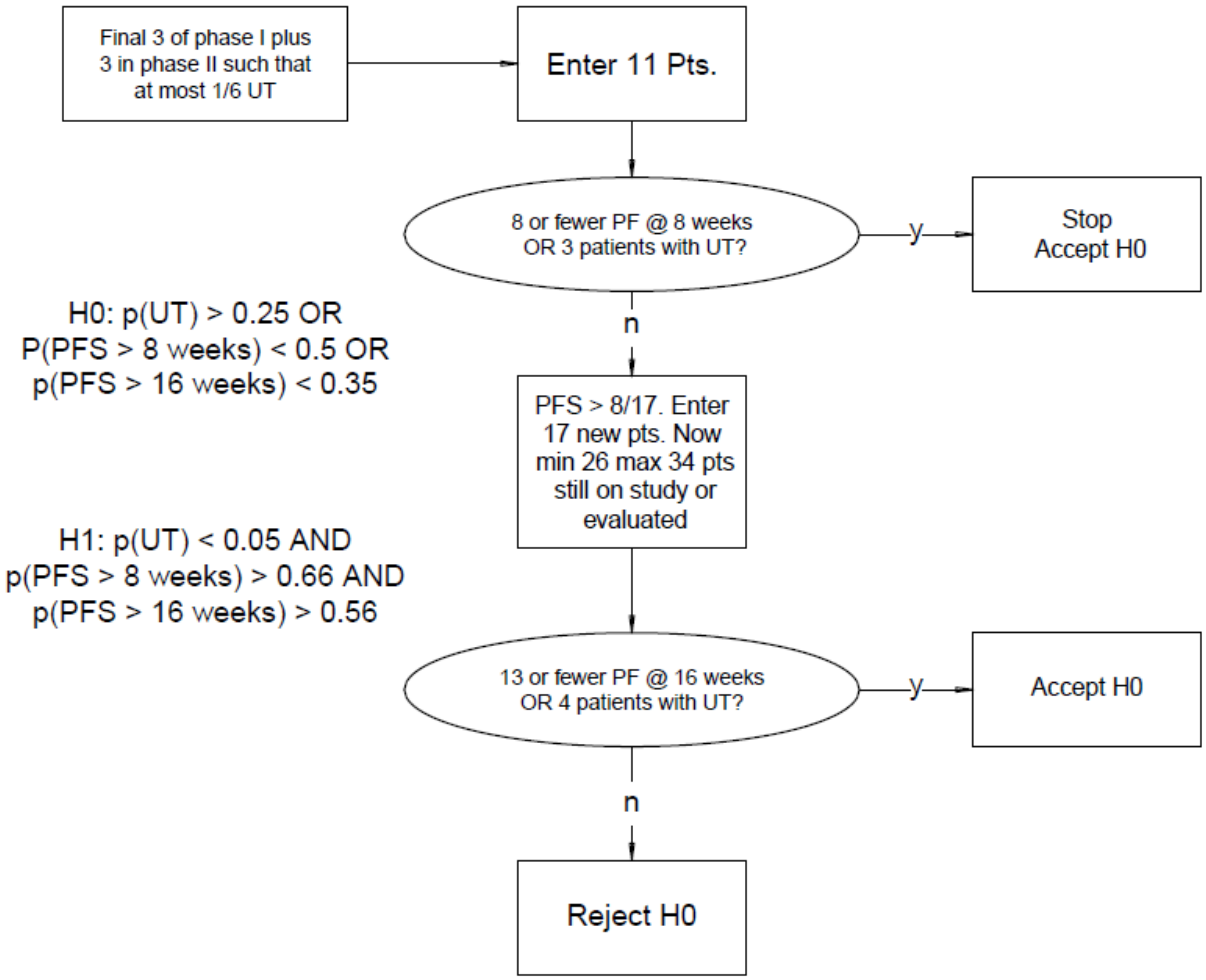
Pregnancy Reporting Form:

- Pregnancy Report Form (a sample is provided by Millennium)

8 STATISTICAL AND QUANTITATIVE ANALYSES

8.1 Statistical Methods

Figure 8-1: Flow diagram of statistical design for the phase II of the study



8.1.1 Definition of Phase II and exploratory endpoints and Analytic Plan

The phase II dose will be determined by an initial standard '3+3' phase I component as described in Section 4.1. Once a RP2D is reached, the phase II will begin.

Definition of phase II primary endpoint: Progression free survival at 8 and 16 weeks using RECIST v 1.1, defined from date of treatment initiation to date of progression according to RECIST or death, or is censored at the date of the last imaging. If patient is not evaluated at 8 and/or 16 weeks then the patient is not considered as alive and progression-free.

Definition of secondary outcomes/endpoints:

1. Composite of unacceptable toxicity at 8 weeks (grade 4 non-hematologic toxicity thought to be definitely, probably or possibly related to study drug)
2. Overall response using RECIST v1.1.

