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Title: A phase II trial of mutation-targeted therapy with sunitinib or everolimus in patients with advanced low-or intermediate grade neuroendocrine tumors of the gastrointestinal tract and pancreas with or without cytoreductive surgery.

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Abstract

Introduction

Finding the optimal management strategy for patients with advanced, metastatic neuroendocrine tumors (NETs) of the gastrointestinal tract and pancreas is a work in progress. Sunitinib and everolimus are currently approved for the treatment of progressive, unresectable, locally advanced or metastatic low-or intermediate grade pancreatic NETs. However, mutation targeted therapy with sunitinib or everolimus has not been studied in this patient population.

Methods and analysis

This prospective, open-label Phase II clinical trial was designed to determine if mutation targeting therapy with sunitinib or everolimus for patients with advanced low- or intermediate grade NETs is more effective than historically expected results with progression-free survival (PFS) as the primary endpoint. Patients ≥18 years of age with progressive, low or intermediate grade locally advanced or metastatic NETs are eligible for this study. Patients will undergo tumor biopsy (if not a surgical candidate) for tumor genotyping. Patients will be assigned to sunitininb or everolimus based on somatic/germline mutations profile. Patients who have disease-progression on either sunitinib or everolimus will cross-over to the other drug. Treatment will continue until disease progression, unacceptable toxicity, or consent withdrawal. Using the proposed criteria, 44 patients will be accrued within each treatment group during a 48 month period (88 total patients for the two treatments), and followed for up to an additional 12 months (60 months total from entry of the first patient) to achieve 80% power in order to test whether there is an improvement in PFS compared to historically expected results, with a 0.10 alpha level one-sided significance test.

Ethics and dissemination

The study protocol was approved by the Institutional Review Board of the National Cancer Institute (NCI-IRB Number 15C0040; iRIS Reference Number 339636). The results will be published in a peer-reviewed journal and shared with the worldwide medical community.

Keywords: neuroendocrine tumors; gastrointestinal tract; pancreas; low-or intermediate grade neuroendocrine tumors; sunitininb; everolimus

Background

Neuroendocrine tumors (NETs) of the gastrointestinal tract (GI) and pancreas are a rare and heterogeneous group of neoplasms with unique tumor biology, natural history, and clinical management issues (1-4). Most NETs are sporadic, but they can be part of familial cancer syndromes such as multiple endocrine neoplasia type 1 (MEN1), neurofibromatosis type 1 (NF1), tuberous sclerosis (TS) or Von Hippel-Lindau (VHL) syndrome (5-9).

While poorly differentiated tumors may exhibit highly aggressive behavior, well-differentiated, low or intermediate grade NETs have a relatively indolent behavior with slow progression (6, 8, 10). As a result of this insidious biological behavior, many patients with well-differentiated, low or intermediate grade NETs have advanced disease at diagnosis, with regional or distant metastasis observed in more than 50% of patients (11, 12). Surgery is the only curative treatment option in patients with localized early stage NETs. The optimal management strategy for patients with advanced NETs is unknown.

Our understanding of the genetic events associated with sporadic and familial NETs has improved considerably over the last 3 decades. Driver oncogene and tumor suppressor genes have been identified in most NETs (13-19). Overall, the majority of NETs have somatic mutations in *MEN1*, the phosphatidylinositol 3-kinase (*PI3K)/AKT/* mammalian target of rapamycin (*mTOR*) signaling pathway (20-24), and or overexpression of growth factors and their receptor such as vascular endothelial growth factor (*VEGF*), *VEGF* receptor (*VEGFR*), platelet-derived growth factor (*PDGF*), and *PDGF* receptor (*PDGFR*) (25-28). A recent study also revealed the presence of somatic mutations in *MEN1, DAXX, ATRX, TSC2, PTEN*, and *PIK3CA* genes in the majority of sporadic pancreatic NETs (22). Moreover, the presence of these

mutations was associated with better survival when compared to patients with NETs, which had wild type *MEN1,* and/or *DAXX/ATRX* (22).

In 2011, sunitinib (multi-tyrosine kinase inhibitor) and everolimus (mTOR signaling pathway inhibitor) were approved by the Food and Drug Administration (FDA) for the treatment of unresectable, metastatic, progressive pancreatic NETs based on the results of Phase III trials demonstrating a significantly improved progression-free survival (PFS) in the treatment vs. placebo arm (11.4 vs. 5.5 months for sunitinib and 11.0 vs. 4.6 months for everolimus) (14, 29). However, there are several important management issues that remain unclear: 1) Is treatment with everolimus/sunitinib beneficial to other groups of patients with advanced NETs, who have NETs of GI tract (also called "carcinoids") and or patients who have cytoreductive surgery? 2) While the choice of targeted therapies in other malignancies is more frequently being driven by the findings of the precise molecular alterations present in the tumor, no such study has been done in NETs. This is particularly important given that the survival of patients with malignant NETs appears to be different based on the driver mutation(s) present in the tumor and low- or intermediate grade tumors can have a relatively indolent growth (22).

The primary objective of this Phase II trial is to determine the PFS in patients with NETs of the GI tract and pancreas treated with sunitinib or everolimus based on tumor genotyping with or without surgical resection. The study was designed to test the hypothesis that an improvement in PFS can be achieved using this mutation targeted treatment strategy when compared to previous studies (14, 29).

Methods and Design

Study population

All patients with NETs of the GI tract and pancreas who meet the following criteria are eligible to participate in this study.

Inclusion Criteria

- Progressive, histologically or cytologically diagnosed low or intermediate grade, neuroendocrine tumors confirmed by the Laboratory of Pathology, National Cancer Institute (NCI).
- Age ≥ 18 years, because no dosing or adverse event data are currently available on the use of Sunitinib and or Everolimus in patients <18 years of age, (children are excluded from this study, but will be eligible for future pediatric trials).
- ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).
- Patients must have normal organ and bone marrow function as defined below:
    - leukocytes ≥3,000/mcL
    - absolute neutrophil count ≥1,500/mcL
    - platelets ≥ institutional lower limit of normal
        - total bilirubin ≤2-fold above institutional upper limit of normal
        - AST(SGOT)/ALT(SGPT) ≤2.5-fold above institutional upper limit of normal
    - creatinine within normal institutional limits

      OR
    - creatinine clearance ≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

- Agreement to use effective contraception while on treatment and for ≥ 3 months after end of treatment, because the effects of Sunitinib and Everolimus on the developing human fetus are unknown. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.
- Must have fully recovered from toxicities of any prior treatment with cytotoxic drugs, radiotherapy, surgery, or other anti-cancer modalities (returned to baseline status as noted before most recent treatment).
- Ability of subject or Legally Authorized Representative to understand and the willingness to sign a written informed consent document.

Exclusion Criteria

- Uncontrolled hypertension (>150/100 mmHg).
- Prior external beam radiation therapy to the target lesion(s) within 1 months prior to enrollment
- Prior systemic chemotherapy or therapy with one of the investigation agents within 1 month prior to enrollment.
- Patients who are receiving any other investigational agents.
- Patients with known brain metastases will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to Sunitinib or Everolimus.

- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- Serious uncontrolled concomitant disease that the investigator feels might compromise study participation.
- Pregnant or nursing patients will be excluded from the study, because the effects of Sunitinib and Everolimus on the developing human fetus are unknown. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Sunitinib or Everolimus, breastfeeding should be discontinued if the mother is treated with Sunitinib or Everolimus.
- Any of the following clinical conditions within the 12 months prior to starting study treatment: myocardial infarction, severe/unstable angina, coronary/peripheral artery bypass graft, congestive heart failure, cerebrovascular accident including transient ischemic attack, pulmonary embolism, ongoing cardiac dysrhythmias of NCI CTCAE grade at least 2, atrial fibrillation of any grade, or QTc interval >450 msec for males or >470 msec for females.
- Clinically significant pulmonary disease (e.g., severe chronic obstructive pulmonary disease [COPD] or asthma).
- Current treatment with therapeutic doses of Coumadin-derivative anticoagulants (low dose Coumadin up to 2 mg PO daily for deep vein thrombosis prophylaxis is allowed).
- Patients with a history of uncontrolled seizures, central nervous system disorders of psychiatric disability judged by the Investigator to be clinically significant precluding informed consent or interfering with compliance for oral drug intake will be excluded from study.

- HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with study agents.
- Lack of physical integrity of the upper gastrointestinal tract or malabsorption syndrome, or the inability to take oral medication

Study Design

In this Phase II trial, patients will undergo cytoreductive surgery for standard of care indications, or an image guided tumor biopsy will be performed for tumor genotyping in patients who do not undergo an operation and who do not have archived tumor tissue samples. Patients who have been potentially rendered disease free at the discretion of the principal investigator after surgical resection will not be assigned to a study medication and will be removed from the study. All other patients must begin study drug within 3 months after surgery/biopsy. Patients with syndromic NETs (e.g., VHL and MEN1) will not undergo tumor biopsy. Based on the tumor genotype or germline mutation status and the results of analysis of the involved cell signaling pathways (**Table 1**), patients will be treated with sunitinib (for mutations in *MEN1/PDGFR/KIT/FLT3*) or everolimus (for mutations in *NF1/PTEN/PI3K/AKT/mTOR/VHL/TP53*) daily in 28 day cycles (**Table 2**). Patients will receive long-acting octreotide for symptoms associated with hormonal hypersecretion. Treatment with sunitinib/everolimus in patients who undergo surgical treatment will begin after recovery from surgery. Patients who have gene mutations not known to be specifically targeted by sunitinib or everolimus or with more than one mutation will be assigned to sunitinib.

If a patient has two or more mutations (one in the everolimus-*MEN1/PDGFR/KIT/FLT3* and another in the sunitinib group- *NF1/PTEN/PI3K/AKT/mTOR/VHL/TP53*), the patient will be assigned to sunitinib and will cross over to the other drug if disease progression develops.

