Phase I Study Evaluating Combination Therapy With the Receptor Tyrosine Kinase Inhibitor PLX3397 and Sirolimus in Patients With Unresectable Sarcoma and Phase II Study in Malignant Peripheral Nerve Sheath Tumors

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INTRODUCTION

Malignant peripheral nerve sheath tumors (MPNST) are soft-tissue tumors with a poor prognosis (1). They are highly aggressive and therapeutically resistant tumors that arise in connective tissue surrounding peripheral nerves. MPNSTs occur in a subset of patients with Neurofibromatosis type 1 (NF1), an autosomal dominant genetic disorder (2, 3). There is a 10% chance that patients with NF1 will develop MPNSTs in their lifetime (4, 5). Despite advances in cancer treatment, MPNSTs typically remain fatal and there is an unmet need to develop new therapeutic strategies in this disease setting.

Growth factor dependent pathways driven specifically by receptor tyrosine kinases (RTKs) have been of particular interest due to their crucial role in tumor progression and survival. Targeting single or multiple RTK pathways using small molecule inhibitors is an attractive therapeutic treatment option for blocking sarcoma cell growth. Such inhibitors have been used in sarcoma patients with some promising results (6, 7). In addition to the RTKs, mTOR (mammalian target of Rapamycin) protein plays a key role in AKT activation and downstream survival signaling. Blocking RTK signaling pathways such as c-Kit and PDGFR using multi-targeted inhibitors like Imatinib mesylate (Imatinib) has been used with some encouraging results in *in vitro* models of MPNSTs (8-10). However, MPNSTs still remain one of the most challenging sarcoma subtypes to treat and novel therapeutic approaches are urgently needed to treat this disease.

MPNSTs have been shown to have gene amplification for receptor tyrosine kinases such as *PDGFRα* as well as *c-Kit* (9, 11). The role of *c-Kit* oncogenic mutations in gastro-intestinal stromal tumors (GIST) is well established (12, 13). MPNSTs contain *PDGFR* and *c-Kit* gene amplifications and mutations within *PDGFRα* have been identified (9). Imatinib, an inhibitor of c-Kit and PDGFR, which is approved for the treatment of GIST, has been shown to be active in patients with plexiform neurofibromas, a slow growing, chemotherapy resistant tumor that develops in patients with NF (14). However, in clinical trials, response rates to imatinib in neurofibromatosis are only in the order of 17%, indicating that alternate signaling pathways must be involved in the growth and development of tumors associated with *NF1* loss (14). Though imatinib has never been formally tested in MPNST, it has been evaluated in patients affected by *NF1* loss who developed GIST. Similar to MPNST patients, the overall prognosis of this patient population is poor, and the response to imatinib in NF1-associated GIST is very low (15).

In a recently reported study, Pexidartinib, also referred to as Turalio®, a tyrosine kinase inhibitor that selectively targets c-FMS and c-KIT receptors, resulted in superior target inhibition and tumor growth suppression when compared to imatinb in *in vitro* and *in vivo* models of MPNST (16). Recently it has also been reported that macrophage infiltration of both mouse and human neurofibromas correlates with disease progression. Macrophages account for almost half of neurofibroma cells. In the Dhh-Cre/Nf1 mouse model of neurofibroma, Turalio® has been shown to cause neurofibroma regressions and to block macrophage infiltration (17). Moreover, addition of TORC1 inhibitor rapamycin to Turalio® resulted in sustained tumor suppression by decreased cell proliferation and macrophage tumor depletion, a marker thought to be critical for tumor progression (18).

These significant pre-clincal findings are the basis of the proposed phase I/II study in patients suffering from MPNST, a disease whose treatment options are limited.

1. STUDY OBJECTIVES

1.1 Primary Objective

Phase 1 study:

a. To determine the recommended phase 2 dose (RP2D) for Turalio® when administered orally in combination with Sirolimus in patients with unresectable or metastatic sarcoma.
b. To evaluate the safety profile of Turalio® with Sirolimus in patients with unresectable or metastatic sarcoma.

Phase 2 study:

a. To determine the preliminary efficacy of Turalio® in combination with Sirolimus in patients with unresectable or metastatic MPNST by determining the median progression free survival.
b. To evaluate the safety profile of Turalio® with Sirolimus at the RP2D in patients with unresectable or metastatic MPNST.

1.2 Secondary Objective

To investigate the objective response rate (ORR) and overall survival (OS) of patients with unresectable malignant peripheral nerve sheath tumors (MPNSTs). Response rate endpoint will be assessed using Response Evaluation Criteria in Solid Tumors (RECIST) 1.1.

1.3 Exploratory Objective

a. Analysis of TORC1/TORC2 signaling pathways and receptor tyrosine kinase activity including c-FMS, c-KIT, AKT, S6, and assessment of macrophage activation.

2. BACKGROUND

Malignant peripheral nerve sheath tumors (MPNSTs) represent up to 10% of adult soft tissue sarcomas. Due to its rarity, few MPNST-specific prospective trials exist, and treatments are largely based on extrapolation from results from other sarcoma subtypes. Since the molecular pathways driving pathogenesis within sarcoma subtypes are distinct, these treatment options are likely suboptimal at best. Targeted therapies that block key pathways known to drive MPNST will likely result in superior tumor responses with limited toxicities.