Definition of exploratory objectives:

1. Changes in STAT1 phosphorylation and MHC1 up regulation in PBMCs from baseline to week 2, 3, 7, EOT.
2. Degree of desmoplasia in archived FFPE tumor samples and change in serum IL-6
3. Degree of nuclear NF-κB p65 and STAT1 in archived FFPE tumor samples

Analytic plan for primary and secondary objectives:

Kaplan Meier curves will be used to estimate the distribution of progression free survival, median survival, and 1-year survival. Proportions, 95% confidence intervals, will be used to characterize composite rate of unacceptable toxicity and overall response rate. All analyses are for estimation only, and no larger inference will be generated given the sample size of the study.

Analytic plan for exploratory objectives:

Pre- and post- treatment phosphorylation changes in pSTAT1 and MHC1 (flow data, the MFIs will be log10) and serum IL-6 will be analyzed by paired t-tests. Degree of desmoplasia and nuclear NF-kB and STAT1 will be quantified using standard immunohistochemical methods. Statistical analysis for western blots and responders vs non-responders tumor immunohistochemistry will be performed using non-parametric Mann-Whitney test and/or paired t-tests.

8.1.2 Sample Size Determination

The design incorporates two conditions not usually treated. First, the final group of patients in the phase I component is considered part of the phase II trial. Second, the phase II part has two end-points, toxicity (UT: unacceptable toxicity) and efficacy. The relation between toxicity and efficacy is explicitly taken into account. We test the composite null hypothesis:

H0: $p(UT) > 0.25$ or $p(PFS > 8 \text{ weeks}) < 0.5$ or $p(PFS > 16 \text{ weeks}) < 0.35$

versus the alternative:

H1: $p(UT) < 0.05$ and $p(PFS > 8 \text{ weeks}) > 0.66$ and $p(PFS > 16 \text{ weeks}) > 0.56$.

Eleven new patients will be treated in the first stage of the phase 2 component. If 3/17 patients have UTs OR if at most 8/17 are alive and PF at 8 weeks the trial will be either be

suspended for excess toxicity or for futility. The decision for early termination (or amendment) is based on these cut points. If either 3 of 17 stage I patients are DLTs OR if at most 8 of 17 are PF at 8 weeks then terminate or hold up. The final decision point, PFS at 16 weeks is not relevant; the number of such patients does not influence the early decision. The confusion seems to relate to the alternative hypothesis which is couched in terms of 'AND'. It does, of course, require both early and late components of the outcome to be defined. Finally, although 3/17 is less than 0.25, this is the cut point, clearly between 0.05 and 0.25. The early PFS cut point 8/17 is less than 0.5 but is only a cut point.

At that point we will consider protocol amendment. Otherwise, 17 new patients will be recruited. If at least 14/34 patients are PF at 16 weeks AND less than 4/34 have UTs the null hypothesis will be rejected and the treatment declared efficacious. Otherwise it will be declared either too toxic or not efficacious.

Further operating characteristics and statistical details can be found in Appendix 12.3

8.1.3 Randomization and Stratification

No randomization or stratification will be used.

8.1.4 Populations for Analysis

Safety Population: All patients who receive at least one dose of both drugs.

Efficacy Population: All patients who receive at least one dose of both drugs.

8.1.5 Demographic and Baseline Characteristics

Descriptive statistics will be used to summarize baseline characteristics of patients.

8.1.6 Safety Analysis

Safety analysis will be performed by the PI and biostatics. Safety data will be reviewed by the Extramural Data Safety Monitoring Committee (EDMSC). Each patient will be monitored closely as detailed in the study calendar. Adverse events will be documented and summarized in table form. Interim analysis of toxicity, outcome and ongoing scientific investigations may be performed every 3 months by the Extramural Data Safety Monitoring Committee (EDSMC). In this capacity the EDSMC will serve as an advisory committee to ERP and FCCC IRB. The EDSMC will review those aspects of this trial that are outlined in the responsibilities section of the ERP Data and Safety Monitoring Plan (DSMP). If the committee decides that changes should be made to this trial, it will make recommendations in writing to the Extramural Research Committee and Division Medical Director and PI which, in turn, have the authority to approve or disapprove these recommendations. These changes will be discussed with the Study Principal Investigator before they are

implemented. These changes may include early termination of accrual. Other changes might include altering the accrual goals or changing the eligibility criteria for the trial.

9 MEASUREMENT OF EFFECT

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[53] 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.

All patients will be evaluable for toxicity from the time of their first treatment with pegylated interferon and ixazomib. Only those patients with evaluable and measurable disease will be enrolled and thus evaluable for objective response. A patient must have had at least one cycle of therapy (28 days) and have their disease re-evaluated to be considered evaluable for response and PFS.

Measurable Disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter for non-nodal lesions and short axis for nodal lesions to be recorded) as > 20 mm by chest x-ray, as > 10 mm with CT scan, or > 10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).
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 reproducible 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.