Patients who develop disease-progression on either sunitinib or everolimus will cross-over to the other drug. Treatment will continue until disease progression, unacceptable treatment-related toxicity, or consent withdrawal. After discontinuation from the study, the subject will be contacted at 3 month intervals to obtain information about subsequent treatment(s) and survival status (**Figure 1).**

Biospecimen collection

Tumor genotyping and germline mutation analysis

Within three months prior to initiation of treatment with the respective study agent (everolimus or sunitinib), patients who meet eligibility criteria, but are not candidates for cytoreductive surgery, will undergo biopsy of the primary tumor or any metastatic site for molecular analyses. If patients are scheduled for an operative intervention for tumor debulking, a portion of the resected tumor will be used for research. If surgical resection is not indicated, patients will undergo a percutaneous core needle biopsy of the tumor under local anesthesia. These percutaneous biopsies will be performed by interventional radiology (under Computed Tomography (CT) scan or ultrasound (US) guidance). If needed, patients will be offered conscious sedation for the biopsy procedure. Sample collection will be performed according to standard operating procedures.

Peripheral blood (30 mL) will be obtained for targeted sequencing and comparison to tumor sequencing results and mutation calls after enrollment and before treatment.

Tumor tissues samples will be examined by standard histology, immunohistochemistry and or in situ hybridization in the Laboratory of Pathology, NCI. Tumor genotyping and germline sequencing for 197 driver genes will be performed in a Clinical Molecular Profiling Core (CLIA) certified genetic laboratory at the NIH-NCI.

Cytoreductive surgery

Cytoreductive surgery will be performed in subjects in whom it is indicated by standard of care. Operative resections will not be performed for research purposes only. Surgery will be done per NIH Clinical Center standard operating procedure. Subjects will sign a separate consent for the surgery.

Treatment agents and dose

Based on the tumor genotype or germline mutation status, patients will be assigned to one of the two study drugs (**Table 1 and Supplemental Table 1**).

Sunitinib

Sunitinib is a multi-kinase inhibitor and inhibits all 3 types of *VEGFR* and several other tyrosine kinase receptors. It is FDA approved for patients with advanced, progressive, unresectable NETs. The most frequent adverse events associated with sunitinib therapy are

diarrhea, nausea, vomiting, rash (hand-foot), asthenia, and fatigue (14). Patients with *MEN1/PDGFR/KIT/FLT3* mutations or other gene mutations will be treated with oral sunitinib at a dose of 37.5 mg once daily (**Table 1**). Patients will take sunitinib once daily in the morning, with or without food, as desired. Treatment will continue until progression of disease, development of an unacceptable toxicity, drug interruption for 3 weeks or longer, or withdrawal of consent. Treatment interruptions and a dose reduction to 25 mg per day will be permitted to manage adverse events, with a subsequent increase in dose if toxicity of grade 2 or higher did not recur.

Everolimus

Everolimus is an *mTOR* inhibitor. *mTOR* is an intracellular serine-threonine kinase that has a role in regulating cell growth, proliferation, apoptosis and angiogensis, and is activated in several cancers. Everolimus is also FDA approved for patients with advanced, progressive, unresectable NETs. Common side effects are stomatitis, rash, diarrhea, fatigue and respiratory tract infection. Previously reported, Grade 3 and 4 toxicities include anemia (6%) and hyperglycemia (5%) (16). Patients with *NF1/PTEN/PI3K/AKT/mTOR/VHL/TP53* mutations will be treated with oral everolimus at a dose of 10 mg once daily (**Table 1**). Treatment will continue until progression of disease, development of unacceptable toxicity, drug interruption for 3 weeks or longer, or withdrawal of consent. Doses will be delayed or reduced if patients have clinically significant adverse events that are considered to be related to the study treatment. In such cases, two reductions in the dose of the study drug will be permitted: an initial reduction to 5 mg daily with a subsequent increase in dose if toxicity of grade 2 or higher did not recur.

Study Endpoints

Primary Endpoint

- PFS on first-line therapy in patients with NETs of the gastrointestinal tract and pancreas treated with sunitinib or everolimus based on tumor genotyping with or without surgical resection.

Secondary Endpoints

- Overall response rate (ORR; i.e., sum of complete response [CR], partial response [PR], and stable disease [SD]) and duration of response
- PFS in patients who undergo cytoreductive surgery with tumor genotype and treatment with sunitinib or everolimus based on tumor genotyping results may represent a potential improvement over published results of treatment with only everolimus/sunitinib or surgical resection only.
- Overall survival and median survival time (MST)
- Relationship between tumor genotype, treatment and PFS
- Safety endpoints (i.e. AEs, clinical laboratory evaluations, ECGs, physical examination findings, and vital sign measurements).

Study calendar and data collection

The screening and on study assessments listed in detail in **Table 2** may be performed within 1 week of the time listed in order to accommodate weekends, holidays, travel delays,

inclement weather and other such unexpected events with the exception of the baseline pregnancy test which must be performed as stated, within 3 days prior to study drug initiation.

Response criteria

For the purposes of this study, patients will be re-evaluated for response every 12 weeks (**Figure 1 and Table 2**). 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).

Criteria for withdrawal of individual subjects

The criteria that take the subject off active protocol therapy include:

- Progressive disease on each study arm
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined in section 3.3
- Investigator discretion
- In all cases, a safety follow up visit will be conducted within approximately 30 days of the last dose of study drug therapy

Statistical analysis and sample size calculation

The primary objective of this trial is to determine PFS of patients with low- or intermediate grade NET who receive first-line targeted therapy with or without cytoreductive surgery. This will provide data to explore whether the PFS may represent a potential improvement over published results with treatment only with everolimus/sunitinib (14, 29). Secondary objectives include evaluation of response and OS as well as safety and exploring the association between tumor genotype and PFS, and response to treatment. If the findings of this study suggest improved PFS from mutation-targeted and or combination treatment, larger randomized trials to confirm the findings will be developed.

Following surgery or tumor biopsy for tumor genotyping, eligible patients will be treated with everolimus or sunitinib. Based on results of the genotyping, enrolled patients will then receive targeted treatment with sunitinib (for mutations in *MEN1/PDGFR/KIT/FTL3* or everolimus (for mutations in *NF1/PTEN/ PI3K/AKT/mTOR/VHL/TP53*). Patients with other different mutations or multiple types of mutations will be assigned to receive sunitinib since almost all of these tumors have elevated levels of VEGF/VEGFR expression.

Results from previously published trials (14, 29) both demonstrated approximately 11 months PFS in patients similar to the ones to be treated on this protocol. Those patients were not assigned to receive treatment on the basis of any tumor genotype information. The study was designed to determine if meaningful improvement in PFS may be obtained using this focused strategy. For purposes of sample size determination, patients will be primarily evaluated based on the treatment received. Thus, within each treatment group, using the method of Brookmeyer and Crowley, with 44 patients accrued during a 48 month period (88 total patients for the two treatments), and followed for up to an additional 12 months (60 months total from entry of the

first patient), there would be 80% power to test whether the median PFS is consistent with 18 months, and greater than 11 months, with a 0.10 alpha level one-sided significance test.

In practice, a Kaplan-Meier curve of PFS will be constructed for each group, and will have the median as well as key time points such as 12 and 18 months estimated, along with appropriate 80% and 95% confidence intervals to explore whether the present results exceed those from prior studies. In addition, the Kaplan-Meier curves for all patients who received either sunitinib and everolimus as their treatments may be combined into one pooled Kaplan-Meier curve if the two curves for these treatments are sufficiently similar to one another ($p>0.30$ by a two-tailed log-rank test). This combined curve will then be evaluated relative to the published median of 11 months PFS for each group to provide a more powerful comparison relative to the historically expected results. In addition, Kaplan-Meier curves limited to the patients who received targeted therapy based solely on mutation status may be constructed for exploratory and descriptive purposes.

Response and overall survival will also be evaluated as secondary endpoints, based on the patients treated with sunitinib or everolimus, using all patients receiving treatment. These results will be descriptive and may consist of fractions of responses as well as Kaplan-Meier curves for survival. The association between genotype and PFS will be explored by evaluating the results within a treatment based on the major genotype categories identified. Safety will be evaluated by tabulating and reporting the distribution of the worst grade of each type of toxicity found, per patient, separately by treatment. Should any particular type of toxicity result in 5 or more patients with grade 3-4 toxicity, a comparison of the distributions of toxicity between the two treatments may be performed using a Cochran-Armitage test for trend.

Because the patients may not end up being assigned in equal proportions to the two treatments, and because the goal is to have 44 evaluable patients who have received each treatment, additional patients beyond the 44 described above may be enrolled on the arm with faster accrual. As such, the study will have an accrual ceiling of 120 patients, with accrual ending when there are 44 evaluable patients on the arm with fewer patients. An amendment may also be needed if a lower proportion of patients than expected receive study drug therapy or if a high portion of treated patients are not evaluable for response. It is anticipated that 20-30 patients per year may enroll onto this trial; thus accrual may be completed in 3-4 years.

Ethics and dissemination

The study protocol was approved by the Institutional Review Board of the National Cancer Institute (NCI-IRB Number 15C0040; iRIS Reference Number 339636). The results will be published in a peer-reviewed journal and shared with the worldwide medical community. ClinicalTrials.gov Identifier: NCT02315625

Discussion

NETs of the GI tract and pancreas are rare and heterogeneous, but clinically important group of neoplasms that arise in the disseminated neuroendocrine cells of the GI tract and the pancreatic islet cells. The annual incidence of NETs has been increasing in the United States and worldwide and was estimated to be 7.8 per 100,000 persons in 2013 (1, 8, 11, 30-32).

NETs are classified into functioning (hormone hypersecreting) or nonfunctioning (clinically “silent”) tumors, based on their ability to produce hormone-associated symptoms.

However, other classification systems with many common themes, such as the distinction of well-differentiated (low and intermediate-grade) from poorly differentiated (high-grade) NETs and the prognostic significance of proliferative rate index have been used over the past 5 decades. The majority of NETs (60-90%) are clinically non-functioning, well-differentiated, slow-growing neoplasms diagnosed, in most instances, incidentally during an unrelated procedure (1, 7, 12, 33-36). As a result of this insidious biological behavior, many patients with NETs have advanced disease at diagnosis, with regional or distant metastasis observed in more than 50% of patients (11, 12). NETs most commonly metastasize to the loco-regional lymph nodes and liver, and 25% to 93% of patients will develop liver metastases during the course of their disease (37, 38).

Surgical resection alone is a valuable treatment option for patients with early-stage disease; however, the extent, timing and effect of surgical intervention for advanced, metastatic NETs remain controversial and difficult to estimate. Although not supported by randomized clinical trial data, currently it is advocated that surgery should be undertaken only if metastatic disease is confined to the liver and if 90% or more of the tumor mass, including liver metastases, can be successfully removed (39). However, most patients will present with multiple bilobar liver metastases, and altogether only 5–10% will have apparently solitary or dominant liver metastases amenable to surgical resection (37, 40). Furthermore, recurrence after surgery is common and a significant number of patients with advanced NETs undergoing debulking surgery will have residual disease and suffer from complications associated with hormonal hypersecretion and or tumor progression (37, 41, 42).

The approval, by the FDA, of sunitinib and everolimus for the treatment of unresectable, locally advanced or metastatic NETs is a remarkable milestone in the field of medical therapy of

malignant NETs (13, 14, 29). However, while the mutation-targeted therapy in other malignancies is driven by the findings of the precise molecular alterations present in the tumor, no such study has been done in malignant NETs. In addition, it is not known if treatment with everolimus or sunitinib may be beneficial to other groups of patients with advanced NETs, including those with carcinoid tumors or as adjuvant therapy for patients who have cytoreductive surgery.

Although improving overall survival is the ultimate goal for any cancer therapy, the variable and at times long survival time in many patients with NETs makes overall survival a less suitable initial end point to study for the proposed strategy of targeted therapy (42, 43). In this regard, using PFS as a primary end point poses less significant scientific challenges and is attractive from both an ethical and feasibility standpoint (42). We believe that such an approach will allow us to generate enough data to determine whether a larger study is warranted using mutation-targeted therapy for patients with NETs. The results from this Phase II open-labeled study could also provide important data for future study questions: 1) Does specific tumor genotype predict response to therapy, 2) Does adjuvant therapy after surgical debulking have any benefit, and 3) What are the long-term side-effects associated with sunitinib or everolimus therapy?

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Figure legends

Figure 1. Study flow chart.

CR - complete response, PR - partial response, SD - stable disease, PD - disease progression

Trial status

Patient recruitment is currently ongoing

ClinicalTrials.gov Identifier

NCT02315625

List of abbreviations

NETs - neuroendocrine tumors of the gastrointestinal tract (GI) and pancreas; MEN1 - multiple endocrine neoplasia type 1; NF1 - neurofibromatosis type 1; TS - tuberous sclerosis; VHL - Von Hippel-Lindau syndrome; *VEGF* - vascular endothelial growth factor; *PI3K* - phosphatidylinositol 3-kinase; *PTEN* - Phosphatase and tensin homolog; *PIP2* - Phosphatidylinositol-4,5-bisphosphate; *PIP3* - phosphatidylinositol-3,4,5-trisphosphate; *AKT* – gene encoding RAC-alpha serine/threonine-protein kinase; TSC - tuberous sclerosis complex; *mTOR* - mammalian target of rapamycin; *FKBP12* - FK506 binding protein; *4E-BP* - eukaryotic translation initiation factor 4E-binding protein; *p70S6K* - p70S6 kinase; DAXX - death-associated protein 6; ATRX - ATP-dependent helicase; FDA - Food and Drug Administration; PFS - progression-free survival; NCI - National Cancer Institute; NIH- National Institutes of Health; ECOG - Eastern Cooperative Oncology Grou; AST/ALT - aspartate transaminase / alanine transaminase; COPD - chronic obstructive pulmonary disease; HIV – human immunodeficiency virus; *TP53* - tumor protein p53; *KIT* - proto-oncogene c-Kit; *FLT3* - Fms-like tyrosine kinase 3; *PDGFR* - platelet-derived growth factor receptors; CLIA - Clinical Molecular Profiling Core; RECIST - Response Evaluation Criteria in Solid Tumors

Competing interests

The authors declare that they have no competing interests

Authors' contributions

EK is the principal investigator. VN is the lead associate investigator. EK and VN conceived, designed and wrote the project proposal and manuscript and will be responsible for study implementation, data collection, management and analysis. SS participated in outlining statistical methods, critical review of the protocol and manuscript, and will participate in data analysis. NN participated in outlining study design, critical review of the protocol and manuscript and will participate in study implementation. CCD and RM are the study coordinators and will be responsible for the clinical data collection and implementation of the study. SM, JY, PM, and KP participated in study development, critical review of the protocol and manuscript and will participate in study implementation and data collection and analysis. All authors reviewed and approved the final manuscript.

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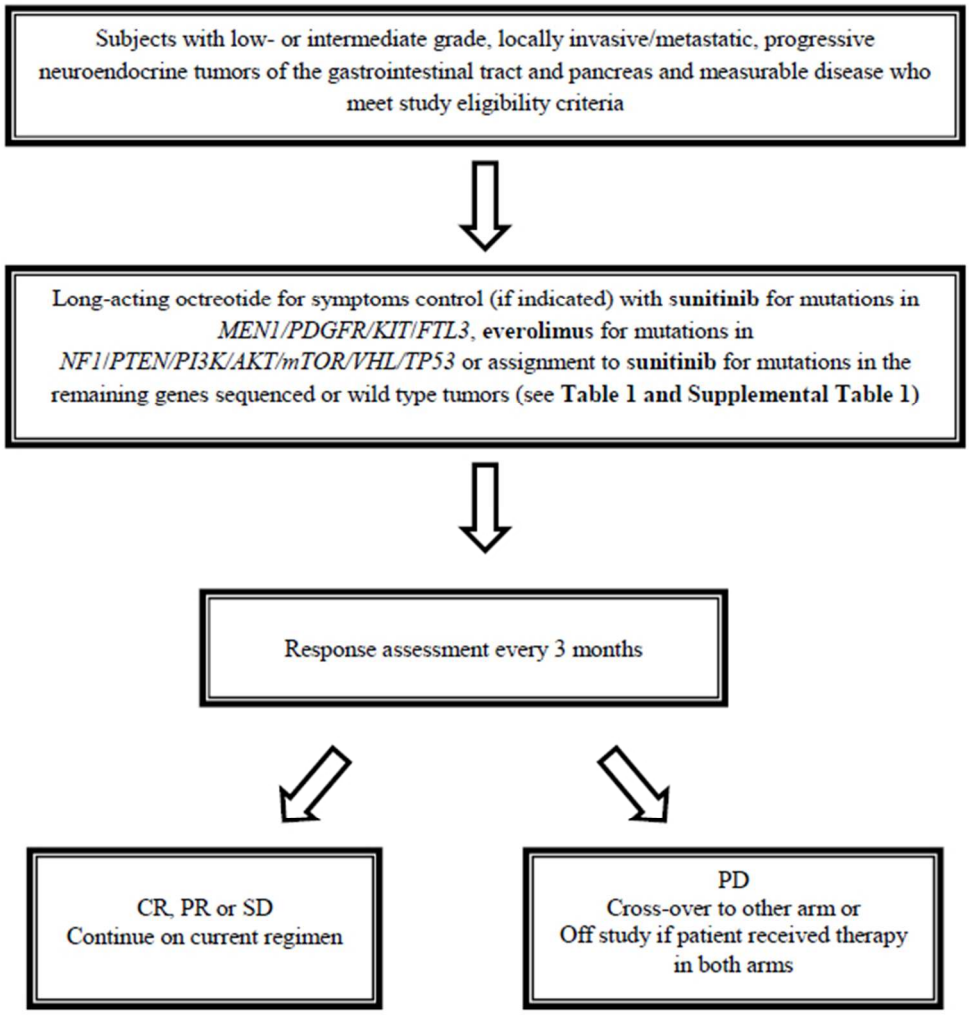
Disclaimer

This is a US government work. The views expressed within this paper do not necessarily reflect those of the US government.



Study flow chart.
CR - complete response, PR - partial response, SD - stable disease, PD - disease progression
173x179mm (96 x 96 DPI)

Table 1. Choice of targeted therapy driven by the findings of the precise molecular alterations based on common mutations that occur in NETs.

Study Agent	Mutations *	Affected Pathways
Everolimus	*PTEN*	*PI3K/AKT/mTOR*
	PI3K	*PI3K/AKT/mTOR*
	AKT	*PI3K/AKT/mTOR*
	mTOR	*PI3K/AKT/mTOR*
	VHL	Hypoxia induced *PI3K/AKT/mTOR*
	TSC1	*PI3K/AKT/mTOR*
	TSC2	*PI3K/AKT/mTOR*
	NF1	*TSC2/mTOR,* Hypoxia induced neoangiogenesis
Sunitinib	*MEN1*	Cell growth, cell cycle and genome instability
	FLT3	Cell survival, proliferation, and differentiation
	PDGFR	Cell proliferation, cell migration, neoangiogenesis
	ATM	Cell survival, cell cycle, DNA repair and apoptosis
	KIT	Cell survival, proliferation, and differentiation
	ATRX	Cell survival, proliferation, and differentiation

*Mutations in genes not listed above or that are wildtype will be treated with sunitinib (see Supplemental Table 1 for list of other genes that will be genotyped). Germline DNA will be obtained for comparison to tumor genotype data for every patient. Also, some patients with known familial cancer syndromes (MEN1 and VHL) will be included in the study and tumor biopsy for the sole purpose of agent selection in these patients will not be performed.