All measurements should be taken and recorded in metric notation using a ruler or calipers.

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.

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-holding techniques, if possible.

PET-CT should not be used as an imaging modality

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement.

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.

Evaluation of Target Lesions

Response	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 diameters of target lesions, taking as reference the baseline sum of diameters.
Progressive Disease (PD)	At least a 20% increase in the sum of 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 progression.
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.

Evaluation of Non-Target Lesions

Response	Evaluation of 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).
Non-CR/ Non-PD [Incomplete response/ Stable Disease (SD)]	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).

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).

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 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.

Progression-Free Survival
PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

10 ADMINISTRATIVE REQUIREMENTS

10.1 Product Complaints

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact MedComm Solutions (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Millennium Quality representative.

For Product Complaints,

- Phone: 1-877-TAKEDA7 (1-877-825-3327)
- E-mail: medicalinformation@tpna.com
- FAX: 1-800-247-8860
- Hours: Mon-Fri, 8 a.m. – 6 p.m. ET

Product complaints in and of themselves are not AEs. If a product complaint results in an SAE, an SAE form should be completed and sent to Millennium Pharmacovigilance (refer to Section 9.2).

10.2 Compliance with Protocol and Protocol Revisions

The study must be conducted as described in this approved protocol. All requests for revisions to the protocol must be provided to ERP. The Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an Amendment, except where necessary to eliminate an immediate hazard(s) to study patients. Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to the ERP. If the revision is an Administrative Letter, Investigators must inform their IRB(s)/IEC(s). ERP will work with Fox Chase Cancer Center Coordinating Center to ensure they are in receipt of all required documents.

10.3 Data Collection

An Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the investigational product.

10.4 Institutional Review Board/Independent Ethics Committee (IRB/IEC)

Before study initiation, the Investigator must have written and dated approval/favorable opinion from the IRB/IEC for the protocol, consent form, patient recruitment materials/process (e.g., advertisements), and any other written information to be provided to patients. The Investigator should also provide the IRB/IEC with a copy of the product labeling, information to be provided to patients, and any updates.

The Investigator should provide the IRB/IEC with reports, updates, and other information (e.g., Safety Updates, Amendments, and Administrative Letters) according to regulatory requirements or Institution procedures. Copies of the initial IRB approval as well as annual re-approvals must be submitted to ERP.

10.5 Records Retention

The Investigator must retain investigational product disposition records and source documents for the maximum period required by applicable regulations and guidelines, or Institution procedures. If the Investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another Investigator, IRB). Notice of such transfer will be given in writing to ERP.

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12 APPENDICES

12.1 Eastern Cooperative Oncology Group (ECOG) Scale for Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all predisease performance without restriction
1	Symptoms but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (eg, light housework, office 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.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5 (6):649-55.

12.2 Cockcroft-Gault Equation

For males:

$$\text{Creatinine Clearance} = \frac{(140 - \text{age[years]} \times \text{weight [kg]})}{72 \times (\text{serum creatinine[mg/dL]})} \quad \text{OR} \quad \frac{(140 - \text{age[years]} \times \text{weight [kg]})}{0.81 \times (\text{serum creatinine[}\mu\text{mol/L]})}$$

For females:

$$\text{Creatinine Clearance} = \frac{0.85\ (140 - \text{age[years]} \times \text{weight [kg]})}{72 \times (\text{serum creatinine[mg/dL]})} \quad \text{OR} \quad \frac{0.85\ (140 - \text{age[years]} \times \text{weight [kg]})}{0.81 \times (\text{serum creatinine[}\mu\text{mol/L]})}$$

Source: Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16(1):31-41.

12.3 Statistical Considerations and Operating Characteristics for Phase II

Operating characteristics of the design depend on four conditions: toxicity being low (0.05) or high (0.25) and PFS at 16 weeks being low (0.35) or high (0.56). They are summarized below:

1. Toxicity = 0.05 and PFS = 0.56. In this case the power of the study is at least 80%. The chance of early stopping (in error) for excess toxicity is less than 1%. The chance for stopping (in error) for futility varies from 8% to 12% depending on the relation of toxicity to PFS (see details below).

2. Toxicity = 0.05, PFS = 0.35. The chance of early stopping for futility varies from 49% to 57%. The chance of declaring treatment efficacious (type I error) varies from 10% to 21%.

3. Toxicity = 0.25 and PFS = 0.35. Type I error varies from less than 1% to about 5%. Chance of stopping early for either excess toxicity or futility varies from about 26% to about 59%.

4. Toxicity = 0.25 and PFS = 0.56. Type I error varies from about 5% to about 10%. Chance of early stopping for excess toxicity is about 27%.

The term 'UT' here means severe toxicity for which, if frequent enough, dictates dose reduction or trial termination. This early stopping design is an extension of the method of Litwin, Wong and Hudes (Statistics in Medicine, 2007,26:4400-4415). Below, the chance that a patient has a UT and also is PF at 16 weeks is represented by 'a'. 'b', 'c' and 'd', similarly, represent the indicated joint probabilities.

Let p = p(PFS > 16 weeks), p1 = p(PFS > 8 weeks) and p2 =p(PFS > 16 weeks | PFS > 8 weeks). Also, let ptox = p(UT in 16 weeks). Let ptox1 = f*ptox = p(UT in initial 8 week period) and ptox2 = (1-f)*ptox = p(UT in 8 through 16 week period| no UT in the initial 8 weeks). Then, b = p – a, c = ptox – a and d = 1 – a – b – c. a1, b1, c1, and d1 are similarly determined from p1 and ptox1. a2,b2,c2 and d2 are determined likewise, from p2 and ptox2.

The table:

P(UT)	1-p(UT)	
a	b	p(PFS > 16 weeks) = 0.56 (under the alternative hypothesis)
c	d	p(PFS < 16 weeks) = 0.44
ptox	1 - ptox	1 (ptox = 0.05 under the alternative hypothesis)

Illustrates how the joint event probabilities a, b, c and d at the end of the study relate. Namely, if we assign a = 0.02 then b, c and d can be determined by subtraction. Here we would get: b = 0.54, c = 0.03 and d = 0.41. For any value of a in (0, 0.05) the other three terms are completely specified. In this design we are considering toxicity to be cumulative. We model the total chance of UT to occur with fraction f in the first interval (0, 8 weeks) and 1-f in the final 8 weeks. We have considered fractions f = 0, 0.25, 0.5, 0.75 and 1.0, i.e. f=0 implies no toxicity in the first 8 weeks and f = 1 implies all toxicity occurs then. The final design is based on f = ½.

P(UT) 1-p(UT)

a1 b1 p(PFS > 8) = 0.66

c1 d1 p(PFS < 8) = 0.34

ptox1 1 – ptox1 1 (ptox1 = ptox2 = 0.025 under the alternative with f = ½) and

P(UT) 1-p(UT)

a2 b2 p(PFS > 16|PFS > 8) = 0.848

c2 d2 p(PFS < 16|PFS > 8) = 0.15

ptox2 1 – ptox2 1

The two tables above characterize the early stopping interval (0, 8 weeks) and final interval (8 weeks, 16 weeks). Numbers in the tables represent the alternative hypothesis. Patients in the initial cohort are represented by the top table (a1, b1, c1, d1) in the initial interval and those who are toxicity and progression free at 8 weeks, hence continue on study are represented by the second table (a2, b2,c2,d2) for their second 8 weeks. Patients recruited into the second cohort, if the study makes it past the first 8 week period, are evaluated only at 16 weeks and are represented by the very first table (a, b, c, d).

The chance of early stopping for excess toxicity, for futility, the chance of final declaration of excess toxicity, the power or type I error were all computed for each of the 4 conditions. Within each of these combinations, the fraction, f, of toxicity born within the first 8 weeks was varied in 5 steps from 0 to 1. We selected the number f = ½ in the belief that toxicity is cumulative and this is the maximum fraction expected to occur in the first half of the trial. Design behavior for f < ½ is 'better' than that for f > ½, so is not reported here in detail.

The table below lists the various operating probabilities as functions of 'a' for each of the four conditions.

Key:

null = 0: PFS at 16 weeks = 0.35

null = 1: PFS at 16 weeks = 0.56

ntox = 0: Toxicity = 0.05

ntox = 1: Toxicity = 0.25

a: probability that PFS > 16 weeks and patient had UT

tox: probability of early stopping for excess toxicity

fut: probability of early stopping for futility

toxend: probability that final decision is 'too toxic'

alpha: type I error, probability of declaring efficacy when either PFS = 0.35 at 16 weeks or toxicity = 0.25

power: probability of declaring efficacy when PFS at 16 weeks is 0.56 and toxicity is 0.05.

null	ntox	a	tox	fut	toxend	alpha	cksum
0	0	0.00	0.0059	0.4938	0.0115	0.2116	1.0000
0	0	0.01	0.0059	0.5105	0.0111	0.1871	1.0000
0	0	0.02	0.0059	0.5271	0.0107	0.1642	1.0000
0	0	0.03	0.0059	0.5437	0.0103	0.1429	1.0000
0	0	0.04	0.0059	0.5602	0.0099	0.1233	1.0000
0	0	0.05	0.0059	0.5766	0.0095	0.1054	1.0000

null	ntox	a	tox	fut	toxend	alpha	cksum
0	1	0.00	0.2690	0.2920	0.3671	0.0493	1.0000
0	1	0.01	0.2690	0.3050	0.3561	0.0451	1.0000
0	1	0.02	0.2690	0.3181	0.3450	0.0410	1.0000
0	1	0.03	0.2690	0.3312	0.3339	0.0369	1.0000
0	1	0.04	0.2690	0.3444	0.3227	0.0329	1.0000