Table 2. Study calendar

Screening for eligibility		**Within 4 weeks prior to enrollment**	• Clinical Assessment[i] • 24 hour urine collection for urine protein, urine creatinine and urine albumin • Serum creatinine • Hemoglobin A1C • HIV antibody • Radiological evaluations[ii] • Histopathological confirmation of progressive, low or intermediate grade NET
		Within 2 weeks prior to enrollment	• 12- lead electrocardiogram[iii] • CBC[iv-a], Chemistriesiv[-b], INR, Lipid panel (fasting)
		Within 3 days prior to enrollment	• Serum or urine HCG (in women of childbearing potential only)
Enrollment			Patient signs consent
Prior to streatment with study drug		**(Surgical Candidates only)** **Month -3 to Day 1**	• Contrast enhanced CT scan (within 4 weeks prior to operation – screening scan may be used if timeframe is met) • Standard pre-operative evaluation based on diagnosis • Cytoreductive Surgery and intraoperative biospecimen collection for gentotyping • Contrast Enhanced CT scan for post operative tumor burden (within 4 weeks prior to study drug initiation) • Routine post surgery care and recovery
		(Subjects not undergoing surgery only) **Month -3 to Day 1**	• Image guided tumor biopsy for genotyping
		All Subjects **Within 4 weeks prior to treatment initiation**	• Hepatitis B and C Evaluation • Echocardiogram (in patients with carcinoid tumors only)
		All Subjects **Within 2 weeks prior to treatment initiation** [Tests need not be repeated if they have been done during the appropriate timeframe at screening]	• Clinical Assessmenti • Chromogranin A, pancreatic polypeptide, and neuron-specific enolase. • Vasoactive intestinal polypeptide (VIP), serotonin (urinary 5-HIAA), gastrin, somatostatin, fasting insulin, C-peptide (proinsulin) and or glucagon only in patients known to have functioning NETs. • CBCiv[-a], Chemistriesiv[-b], and TFTsiv[-c] • Urinalysis • Cardiac Evaluation (in patients that present with cardiac or pulmonary risk factors) • CT CAP or MRIvii • 12 lead ECGiii • 30 mL of peripheral blood f (red top tubes) or research (see section Error! Reference source not found.)
		Women of childbearing potential only **Within 3 days of study drug initiation**	• Urine or serum HCG
Treatment based on tumor genotyping	**Cycle 1 (28 days)**	**Day 14**	• Clinical assessmenti • CBCiv[-a] and Chemistriesiv[-b], • Urinalysis
	Cycle 2 (28 days)	**Day 1**	• Clinical assessmenti • CBCiv[-a], Chemistriesiv[-b], and TFTsiv[-c] • Urine or serum HCG in women of childbearing potential • Urinalysis • 12 lead ECGiii
		Day 14	• CBC iv[-a], • Chemistriesiv[-b], • Urinalysis
	Cycle N (28 days)	**Day 1**	• Clinical assessmenti • CBCiv[-a], Chemistriesiv[-b], and TFTsiv[-c] • Urine or serum HCG in women of childbearing potential • Urinalysis • 12 lead ECGiii • Radiological evaluation of treatment responsev
Final/Early termination visit[vi]			• Clinical assessmenti

	• CBCiv[-a], Chemistriesiv[-b], and TFTsiv[-c] • Urine or serum HCG in women of childbearing potential • Urinalysis • Radiological evaluation of treatment responsev (not applicable for patients that have progressed on 2 drugs)
Long Term Follow up	Telephone contact every 3 months to determine anti-cancer therapy and survival status
Concomitant Medications	Throughout study
Adverse Events	Throughout study

[i] Clinical assessment: Complete history and physical examination including height, weight, vital signs (incl blood pressure, pulse) and ECOG at screening, baseline, Day 1 and 15 of Cycle 1, and then on Day 1 of each subsequent cycle. 12L ECG to be completed within 2 weeks prior to treatment and then at the end of each cycle prior to starting next cycle of therapy

[ii] Radiological Evaluations to be completed as part of the screening.

- Brain MRI or CT
- Contrast CT scan or MRI of the chest, abdomen and pelvis (CT C/A/P) for the purpose of tumor burden and tumor volumetric measurement
- Bone scan for patients in whom bone metastases are suspected
- FDG PET scan

[iii] 12L ECG to be completed within 2 weeks prior to treatment and then at the end of each cycle prior to starting next cycle of therapy.

[iv] Laboratory Evaluations:

a. CBC with differential and platelets to be completed within 2 weeks prior to enrollment, within 2 weeks prior to treatment, then every 2 weeks for the first 2 cycles and then every 4 weeks thereafter.
b. Chemistries: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Total Protein to be completed within 2 weeks prior to enrollment, within 2 weeks prior to treatment, then every 2 weeks for the first 2 cycles and then every 4 weeks thereafter.
c. TFTs: Free T3, TSH to be done within 2 weeks prior to treatment then every 4 weeks thereafter.

[v] A CT or MRI of the chest/abdomen/pelvis to reassess treatment response will be done at baseline, at 3 months after treatment initiation and then every 3 months. MRI can be substituted for CT scan at the discretion of the investigator as some lesions such as hepatic metastasis are best visualized on MRI.

[vi] Final/early termination visit will occur approximately 30 days after the last dose of study drug

Supplemental Table 1. Panel of 197 genes tested.

Genes							
ABL1	*CD34*	*DMBT1*	*FOXL2*	*KRAS*	*NF2*	*RPS15*	*SUFU*
ACN9	*CDC73*	*DNMT3A*	*FZR1*	*KRT20*	*NOTCH1*	*RUNX1*	*SYP*
ACVR1B	*CDH1*	*DPYD*	*GATA1*	*MAGI1*	*NOTCH2*	*SCLC1*	*TCF7L2*
AKT1	*CDK4*	*EGF*	*GATA2*	*MAP2K4*	*NPM1*	*SDHA*	*TET2*
ALK	*CDKN2A*	*EGFR*	*GATA3*	***MEN1***	*NRAS*	*SDHAF1*	*TFE3*
APC	*CDX2*	*EGR3*	*GFAP*	*MET*	*PBRM1*	*SDHAF2*	*TFEB*
ARHGEF2	*CEACAM7*	*EIF4G2*	*GNA11*	*MGMT*	*PCNA*	*SDHB*	*TMEM97*
ARID1A	*CEBPA*	*EML4*	*GNAQ*	*MIB1*	*PDGFRA*	*SDHC*	*TNFAIP3*
ASXL1	*CES3*	*ENO1*	*GNAS*	*MKI67*	*PDZD4*	*SDHD*	*TNFSF13*
ATM	*CHDM*	*ENO2*	*GRP*	*MLH1*	*PGR*	*SELT*	***TP53***
ATRX	*CRLF2*	*ERBB2*	*GSTM1*	*MPL*	*PHOX2B*	*SETD2*	*TPD52L2*
BAG3	*CSF1R*	*ERBB3*	*HIVEP3*	*MSH2*	***PIK3CA***	*SLC38A1*	*TPM4*
BAI3	*CSF3*	*ERBB4*	*HNF1A*	*MSH6*	*PMS2*	*SLC6A2*	***TSC1***
BAP1	*CTNNA1*	*ERG*	*HRAS*	*MUC1*	*PPP2R1A*	*SLTM*	***TSC2***
BCAN	*CTNNB1*	*EZH2*	*IDH1*	*MUC17*	*PRCC*	*SMAD4*	*TSHR*
BCL2	*CUL2*	*F10*	*IDH2*	*MUC2*	*PRKAR1A*	*SMARCA4*	*TYK2*
BRAF	*CYLD*	*FAM123B*	*IFNA1*	*MUC3A*	*PTCH1*	*SMARCB1*	***VHL***
BRCA1	*CYP1A1*	*FBXW7*	*IGKV1D-43*	*MUTYH*	***PTEN***	*SMO*	*VIM*
BRCA2	***DAXX***	*FGFR1*	*IL2*	*MVP*	*PTGS2*	*SMOX*	*WT1*
CA12	*DCC*	*FGFR2*	*ITGB5*	*MYC*	*PTPN11*	*SMUG1*	*WTS*
CA9	*DES*	*FGFR3*	*JAK1*	*MYD88*	*RB1*	*SOCS1*	*XRCC1*
CALU	*DIRAS1*	*FH*	*JAK2*	*NAT1*	*REEP5*	*SRC*	*ZNF135*
CARD11	*DIRC2*	*FHIT*	*JAK3*	*NAT2*	*RET*	*SST*	
CBL	*DKK3*	*FLCN*	*KDR*	*NES*	*RNF139*	*STC1*	
CCND1	*DLD*	***FLT3***	*KIT*	***NF1***	*RNF2*	*STK11*	

Genes implicated in NETs are in bold

BMJ Open

Mutation-targeted therapy with sunitinib or everolimus in patients with advanced low-or intermediate grade neuroendocrine tumors of the gastrointestinal tract and pancreas with or without cytoreductive surgery: protocol for a Phase II clinical trial.

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Title: Mutation-targeted therapy with sunitinib or everolimus in patients with advanced low-or intermediate grade neuroendocrine tumors of the gastrointestinal tract and pancreas with or without cytoreductive surgery: protocol for a Phase II clinical trial.

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Abstract

Introduction

Finding the optimal management strategy for patients with advanced, metastatic neuroendocrine tumors (NETs) of the gastrointestinal tract and pancreas is a work in progress. Sunitinib and everolimus are currently approved for the treatment of progressive, unresectable, locally advanced or metastatic low-or intermediate grade pancreatic NETs. However, mutation targeted therapy with sunitinib or everolimus has not been studied in this patient population.