0	1	0.05	0.2690	0.3576	0.3115	0.0291	1.0000
0	1	0.06	0.2690	0.3708	0.3003	0.0255	1.0000
0	1	0.07	0.2690	0.3840	0.2892	0.0221	1.0000
0	1	0.08	0.2690	0.3972	0.2781	0.0189	1.0000
0	1	0.09	0.2690	0.4103	0.2671	0.0161	1.0000
0	1	0.10	0.2690	0.4233	0.2562	0.0134	1.0000
0	1	0.11	0.2690	0.4361	0.2454	0.0111	1.0000
0	1	0.12	0.2690	0.4489	0.2347	0.0091	1.0000
0	1	0.13	0.2690	0.4615	0.2241	0.0073	1.0000
0	1	0.14	0.2690	0.4739	0.2137	0.0058	1.0000
0	1	0.15	0.2690	0.4860	0.2035	0.0045	1.0000
0	1	0.16	0.2690	0.4980	0.1935	0.0035	1.0000
0	1	0.17	0.2690	0.5097	0.1837	0.0026	1.0000
0	1	0.18	0.2690	0.5212	0.1741	0.0020	1.0000
0	1	0.19	0.2690	0.5324	0.1648	0.0014	1.0000
0	1	0.20	0.2690	0.5433	0.1557	0.0010	1.0000
0	1	0.21	0.2690	0.5540	0.1468	0.0007	1.0000
0	1	0.22	0.2690	0.5643	0.1382	0.0005	1.0000
0	1	0.23	0.2690	0.5742	0.1299	0.0003	1.0000
0	1	0.24	0.2690	0.5839	0.1219	0.0002	1.0000
0	1	0.25	0.2690	0.5932	0.1141	0.0001	1.0000

null	ntox	a	tox	fut	toxend	power	cksum
1	0	0.00	0.0059	0.0817	0.0234	0.8759	1.0000
1	0	0.01	0.0059	0.0888	0.0231	0.8653	1.0000

1	0	0.02	0.0059	0.0963	0.0228	0.8535	1.0000
1	0	0.03	0.0059	0.1042	0.0225	0.8404	1.0000
1	0	0.04	0.0059	0.1125	0.0222	0.8259	1.0000
1	0	0.05	0.0059	0.1213	0.0219	0.8099	1.0000

null	ntox	a	tox	fut	toxend	alpha	cksum
1	1	0.00	0.2690	0.0247	0.6054	0.1010	1.0000
1	1	0.01	0.2690	0.0279	0.6021	0.1011	1.0000
1	1	0.02	0.2690	0.0313	0.5985	0.1012	1.0000
1	1	0.03	0.2690	0.0351	0.5947	0.1012	1.0000
1	1	0.04	0.2690	0.0391	0.5907	0.1011	1.0000
1	1	0.05	0.2690	0.0435	0.5864	0.1009	1.0000
1	1	0.06	0.2690	0.0483	0.5818	0.1006	1.0000
1	1	0.07	0.2690	0.0533	0.5770	0.1002	1.0000
1	1	0.08	0.2690	0.0587	0.5718	0.0997	1.0000
1	1	0.09	0.2690	0.0645	0.5664	0.0990	1.0000
1	1	0.10	0.2690	0.0706	0.5607	0.0981	1.0000
1	1	0.11	0.2690	0.0771	0.5548	0.0970	1.0000
1	1	0.12	0.2690	0.0840	0.5485	0.0957	1.0000
1	1	0.13	0.2690	0.0912	0.5419	0.0941	1.0000
1	1	0.14	0.2690	0.0988	0.5350	0.0923	1.0000
1	1	0.15	0.2690	0.1068	0.5278	0.0901	1.0000
1	1	0.16	0.2690	0.1152	0.5203	0.0877	1.0000
1	1	0.17	0.2690	0.1239	0.5126	0.0850	1.0000
1	1	0.18	0.2690	0.1330	0.5045	0.0819	1.0000

1	1	0.19	0.2690	0.1425	0.4962	0.0786	1.0000
1	1	0.20	0.2690	0.1522	0.4876	0.0749	1.0000
1	1	0.21	0.2690	0.1624	0.4787	0.0710	1.0000
1	1	0.22	0.2690	0.1728	0.4696	0.0668	1.0000
1	1	0.23	0.2690	0.1836	0.4602	0.0625	1.0000
1	1	0.24	0.2690	0.1947	0.4506	0.0580	1.0000
1	1	0.25	0.2690	0.2060	0.4408	0.0533	1.0000

Our analysis above is based on the assumption that toxicity is judged at 8 weeks and again at 16 weeks. In fact, the phase I portion of the study will not last beyond 4 weeks for each patient, so that the initial 3 patients, carried over from phase I, could become UT at 8 weeks. The tables above assume that the number of UTs at 8 weeks (not 4 weeks) is at most one. To account for this possibility, we re-computed the table assuming no restriction on the first 6 patients, but using only the early stopping rules for toxicity at 8 weeks. Thus the initial phase II cohort of all 17 patients in this re-computation are all treated alike. The results are shown in the next table. While the table above allows a smaller chance of toxicity than the actual trial, the table below allows a greater chance of toxicity in the initial phase II cohort. The main difference in the two computations is that the chance of eacly stopping for UT is about 9% higher under the re-computation conditions (35%) than it is under the top table (27%).

Operating characteristics of design with no restriction on the first 6 patients other than those tested at 8 or 16 weeks.

null	ntox	a	tox	fut	toxend	alpha	cksum
0	0	0.00	0.0082	0.4937	0.0114	0.2106	1.0000
0	0	0.01	0.0082	0.5103	0.0110	0.1862	1.0000
0	0	0.02	0.0082	0.5269	0.0106	0.1633	1.0000
0	0	0.03	0.0082	0.5434	0.0102	0.1421	1.0000
0	0	0.04	0.0082	0.5599	0.0098	0.1227	1.0000
0	0	0.05	0.0082	0.5762	0.0094	0.1049	1.0000

null	ntox	a	tox	fut	toxend	alpha	cksum
0	1	0.00	0.3591	0.2586	0.3197	0.0429	1.0000
0	1	0.01	0.3591	0.2700	0.3100	0.0393	1.0000
0	1	0.02	0.3591	0.2814	0.3003	0.0357	1.0000
0	1	0.03	0.3591	0.2929	0.2905	0.0321	1.0000
0	1	0.04	0.3591	0.3045	0.2807	0.0286	1.0000
0	1	0.05	0.3591	0.3161	0.2709	0.0253	1.0000
0	1	0.06	0.3591	0.3277	0.2612	0.0221	1.0000
0	1	0.07	0.3591	0.3392	0.2514	0.0192	1.0000
0	1	0.08	0.3591	0.3507	0.2417	0.0164	1.0000
0	1	0.09	0.3591	0.3622	0.2321	0.0139	1.0000
0	1	0.10	0.3591	0.3735	0.2225	0.0117	1.0000
0	1	0.11	0.3591	0.3848	0.2131	0.0096	1.0000
0	1	0.12	0.3591	0.3959	0.2038	0.0079	1.0000
0	1	0.13	0.3591	0.4069	0.1945	0.0063	1.0000
0	1	0.14	0.3591	0.4177	0.1855	0.0050	1.0000
0	1	0.15	0.3591	0.4283	0.1766	0.0039	1.0000
0	1	0.16	0.3591	0.4388	0.1679	0.0030	1.0000
0	1	0.17	0.3591	0.4490	0.1593	0.0023	1.0000
0	1	0.18	0.3591	0.4590	0.1510	0.0017	1.0000
0	1	0.19	0.3591	0.4688	0.1428	0.0012	1.0000
0	1	0.20	0.3591	0.4783	0.1349	0.0009	1.0000
0	1	0.21	0.3591	0.4875	0.1272	0.0006	1.0000
0	1	0.22	0.3591	0.4965	0.1197	0.0004	1.0000
0	1	0.23	0.3591	0.5051	0.1125	0.0003	1.0000

0	1	0.24	0.3591	0.5135	0.1055	0.0002	1.0000
0	1	0.25	0.3591	0.5216	0.0988	0.0001	1.0000

null	ntox	a	tox	fut	toxend	power	cksum
1	0	0.00	0.0082	0.0821	0.0233	0.8732	1.0000
1	0	0.01	0.0082	0.0892	0.0230	0.8626	1.0000
1	0	0.02	0.0082	0.0967	0.0227	0.8508	1.0000
1	0	0.03	0.0082	0.1047	0.0224	0.8377	1.0000
1	0	0.04	0.0082	0.1130	0.0221	0.8232	1.0000
1	0	0.05	0.0082	0.1218	0.0218	0.8072	1.0000

null	ntox	a	tox	fut	toxend	alpha	cksum
1	1	0.00	0.3591	0.0222	0.5301	0.0886	1.0000
1	1	0.01	0.3591	0.0251	0.5271	0.0887	1.0000
1	1	0.02	0.3591	0.0281	0.5240	0.0888	1.0000
1	1	0.03	0.3591	0.0315	0.5206	0.0887	1.0000
1	1	0.04	0.3591	0.0351	0.5170	0.0887	1.0000
1	1	0.05	0.3591	0.0391	0.5132	0.0885	1.0000
1	1	0.06	0.3591	0.0433	0.5091	0.0882	1.0000
1	1	0.07	0.3591	0.0478	0.5048	0.0878	1.0000
1	1	0.08	0.3591	0.0526	0.5003	0.0874	1.0000
1	1	0.09	0.3591	0.0577	0.4955	0.0867	1.0000
1	1	0.10	0.3591	0.0632	0.4904	0.0859	1.0000
1	1	0.11	0.3591	0.0690	0.4851	0.0849	1.0000
1	1	0.12	0.3591	0.0751	0.4795	0.0838	1.0000