Methods and analysis

This prospective, open-label Phase II clinical trial was designed to determine if mutation targeting therapy with sunitinib or everolimus for patients with advanced low- or intermediate grade NETs is more effective than historically expected results with progression-free survival (PFS) as the primary endpoint. Patients ≥18 years of age with progressive, low or intermediate grade locally advanced or metastatic NETs are eligible for this study. Patients will undergo tumor biopsy (if not a surgical candidate) for tumor genotyping. Patients will be assigned to sunitininb or everolimus based on somatic/germline mutations profile. Patients who have disease-progression on either sunitinib or everolimus will cross-over to the other drug. Treatment will continue until disease progression, unacceptable toxicity, or consent withdrawal. Using the proposed criteria, 44 patients will be accrued within each treatment group during a 48 month period (88 total patients for the two treatments), and followed for up to an additional 12 months (60 months total from entry of the first patient) to achieve 80% power in order to test whether there is an improvement in PFS compared to historically expected results, with a 0.10 alpha level one-sided significance test.

Ethics and dissemination

The study protocol was approved by the Institutional Review Board of the National Cancer Institute (NCI-IRB Number 15C0040; iRIS Reference Number 339636). The results will be published in a peer-reviewed journal and shared with the worldwide medical community.

Keywords: neuroendocrine tumors; gastrointestinal tract; pancreas; low-or intermediate grade neuroendocrine tumors; sunitininb; everolimus

Background

Neuroendocrine tumors (NETs) of the gastrointestinal tract (GI) and pancreas are a rare and heterogeneous group of neoplasms with unique tumor biology, natural history, and clinical management issues (1-4). Most NETs are sporadic, but they can be part of familial cancer syndromes such as multiple endocrine neoplasia type 1 (MEN1), neurofibromatosis type 1 (NF1), tuberous sclerosis (TS) or Von Hippel-Lindau (VHL) syndrome (5-9).

While poorly differentiated tumors may exhibit highly aggressive behavior, well-differentiated, low or intermediate grade NETs have a relatively indolent behavior with slow progression (6, 8, 10). As a result of this insidious biological behavior, many patients with well-differentiated, low or intermediate grade NETs have advanced disease at diagnosis, with regional or distant metastasis observed in more than 50% of patients (11, 12). Surgery is the only curative treatment option in patients with localized early stage NETs. The optimal management strategy for patients with advanced NETs is unknown.

Our understanding of the genetic events associated with sporadic and familial NETs has improved considerably over the last 3 decades. Driver oncogene and tumor suppressor genes have been identified in most NETs (13-19). Overall, the majority of NETs have somatic mutations in *MEN1*, the phosphatidylinositol 3-kinase (*PI3K)/AKT/* mammalian target of rapamycin (*mTOR*) signaling pathway (20-24), and or overexpression of growth factors and their receptor such as vascular endothelial growth factor (*VEGF*), *VEGF* receptor (*VEGFR*), platelet-derived growth factor (*PDGF*), and *PDGF* receptor (*PDGFR*) (25-28). A recent study also revealed the presence of somatic mutations in *MEN1, DAXX, ATRX, TSC2, PTEN*, and *PIK3CA* genes in the majority of sporadic pancreatic NETs (22). Moreover, the presence of these

mutations was associated with better survival when compared to patients with NETs, which had wild type *MEN1,* and/or *DAXX/ATRX* (22).

In 2011, sunitinib (multi-tyrosine kinase inhibitor) and everolimus (mTOR signaling pathway inhibitor) were approved by the Food and Drug Administration (FDA) for the treatment of unresectable, metastatic, progressive pancreatic NETs based on the results of Phase III trials demonstrating a significantly improved progression-free survival (PFS) in the treatment vs. placebo arm (11.4 vs. 5.5 months for sunitinib and 11.0 vs. 4.6 months for everolimus) (14, 29). However, there are several important management issues that remain unclear: 1) Is treatment with everolimus/sunitinib beneficial to other groups of patients with advanced NETs, who have NETs of GI tract (also called "carcinoids") and or patients who have cytoreductive surgery? 2) While the choice of targeted therapies in other malignancies is more frequently being driven by the findings of the precise molecular alterations present in the tumor, no such study has been done in NETs. This is particularly important given that the survival of patients with malignant NETs appears to be different based on the driver mutation(s) present in the tumor and low- or intermediate grade tumors can have a relatively indolent growth (22).

The primary objective of this Phase II trial is to determine the PFS in patients with NETs of the GI tract and pancreas treated with sunitinib or everolimus based on tumor genotyping with or without surgical resection. The study was designed to test the hypothesis that an improvement in PFS can be achieved using this mutation targeted treatment strategy when compared to previous studies (14, 29).

Methods and Design

Study population

All patients with NETs of the GI tract and pancreas who meet the following criteria are eligible to participate in this study.

Inclusion Criteria

- Progressive, histologically or cytologically diagnosed low or intermediate grade, neuroendocrine tumors confirmed by the Laboratory of Pathology, National Cancer Institute (NCI).
- Age ≥ 18 years, because no dosing or adverse event data are currently available on the use of Sunitinib and or Everolimus in patients <18 years of age, (children are excluded from this study, but will be eligible for future pediatric trials).
- ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).
- Patients must have normal organ and bone marrow function as defined below:
    - leukocytes ≥3,000/mcL
    - absolute neutrophil count ≥1,500/mcL
    - platelets ≥ institutional lower limit of normal
        - total bilirubin ≤2-fold above institutional upper limit of normal
        - AST(SGOT)/ALT(SGPT) ≤2.5-fold above institutional upper limit of normal
    - creatinine within normal institutional limits

      OR
    - creatinine clearance ≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

- Agreement to use effective contraception while on treatment and for ≥ 3 months after end of treatment, because the effects of Sunitinib and Everolimus on the developing human fetus are unknown. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.
- Must have fully recovered from toxicities of any prior treatment with cytotoxic drugs, radiotherapy, surgery, or other anti-cancer modalities (returned to baseline status as noted before most recent treatment).
- Ability of subject or Legally Authorized Representative to understand and the willingness to sign a written informed consent document.

Exclusion Criteria

- Uncontrolled hypertension (>150/100 mmHg).
- Prior external beam radiation therapy to the target lesion(s) within 1 months prior to enrollment
- Prior systemic chemotherapy or therapy with one of the investigation agents within 1 month prior to enrollment.
- Patients who are receiving any other investigational agents.
- Patients with known brain metastases will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to Sunitinib or Everolimus.

- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- Serious uncontrolled concomitant disease that the investigator feels might compromise study participation.
- Pregnant or nursing patients will be excluded from the study, because the effects of Sunitinib and Everolimus on the developing human fetus are unknown. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Sunitinib or Everolimus, breastfeeding should be discontinued if the mother is treated with Sunitinib or Everolimus.
- Any of the following clinical conditions within the 12 months prior to starting study treatment: myocardial infarction, severe/unstable angina, coronary/peripheral artery bypass graft, congestive heart failure, cerebrovascular accident including transient ischemic attack, pulmonary embolism, ongoing cardiac dysrhythmias of NCI CTCAE grade at least 2, atrial fibrillation of any grade, or QTc interval >450 msec for males or >470 msec for females.
- Clinically significant pulmonary disease (e.g., severe chronic obstructive pulmonary disease [COPD] or asthma).
- Current treatment with therapeutic doses of Coumadin-derivative anticoagulants (low dose Coumadin up to 2 mg PO daily for deep vein thrombosis prophylaxis is allowed).
- Patients with a history of uncontrolled seizures, central nervous system disorders of psychiatric disability judged by the Investigator to be clinically significant precluding informed consent or interfering with compliance for oral drug intake will be excluded from study.

- HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with study agents.
- Lack of physical integrity of the upper gastrointestinal tract or malabsorption syndrome, or the inability to take oral medication

Study Design

In this prospective, open-label, Phase II, clinical trial patients will undergo cytoreductive surgery for standard of care indications, or an image guided tumor biopsy will be performed for tumor genotyping in patients who do not undergo an operation and who do not have archived tumor tissue samples. Patients who have been potentially rendered disease free at the discretion of the principal investigator after surgical resection will not be assigned to a study medication and will be removed from the study. All other patients must begin study drug within 3 months after surgery/biopsy. Patients with syndromic NETs (e.g., VHL and MEN1) will not undergo tumor biopsy. Based on the tumor genotype or germline mutation status and the results of analysis of the involved cell signaling pathways (**Table 1**), patients will be treated with sunitinib (for mutations in *MEN1/PDGFR/KIT/FLT3*) or everolimus (for mutations in *NF1/PTEN/PI3K/AKT/mTOR/VHL/TP53*) daily in 28 day cycles (**Table 2**). Patients will receive long-acting octreotide for symptoms associated with hormonal hypersecretion. Treatment with sunitinib/everolimus in patients who undergo surgical treatment will begin after recovery from surgery. Patients who have gene mutations not known to be specifically targeted by sunitinib or everolimus or with more than one mutation will be assigned to sunitinib.

If a patient has two or more mutations (one in the everolimus-*MEN1/PDGFR/KIT/FLT3* and another in the sunitinib group- *NF1/PTEN/PI3K/AKT/mTOR/VHL/TP53*), the patient will be assigned to sunitinib and will cross over to the other drug if disease progression develops.

Patients who develop disease-progression on either sunitinib or everolimus will cross-over to the other drug. Treatment will continue until disease progression, unacceptable treatment-related toxicity, or consent withdrawal. After discontinuation from the study, the subject will be contacted at 3 month intervals to obtain information about subsequent treatment(s) and survival status (**Figure 1).**

The study will be conducted at the NIH Clinical Center. Patients from all over the world can be screened for eligibility; however, in order to participate the patient must travel to NIH as this is a single site study.

Biospecimen collection

Tumor genotyping and germline mutation analysis

Within three months prior to initiation of treatment with the respective study agent (everolimus or sunitinib), patients who meet eligibility criteria, but are not candidates for cytoreductive surgery, will undergo biopsy of the primary tumor or any metastatic site for molecular analyses. If patients are scheduled for an operative intervention for tumor debulking, a portion of the resected tumor will be used for research. If surgical resection is not indicated, patients will undergo a percutaneous core needle biopsy of the tumor under local anesthesia. These percutaneous biopsies will be performed by interventional radiology (under Computed Tomography (CT) scan or ultrasound (US) guidance). If needed, patients will be offered

conscious sedation for the biopsy procedure. Sample collection will be performed according to standard operating procedures.

Peripheral blood (30 mL) will be obtained for targeted sequencing and comparison to tumor sequencing results and mutation calls after enrollment and before treatment.

Tumor tissues samples will be examined by standard histology, immunohistochemistry and or in situ hybridization in the Laboratory of Pathology, NCI. Tumor genotyping and germline sequencing for 197 driver genes will be performed in a Clinical Molecular Profiling Core (CLIA) certified genetic laboratory at the NIH-NCI.

Cytoreductive surgery

Cytoreductive surgery will be performed in subjects in whom it is indicated by standard of care. Operative resections will not be performed for research purposes only. Surgery will be done per NIH Clinical Center standard operating procedure. Subjects will sign a separate consent for the surgery.

Treatment agents and dose

Based on the tumor genotype or germline mutation status, patients will be assigned to one of the two study drugs (**Table 1 and Supplemental Table 1**).

Sunitinib

Sunitinib is a multi-kinase inhibitor and inhibits all 3 types of *VEGFR* and several other tyrosine kinase receptors. It is FDA approved for patients with advanced, progressive, unresectable NETs. The most frequent adverse events associated with sunitinib therapy are diarrhea, nausea, vomiting, rash (hand-foot), asthenia, and fatigue (14). Patients with *MEN1/PDGFR/KIT/FLT3* mutations or other gene mutations will be treated with oral sunitinib at a dose of 37.5 mg once daily (**Table 1**). Patients will take sunitinib once daily in the morning, with or without food, as desired. Treatment will continue until progression of disease, development of an unacceptable toxicity, drug interruption for 3 weeks or longer, or withdrawal of consent. Treatment interruptions and a dose reduction to 25 mg per day will be permitted to manage adverse events, with a subsequent increase in dose if toxicity of grade 2 or higher did not recur.

Everolimus

Everolimus is an *mTOR* inhibitor. *mTOR* is an intracellular serine-threonine kinase that has a role in regulating cell growth, proliferation, apoptosis and angiogensis, and is activated in several cancers. Everolimus is also FDA approved for patients with advanced, progressive, unresectable NETs. Common side effects are stomatitis, rash, diarrhea, fatigue and respiratory tract infection. Previously reported, Grade 3 and 4 toxicities include anemia (6%) and hyperglycemia (5%) (16). Patients with *NF1/PTEN/PI3K/AKT/mTOR/VHL/TP53* mutations will be treated with oral everolimus at a dose of 10 mg once daily (**Table 1**). Treatment will continue until progression of disease, development of unacceptable toxicity, drug interruption for 3 weeks or longer, or withdrawal of consent. Doses will be delayed or reduced if patients have clinically significant adverse events that are considered to be related to the study treatment. In such cases,

two reductions in the dose of the study drug will be permitted: an initial reduction to 5 mg daily with a subsequent increase in dose if toxicity of grade 2 or higher did not recur.

Study Endpoints

Primary Endpoint

- PFS on first-line therapy in patients with NETs of the gastrointestinal tract and pancreas treated with sunitinib or everolimus based on tumor genotyping with or without surgical resection.

Secondary Endpoints

- Overall response rate (ORR; i.e., sum of complete response [CR], partial response [PR], and stable disease [SD]) and duration of response
- PFS in patients who undergo cytoreductive surgery with tumor genotype and treatment with sunitinib or everolimus based on tumor genotyping results may represent a potential improvement over published results of treatment with only everolimus/sunitinib or surgical resection only.
- Overall survival and median survival time (MST)
- Relationship between tumor genotype, treatment and PFS
- Safety endpoints (i.e. AEs, clinical laboratory evaluations, ECGs, physical examination findings, and vital sign measurements).

Study calendar,data collection and monitoring adherence

The screening and on study assessments listed in detail in **Table 2** may be performed within 1 week of the time listed in order to accommodate weekends, holidays, travel delays, inclement weather and other such unexpected events with the exception of the baseline pregnancy test which must be performed as stated, within 3 days prior to study drug initiation.

Face-to-face adherence reminder sessions will take place at the initial product dispensing and each study visit thereafter. This session will include: the importance of following study guidelines; instructions about taking study pills including dose timing, storage, and importance of taking pills whole, and what to do in the event of a missed dose; instructions about the purpose, use, and care of the medication event monitoring system and bottle; notification that there will be a pill count at every study visit; importance of calling the providers if experiencing problems possibly related to study product such as symptoms and lost pills.

Response criteria

For the purposes of this study, patients will be re-evaluated for response every 12 weeks (**Figure 1 and Table 2**). 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).

Criteria for withdrawal of individual subjects

The criteria that take the subject off active protocol therapy include:

- Progressive disease on each study arm
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined in section 3.3
- Investigator discretion
- In all cases, a safety follow up visit will be conducted within approximately 30 days of the last dose of study drug therapy

Statistical analysis and sample size calculation

The primary objective of this trial is to determine PFS of patients with low- or intermediate grade NET who receive first-line targeted therapy with or without cytoreductive surgery. This will provide data to explore whether the PFS may represent a potential improvement over published results with treatment only with everolimus/sunitinib (14, 29). Secondary objectives include evaluation of response and OS as well as safety and exploring the association between tumor genotype and PFS, and response to treatment. If the findings of this study suggest improved PFS from mutation-targeted and or combination treatment, larger randomized trials to confirm the findings will be developed.

Following surgery or tumor biopsy for tumor genotyping, eligible patients will be treated with everolimus or sunitinib. Based on results of the genotyping, enrolled patients will then receive targeted treatment with sunitinib (for mutations in *MEN1/PDGFR/KIT/FTL3* or everolimus (for mutations in *NF1/PTEN/ PI3K/AKT/mTOR/VHL/TP53*). Patients with other different mutations or multiple types of mutations will be assigned to receive sunitinib since almost all of these tumors have elevated levels of VEGF/VEGFR expression.

Results from previously published trials (14, 29) both demonstrated approximately 11 months PFS in patients similar to the ones to be treated on this protocol. Those patients were not assigned to receive treatment on the basis of any tumor genotype information. The study was designed to determine if meaningful improvement in PFS may be obtained using this focused strategy. For purposes of sample size determination, patients will be primarily evaluated based on the treatment received. Thus, within each treatment group, using the method of Brookmeyer and Crowley, with 44 patients accrued during a 48 month period (88 total patients for the two treatments), and followed for up to an additional 12 months (60 months total from entry of the first patient), there would be 80% power to test whether the median PFS is consistent with 18 months, and greater than 11 months, with a 0.10 alpha level one-sided significance test.

In practice, a Kaplan-Meier curve of PFS will be constructed for each group, and will have the median as well as key time points such as 12 and 18 months estimated, along with appropriate 80% and 95% confidence intervals to explore whether the present results exceed those from prior studies. In addition, the Kaplan-Meier curves for all patients who received either sunitinib and everolimus as their treatments may be combined into one pooled Kaplan-Meier curve if the two curves for these treatments are sufficiently similar to one another ($p>0.30$ by a two-tailed log-rank test). This combined curve will then be evaluated relative to the published median of 11 months PFS for each group to provide a more powerful comparison relative to the historically expected results. In addition, Kaplan-Meier curves limited to the patients who received targeted therapy based solely on mutation status may be constructed for exploratory and descriptive purposes.

Response and overall survival will also be evaluated as secondary endpoints, based on the patients treated with sunitinib or everolimus, using all patients receiving treatment. These results

will be descriptive and may consist of fractions of responses as well as Kaplan-Meier curves for survival. The association between genotype and PFS will be explored by evaluating the results within a treatment based on the major genotype categories identified. Safety will be evaluated by tabulating and reporting the distribution of the worst grade of each type of toxicity found, per patient, separately by treatment. Should any particular type of toxicity result in 5 or more patients with grade 3-4 toxicity, a comparison of the distributions of toxicity between the two treatments may be performed using a Cochran-Armitage test for trend.

Because the patients may not end up being assigned in equal proportions to the two treatments, and because the goal is to have 44 evaluable patients who have received each treatment, additional patients beyond the 44 described above may be enrolled on the arm with faster accrual. As such, the study will have an accrual ceiling of 120 patients, with accrual ending when there are 44 evaluable patients on the arm with fewer patients. An amendment may also be needed if a lower proportion of patients than expected receive study drug therapy or if a high portion of treated patients are not evaluable for response. It is anticipated that 20-30 patients per year may enroll onto this trial; thus accrual may be completed in 3-4 years.

Ethics and dissemination

The study protocol was approved by the Institutional Review Board of the National Cancer Institute (NCI-IRB Number 15C0040; iRIS Reference Number 339636). The results will be published in a peer-reviewed journal and shared with the worldwide medical community. ClinicalTrials.gov Identifier: NCT02315625

Discussion

NETs of the GI tract and pancreas are rare and heterogeneous, but clinically important group of neoplasms that arise in the disseminated neuroendocrine cells of the GI tract and the pancreatic islet cells. The annual incidence of NETs has been increasing in the United States and worldwide and was estimated to be 7.8 per 100,000 persons in 2013 (1, 8, 11, 30-32).