1	1	0.13	0.3591	0.0815	0.4737	0.0824	1.0000
1	1	0.14	0.3591	0.0882	0.4676	0.0807	1.0000
1	1	0.15	0.3591	0.0953	0.4612	0.0789	1.0000
1	1	0.16	0.3591	0.1027	0.4546	0.0767	1.0000
1	1	0.17	0.3591	0.1105	0.4478	0.0743	1.0000
1	1	0.18	0.3591	0.1185	0.4407	0.0716	1.0000
1	1	0.19	0.3591	0.1268	0.4333	0.0686	1.0000
1	1	0.20	0.3591	0.1355	0.4257	0.0654	1.0000
1	1	0.21	0.3591	0.1445	0.4179	0.0620	1.0000
1	1	0.22	0.3591	0.1537	0.4098	0.0583	1.0000
1	1	0.23	0.3591	0.1632	0.4016	0.0545	1.0000
1	1	0.24	0.3591	0.1730	0.3931	0.0505	1.0000
1	1	0.25	0.3591	0.1830	0.3845	0.0465	1.0000

(Source document for sample collection times/comments – PSLmaster signature sheet in CTO)

IRB 14-052

GU-071: Phase I/II study of ixazomib with pegylated IFN-alpha 2b (pIFN) in metastatic renal cell carcinoma (mRCC)

Ixazomib is an orally administered proteasome inhibitor and can neutralize NF-κB pro-survival signaling preventing the induction of antioxidant enzymes in the cell. IFN induces reactive oxygen species accumulation to toxic levels when NF-κB is disabled and this can lead to mitochondrial dysfunction and necrotic death. IFN-α2b is administered by subcutaneous injection weekly for 8 weeks. Ixazomib is orally administered weeks 2, 3, 4, 6, 7, 8. ***A cycle is 28-days.***
Phase I – dose escalation of both ixazomib and IFN-α2b .
Phase II – expansion of the recommended combination doses.

PBMC – Used to examine STAT1 phosphorylation and MHC class I up-regulation (*indicator of IFN activity)* by flow cytometry and *p*STAT1 by western blot. Dr. Sid Balachandran lab will perform Western. PSL will stain flow tubes and take to Clin. Path. to acquire. Dr. Kerry Campbell will provide antibodies and analyze flow. *Follow PBMC procedure.*

IL-6 sera levels – Clot and spin at 2000 x g 15min., aliquot and freeze at -70°C. At the end of the study Cukierman lab will purchase IL-6 kit . PSL, in conjunction with Cukierman lab, will perform ELISA.

Archival tissue – Look for activated tissue stroma (desmoplasia); IHC for NF-κB p65 and phoso-STAT1.
Pathology will be reviewed By Dr. Essel Al-Saleem and she select an appropriate block to cut.
If a biopsy or additional surgery is performed during treatment, unstained slides will be prepared on the "on-treatment/EOT tissue" , if patient has consented.

nt name	**#**	**:** ***mL)gtt*** ***procedure*:** **Time**	***L) rtt*** ***L) aliquots*** **Time**	**val tissue** ***n) unstained sections on ides*** ***ogy report*** ***tional tissue*** ***____ No ____***
1 Day 1 Pre 0hr	***CRU*** ***1, 2, 3, 4***			
Day 8 Pre 0hr	***2***			
Day 15 Pre 0hr	***3***			
2 Day 1 Pre 0hr	***5, 6, 7, 8***			
Day 15 Pre 0hr	***7***			
3 Day 1 Pre 0hr	***9, 10, 11, 12***			
4 Day 1 Pre 0hr	***13, 14, 15, 16***			
6 Day 1 Pre 0hr	***25, 26, 27, 28***			
8 Day 1 Pre 0hr	***33, 34, 35, 36***			
10 Day 1 Pre 0hr	***41, 42, 43, 44***			
12 Day 1 Pre 0hr	***49, 50, 51, 52***			
14 Day 1 Pre 0hr	***56, 57, 58, 59***			
16 Day 1 Pre 0hr	***64, 65, 66, 67***			
f Study				***atment/EOT Optional***

IRB 14-052

GU-071: Phase I/II study of ixazomib with pegylated IFN-alpha 2b (pIFN) in metastatic renal cell carcinoma (mRCC)

Patient name _____ Study # _____
Mr# _____ Date _____
Cycle 1 Day 1*(week 1)* Baseline ___ Cycle 1 Day 8*(week 2)* ___ Cycle 1 Day 15*(week 3)* ___ Cycle 2 Day 15*(week 7)* ___
_____ # gtt _____ mL blood

PBMC Procedure:

1. **Dilute blood 1:1 with PBS (1-50mL conical) and layer over 15mL Ficoll-Paque (1-50mL conical).**
2. **Centrifuge 500 x g (1500rpm) 30min room temperature - *Brake off.***
3. **Collect PBMC into clean 50mL conical, fill with PBS and spin 2000rpm 5min.**
4. **Remove and discard supernatant; lyse rbcs with equal amounts (5-10mL) 0.2% NaCl followed by 1.6% NaCl.**
5. **Remove a portion of cells to count, determine viability with Trypan blue exclusion, then spin at 2000rpm 5min.**
6. **Remove supernatant and discard, resuspend pellet in PBS to2 x 10^6 cells/mL.**
7. **Place 0.5mL of the 2 x 10^6 cells/mL suspension into each of three labeled flow tubes – *p*STAT1, MHC class I and isotype control.**
8. **Respin remaining suspended cells, remove supernatant , resupend pellet in 500μL PBS and transfer to 2mL microfuge tube, rinse original tube with 500μL PBS and add to microfuge tube.**
9. **Spin microfuge tube at 2000rpm in microfuge, pour-off and blot tube dry on paper towel, then *freeze pellet on dry ice* and place at -70°C for storage.**

Count cells #/25 squares _____

Volume _____

Total cells _____ (2 x 10,000 x #cells/25 squares x volume = total cells)/2 x 10^6 = _____ mL to resuspend cells

 # cells/flow tube _____ # tubes _____

 # cells in frozen pellet _____ (minimum 3 x 10^6 cells in pellet)

FOX CHASE CANCER CENTER
TEMPLE HEALTH

**** TREATING NURSE SOURCE DOCUMENT ONLY**

Protocol #: IRB__14-052____

Subject ID and Study #_____

Patient name_____ Mr#_____

POINTS OF BLOOD DRAWS	IFICATION OF TIMEPOINTS	mL)gtt procedure: Time	nL) rtt L) aliquots Time	ES INITIALS
1 Day 1 Pre 0hr	*1, 2, 3, 4*			
Day 8 Pre 0hr	*2*			
Day 15 Pre 0hr	*3*			
2 Day 1 Pre 0hr	*5, 6, 7, 8*			
Day 15 Pre 0hr	*7*			
3 Day 1 Pre 0hr	*9, 10, 11, 12*			
4 Day 1 Pre 0hr	*13, 14, 15, 16*			
8 Day 1 Pre 0hr	*17, 18, 19, 20*			
10 Day 1 Pre 0hr	*25, 26, 27, 28*			
12 Day 1 Pre 0hr	*33, 34, 35, 36*			
14 Day 1 Pre 0hr	*41, 42, 43, 44*			
16 Day 1 Pre 0hr	*49, 50, 51, 52*			
f Study				

Initials _____ Signature _____ Initials _____ Signature _____

Initials _____ Signature _____ Initials _____ Signature _____

Form Adopted 11-03

FOX CHASE CANCER CENTER
TEMPLE HEALTH

To:_____

Fax number:_____

From: Fox Chase Cancer Center Protocol Department
Daniel Geynisman MD/Lois Malizzia RN

Date:_____

RE: REQUEST OF PATHOLOGY BLOCK/SLIDES (IRB 14-052)

Patient name:_____ Date:_____
(Patient consent obtained and attached.)

To Whom It May Concern:

The above patient has pathology material at your institution. This patient is interested in a clinical trial at Fox Chase Cancer Center. To meet the protocol requirements the patient is requested to submit an H&E recut and 10(4μm) unstained sections cut from a block representative of tumor and placed on charged immunohistochemistry slides. The slides should be labeled with the institutional surgical pathology number. A copy of the institutional pathology report and consultative pathology reports (if available) must accompany the submitted material.
Please Fed Ex these materials to the following address as soon as possible. A Fed Ex account number can be obtained, if required, by contacting Kathy Alpaugh at the number below.

Kathy Alpaugh, PhD
Director Protocol Support Laboratory
Fox Chase Cancer Center
333 Cottman Avenue Ave. P2011
Philadelphia, PA 19111

Telephone: (215) 214-1634 Fax: (215) 214-1635
e-mail: RK_Alpaugh@fccc.edu

If there are any questions concerning this request, please call Lois Malizzia at (215) 728-5311.

PATIENT (or legal representative) CONSENT FOR RELEASE OF PATHOLOGY MATERIAL:

I _____give consent to have pathology block(s) or
(Patient name or Legal Representative)
slides, accompanied by any written reports, to be released to the Fox Chase Cancer Center to be used for evaluation related to the above indicated clinical trial(s). I understand that in sending this tissue it may exhaust the supply of diagnostic tissue available and preclude any chance for possible further testing. I release the sending institution from any responsibility or liability for future care that may depend on this tissue.

Patient signature or legal representative_____

Date _____ Witness _____