NETs are classified into functioning (hormone hypersecreting) or nonfunctioning (clinically "silent") tumors, based on their ability to produce hormone-associated symptoms. However, other classification systems with many common themes, such as the distinction of well-differentiated (low and intermediate-grade) from poorly differentiated (high-grade) NETs and the prognostic significance of proliferative rate index have been used over the past 5 decades. The majority of NETs (60-90%) are clinically non-functioning, well-differentiated, slow-growing neoplasms diagnosed, in most instances, incidentally during an unrelated procedure (1, 7, 12, 33-36). As a result of this insidious biological behavior, many patients with NETs have advanced disease at diagnosis, with regional or distant metastasis observed in more than 50% of patients (11, 12). NETs most commonly metastasize to the loco-regional lymph nodes and liver, and 25% to 93% of patients will develop liver metastases during the course of their disease (37, 38).

Surgical resection alone is a valuable treatment option for patients with early-stage disease; however, the extent, timing and effect of surgical intervention for advanced, metastatic NETs remain controversial and difficult to estimate. Although not supported by randomized clinical trial data, currently it is advocated that surgery should be undertaken only if metastatic disease is confined to the liver and if 90% or more of the tumor mass, including liver metastases, can be successfully removed (39). However, most patients will present with multiple bilobar

liver metastases, and altogether only 5–10% will have apparently solitary or dominant liver metastases amenable to surgical resection (37, 40). Furthermore, recurrence after surgery is common and a significant number of patients with advanced NETs undergoing debulking surgery will have residual disease and suffer from complications associated with hormonal hypersecretion and or tumor progression (37, 41, 42).

The approval, by the FDA, of sunitinib and everolimus for the treatment of unresectable, locally advanced or metastatic NETs is a remarkable milestone in the field of medical therapy of malignant NETs (13, 14, 29). However, while the mutation-targeted therapy in other malignancies is driven by the findings of the precise molecular alterations present in the tumor, no such study has been done in malignant NETs. In addition, it is not known if treatment with everolimus or sunitinib may be beneficial to other groups of patients with advanced NETs, including those with carcinoid tumors or as adjuvant therapy for patients who have cytoreductive surgery.

Although improving overall survival is the ultimate goal for any cancer therapy, the variable and at times long survival time in many patients with NETs makes overall survival a less suitable initial end point to study for the proposed strategy of targeted therapy (42, 43). In this regard, using PFS as a primary end point poses less significant scientific challenges and is attractive from both an ethical and feasibility standpoint (42). We believe that such an approach will allow us to generate enough data to determine whether a larger study is warranted using mutation-targeted therapy for patients with NETs. The results from this Phase II open-labeled study could also provide important data for future study questions: 1) Does specific tumor genotype predict response to therapy, 2) Does adjuvant therapy after surgical debulking have any

benefit, and 3) What are the long-term side-effects associated with sunitinib or everolimus therapy?

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Figure legends

Figure 1. Study flow chart.

CR - complete response, PR - partial response, SD - stable disease, PD - disease progression

Trial status

Patient recruitment is currently ongoing

ClinicalTrials.gov Identifier

NCT02315625

List of abbreviations

NETs - neuroendocrine tumors of the gastrointestinal tract (GI) and pancreas; MEN1 - multiple endocrine neoplasia type 1; NF1 - neurofibromatosis type 1; TS - tuberous sclerosis; VHL - Von Hippel-Lindau syndrome; *VEGF* - vascular endothelial growth factor; *PI3K* - phosphatidylinositol 3-kinase; *PTEN* - Phosphatase and tensin homolog; *PIP2* - Phosphatidylinositol-4,5-bisphosphate; *PIP3* - phosphatidylinositol-3,4,5-trisphosphate; *AKT* – gene encoding RAC-alpha serine/threonine-protein kinase; TSC - tuberous sclerosis complex; *mTOR* - mammalian target of rapamycin; *FKBP12* - FK506 binding protein; *4E-BP* - eukaryotic translation initiation factor 4E-binding protein; *p70S6K* - p70S6 kinase; DAXX - death-associated protein 6; ATRX - ATP-dependent helicase; FDA - Food and Drug Administration; PFS - progression-free survival; NCI - National Cancer Institute; NIH- National Institutes of Health; ECOG - Eastern Cooperative Oncology Grou; AST/ALT - aspartate transaminase / alanine transaminase; COPD - chronic obstructive pulmonary disease; HIV – human immunodeficiency virus; *TP53* - tumor protein p53; *KIT* - proto-oncogene c-Kit; *FLT3* - Fms-like tyrosine kinase 3; *PDGFR* - platelet-derived growth factor receptors; CLIA - Clinical Molecular Profiling Core; RECIST - Response Evaluation Criteria in Solid Tumors

Competing interests

The authors declare that they have no competing interests

Authors' contributions

EK is the principal investigator. VN is the lead associate investigator. EK and VN conceived, designed and wrote the project proposal and manuscript and will be responsible for study implementation, data collection, management and analysis. SS participated in outlining statistical methods, critical review of the protocol and manuscript, and will participate in data analysis. NN participated in outlining study design, critical review of the protocol and manuscript and will participate in study implementation. CCD and RM are the study coordinators and will be responsible for the clinical data collection and implementation of the study. SM, JY, PM, and KP participated in study development, critical review of the protocol and manuscript and will participate in study implementation and data collection and analysis. All authors reviewed and approved the final manuscript.

Organizational structure and responsibilities

- **Principal Investigator and Research Physician**
    - Design and conduct of study
    - Preparation of protocol and revisions with assistance of PSO
    - Preparation of investigators brochure (IB) and CRFs [Case Report Forms]
    - Publication of study reports
- **Scientific Review committee (SRC)**
    - Agreement of final protocol

- Reviewing progress of study and if necessary agreeing changes to the protocol and/or investigators brochure to facilitate the smooth running of the study.

- **Protocol Support Office (PSO)**
  - Study planning
  - Organization of scientific review committee meetings
  - Provide annual reports to IRB committee
  - SUSAR [Serious unexpected suspected adverse events] reporting to sponsors
  - Responsible for trial master file
  - Budget administration and contractual issues
  - Advice for the lead investigator
- **Clinical Trials Office**
  - Data verification
  - Organisation of central blood sample collection
  - Management of site visits and audits
  - Recruitment of patients and liaising with principle investigator
- **Data Manager**
  - Maintenance of trial IT system and data entry
  - Data verification
- **Lead Investigator**
  - Lead investigator will be responsible for identification, recruitment, data collection and completion of CRFs, along with follow up of study patients and adherence to study protocol and investigators brochure.

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Disclaimer

This is a US government work. The views expressed within this paper do not necessarily reflect those of the US government.

Table 1. Choice of targeted therapy driven by the findings of the precise molecular alterations based on common mutations that occur in NETs.

Study Agent	Mutations *	Affected Pathways
Everolimus	*PTEN*	*PI3K/AKT/mTOR*
	PI3K	*PI3K/AKT/mTOR*
	AKT	*PI3K/AKT/mTOR*
	mTOR	*PI3K/AKT/mTOR*
	VHL	Hypoxia induced *PI3K/AKT/mTOR*
	TSC1	*PI3K/AKT/mTOR*
	TSC2	*PI3K/AKT/mTOR*
	NF1	*TSC2/mTOR,* Hypoxia induced neoangiogenesis
Sunitinib	*MEN1*	Cell growth, cell cycle and genome instability
	FLT3	Cell survival, proliferation, and differentiation
	PDGFR	Cell proliferation, cell migration, neoangiogenesis
	ATM	Cell survival, cell cycle, DNA repair and apoptosis
	KIT	Cell survival, proliferation, and differentiation
	ATRX	Cell survival, proliferation, and differentiation

*Mutations in genes not listed above or that are wildtype will be treated with sunitinib (see Supplemental Table 1 for list of other genes that will be genotyped). Germline DNA will be obtained for comparison to tumor genotype data for every patient. Also, some patients with known familial cancer syndromes (MEN1 and VHL) will be included in the study and tumor biopsy for the sole purpose of agent selection in these patients will not be performed.

Table 2. Study calendar

Screening for eligibility		**Within 4 weeks prior to enrollment**	• Clinical Assessment[i] • 24 hour urine collection for urine protein, urine creatinine and urine albumin • Serum creatinine • Hemoglobin A1C • HIV antibody • Radiological evaluations[ii] • Histopathological confirmation of progressive, low or intermediate grade NET
		Within 2 weeks prior to enrollment	• 12- lead electrocardiogram[iii] • CBC[iv-a], Chemistriesiv[-b], INR, Lipid panel (fasting)
		Within 3 days prior to enrollment	• Serum or urine HCG (in women of childbearing potential only)
Enrollment			Patient signs consent
Prior to streatment with study drug		**(Surgical Candidates only)** **Month -3 to Day 1**	• Contrast enhanced CT scan (within 4 weeks prior to operation – screening scan may be used if timeframe is met) • Standard pre-operative evaluation based on diagnosis • Cytoreductive Surgery and intraoperative biospecimen collection for gentotyping • Contrast Enhanced CT scan for post operative tumor burden (within 4 weeks prior to study drug initiation) • Routine post surgery care and recovery
		(Subjects not undergoing surgery only) **Month -3 to Day 1**	• Image guided tumor biopsy for genotyping
		All Subjects **Within 4 weeks prior to treatment initiation**	• Hepatitis B and C Evaluation • Echocardiogram (in patients with carcinoid tumors only)
		All Subjects **Within 2 weeks prior to treatment initiation** [Tests need not be repeated if they have been done during the appropriate timeframe at screening]	• Clinical Assessmenti • Chromogranin A, pancreatic polypeptide, and neuron-specific enolase. • Vasoactive intestinal polypeptide (VIP), serotonin (urinary 5-HIAA), gastrin, somatostatin, fasting insulin, C-peptide (proinsulin) and or glucagon only in patients known to have functioning NETs. • CBCiv[-a], Chemistriesiv[-b], and TFTsiv[-c] • Urinalysis • Cardiac Evaluation (in patients that present with cardiac or pulmonary risk factors) • CT CAP or MRI[v]ii • 12 lead ECGiii • 30 mL of peripheral blood f (red top tubes) or research (see section Error! Reference source not found.)
		Women of childbearing potential only **Within 3 days of study drug initiation**	• Urine or serum HCG
Treatment based on tumor genotyping	**Cycle 1 (28 days)**	**Day 14**	• Clinical assessmenti • CBCiv[-a] and Chemistriesiv[-b], • Urinalysis
	Cycle 2 (28 days)	**Day 1**	• Clinical assessmenti • CBCiv[-a], Chemistriesiv[-b], and TFTsiv[-c] • Urine or serum HCG in women of childbearing potential • Urinalysis • 12 lead ECGiii
		Day 14	• CBC iv[-a], • Chemistriesiv[-b], • Urinalysis
	Cycle N (28 days)	**Day 1**	• Clinical assessmenti • CBCiv[-a], Chemistriesiv[-b], and TFTsiv[-c] • Urine or serum HCG in women of childbearing potential • Urinalysis • 12 lead ECGiii • Radiological evaluation of treatment responsev
Final/Early termination visit[vi]			• Clinical assessmenti • CBCiv[-a], Chemistriesiv[-b], and TFTsiv[-c]

	• Urine or serum HCG in women of childbearing potential • Urinalysis • Radiological evaluation of treatment responsev (not applicable for patients that have progressed on 2 drugs)
Long Term Follow up	Telephone contact every 3 months to determine anti-cancer therapy and survival status
Concomitant Medications	Throughout study
Adverse Events	Throughout study

[i] Clinical assessment: Complete history and physical examination including height, weight, vital signs (incl blood pressure, pulse) and ECOG at screening, baseline, Day 1 and 15 of Cycle 1, and then on Day 1 of each subsequent cycle. 12L ECG to be completed within 2 weeks prior to treatment and then at the end of each cycle prior to starting next cycle of therapy

[ii] Radiological Evaluations to be completed as part of the screening.

- Brain MRI or CT
- Contrast CT scan or MRI of the chest, abdomen and pelvis (CT C/A/P) for the purpose of tumor burden and tumor volumetric measurement
- Bone scan for patients in whom bone metastases are suspected
- FDG PET scan

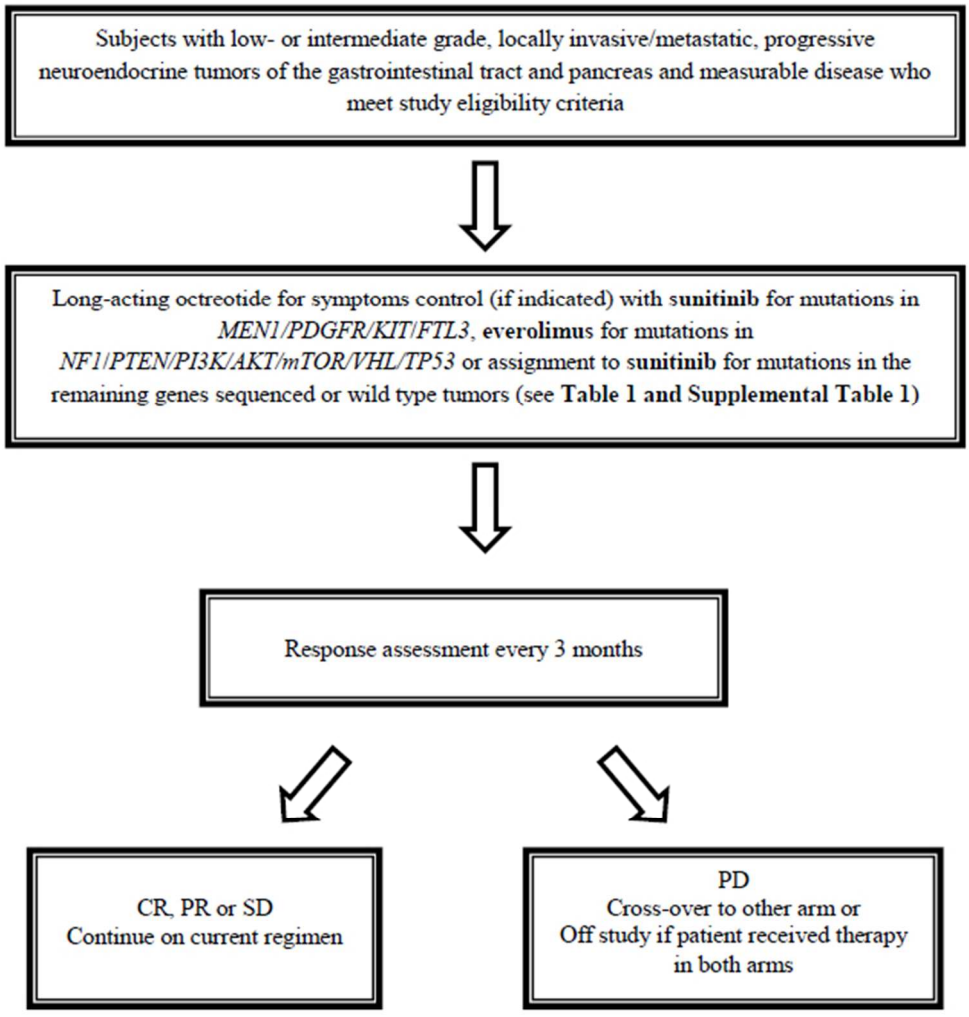
[iii] 12L ECG to be completed within 2 weeks prior to treatment and then at the end of each cycle prior to starting next cycle of therapy.

[iv] Laboratory Evaluations:

a. CBC with differential and platelets to be completed within 2 weeks prior to enrollment, within 2 weeks prior to treatment, then every 2 weeks for the first 2 cycles and then every 4 weeks thereafter.
b. Chemistries: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Total Protein to be completed within 2 weeks prior to enrollment, within 2 weeks prior to treatment, then every 2 weeks for the first 2 cycles and then every 4 weeks thereafter.
c. TFTs: Free T3, TSH to be done within 2 weeks prior to treatment then every 4 weeks thereafter.

[v] A CT or MRI of the chest/abdomen/pelvis to reassess treatment response will be done at baseline, at 3 months after treatment initiation and then every 3 months. MRI can be substituted for CT scan at the discretion of the investigator as some lesions such as hepatic metastasis are best visualized on MRI.

[vi] Final/early termination visit will occur approximately 30 days after the last dose of study drug



Study flow chart.
CR - complete response, PR - partial response, SD - stable disease, PD - disease progression
173x179mm (96 x 96 DPI)

Supplemental Table 1. Panel of 197 genes tested.

Genes							
ABL1	*CD34*	*DMBT1*	*FOXL2*	*KRAS*	*NF2*	*RPS15*	*SUFU*
ACN9	*CDC73*	*DNMT3A*	*FZR1*	*KRT20*	*NOTCH1*	*RUNX1*	*SYP*
ACVR1B	*CDH1*	*DPYD*	*GATA1*	*MAGI1*	*NOTCH2*	*SCLC1*	*TCF7L2*
AKT1	*CDK4*	*EGF*	*GATA2*	*MAP2K4*	*NPM1*	*SDHA*	*TET2*
ALK	*CDKN2A*	*EGFR*	*GATA3*	***MEN1***	*NRAS*	*SDHAF1*	*TFE3*
APC	*CDX2*	*EGR3*	*GFAP*	*MET*	*PBRM1*	*SDHAF2*	*TFEB*
ARHGEF2	*CEACAM7*	*EIF4G2*	*GNA11*	*MGMT*	*PCNA*	*SDHB*	*TMEM97*
ARID1A	*CEBPA*	*EML4*	*GNAQ*	*MIB1*	*PDGFRA*	*SDHC*	*TNFAIP3*
ASXL1	*CES3*	*ENO1*	*GNAS*	*MKI67*	*PDZD4*	*SDHD*	*TNFSF13*
ATM	*CHDM*	*ENO2*	*GRP*	*MLH1*	*PGR*	*SELT*	***TP53***
ATRX	*CRLF2*	*ERBB2*	*GSTM1*	*MPL*	*PHOX2B*	*SETD2*	*TPD52L2*
BAG3	*CSF1R*	*ERBB3*	*HIVEP3*	*MSH2*	***PIK3CA***	*SLC38A1*	*TPM4*
BAI3	*CSF3*	*ERBB4*	*HNF1A*	*MSH6*	*PMS2*	*SLC6A2*	***TSC1***
BAP1	*CTNNA1*	*ERG*	*HRAS*	*MUC1*	*PPP2R1A*	*SLTM*	***TSC2***
BCAN	*CTNNB1*	*EZH2*	*IDH1*	*MUC17*	*PRCC*	*SMAD4*	*TSHR*
BCL2	*CUL2*	*F10*	*IDH2*	*MUC2*	*PRKAR1A*	*SMARCA4*	*TYK2*
BRAF	*CYLD*	*FAM123B*	*IFNA1*	*MUC3A*	*PTCH1*	*SMARCB1*	***VHL***
BRCA1	*CYP1A1*	*FBXW7*	*IGKV1D-43*	*MUTYH*	***PTEN***	*SMO*	*VIM*
BRCA2	***DAXX***	*FGFR1*	*IL2*	*MVP*	*PTGS2*	*SMOX*	*WT1*
CA12	*DCC*	*FGFR2*	*ITGB5*	*MYC*	*PTPN11*	*SMUG1*	*WTS*
CA9	*DES*	*FGFR3*	*JAK1*	*MYD88*	*RB1*	*SOCS1*	*XRCC1*
CALU	*DIRAS1*	*FH*	*JAK2*	*NAT1*	*REEP5*	*SRC*	*ZNF135*
CARD11	*DIRC2*	*FHIT*	*JAK3*	*NAT2*	*RET*	*SST*	
CBL	*DKK3*	*FLCN*	*KDR*	*NES*	*RNF139*	*STC1*	
CCND1	*DLD*	***FLT3***	*KIT*	***NF1***	*RNF2*	*STK11*	

Genes implicated in NETs are in bold