Growth factor-dependent pathways driven by receptor tyrosine kinases (RTKs) play a crucial role in tumor progression and survival. Targeting RTKs to abrogate sarcoma growth remains an attractive proposition, but the challenge of pursuing the correct combination of pathways remains unsolved. mTOR activation is critical for sarcoma growth but monotherapy has shown little clinical benefit. Advanced sarcoma patients with stable disease when treated with Ridaforolimus, an mTOR inhibitor, resulted in a tumor response of 1.3% (19). This may in part be due to the release of a negative feedback

mechanism identified in several cancers by activation of AKT through RTKs (20). We have reported that this survival mechanism is mediated predominantly through both IGF-1R and PDGFR signaling and that dual inhibition of mTOR and RTKs (including IGF-1R or PDGFR) result in a greater degree of growth inhibition compared to inhibition of either pathway alone (21). We showed that this relationship between mTOR targeting and AKT activation was most striking in synovial cell sarcoma cells where PDGFRα is highly expressed. This would suggest that the interaction between mTOR and AKT is intricately context dependent and based on the RTK expressed by the sarcoma cell. The overall desired effect with these combinations is to inhibit both mTOR and pAKT by targeting the RTK that is activated upon mTOR inhibition. Multiple signaling pathways play a role in MPNST growth, and include c-KIT, PDGFR, mTOR, and c-FMS. c-FMS, a RTK involved in macrophage activation and tumor infiltration has also been shown to play a role in tumor growth and maintenance (22). It is likely that these pathways are not mutually exclusive and function in concert to propagate cancer growth in MPNSTs. Thus developing ways to block these multiple pathways could result in an improved therapeutic effect.

Release of negative feedback mechanism by mTOR inhibition is blocked by Turalio®. To identify sarcoma subtypes that may be susceptible to Turalio®, our group screened multiple cell lines for c-FMS and c-KIT expression. Only cell lines from MPNST and GIST expressed c-KIT. Turalio® showed activity in *in vitro* cell viability assays in GIST and MPNST cell lines and blocked c-KIT and AKT phosphorylation in a dose dependent manner (18). MPNST cells treated with Sirolimus blocked p-S6, but resulted in increased p-KIT, p-AKT, and p-PDGFRβ likely through the release of the negative feedback mechanism (Fig. 1, 18). Co-treatment with Turalio® or imatinib reversed this effect and resulted in increased apoptosis, as indicated by PARP cleavage (Fig. 1, 18). In cell viability assays, Turalio® and Sirolimus had potent activity compared to either agents alone, or with the combination of imatinib and Sirolimus (18).

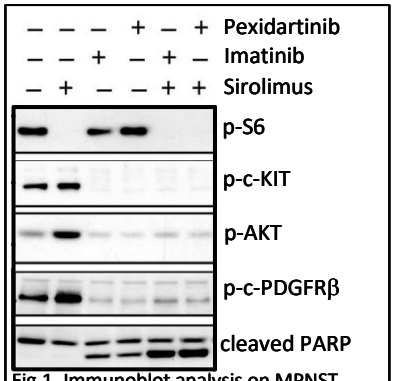

Fig.1. Immunoblot analysis on MPNST cell lysates treated with PLX, Imatinib (imat), and/or sirolimus. [18]

Turalio® and Sirolimus suppress tumor growth in an MPNST xenograft model. Turalio® in combination with Sirolimus resulted in sustained inhibition of target RTKs as well as tumor growth even after treatment was discontinued (Fig. 2, 18). In contrast, combination treatment with imatinib was less effective in controlling tumor growth (18).

Turalio® and Sirolimus block macrophage tumor infiltration. Macrophage infiltration within neurofibromas correlates with disease progression in both animal models and humans. In a mouse neurofiborma model, Turalio® blocked macrophage infiltration and caused neurofibroma regressions (17). Macrophage infiltration, as detected by immunohistochemistry (IHC) using Iba-1 specific antibody, in MPNST xenografts indicated potent inhibition when mice were treated with Turalio® and Sirolimus as compared to treatment with imatinib and/or Sirolimus (Fig. 3A). Furthermore, Turalio® and Sirolimus treatment resulted in sustained inhibition of macrophage tumor infiltration even one week after treatment was discontinued (Fig. 3B, 18).

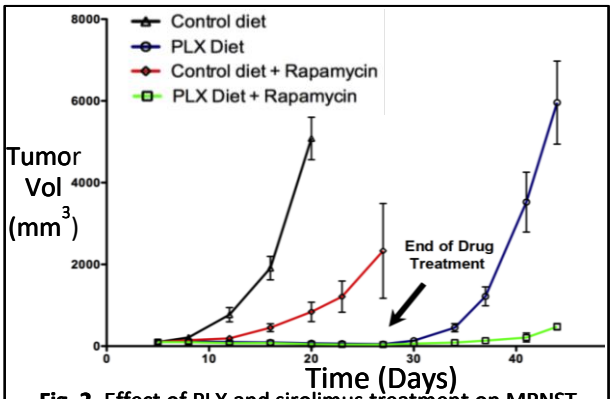

Fig. 2. Effect of PLX and sirolimus treatment on MPNST xenografts. Treatment with PLX alone, or in combination with sirolimus was stopped on day 24 as depicted by the arrow. [18]

Rationale - MPNSTs are fatal tumors that are resistant to chemotherapy and lack treatment options. As mentioned previously, multiple RTK including c-KIT, PDGFR, c-FMS, and mTOR play a critical role in MPNST pathogenesis and survival. However, targeted mono-therapies which include erlotinib, sorafenib, imatinib, and dasatinib have failed to yield an appreciable response in this disease (23, 24). Similarly, mTOR inhibitors have been ineffective in controlling sarcoma growth in clinical studies (23). In order to effectively target MPNSTs, both mTOR and the RTK that it activates as a result of mTOR inhibition, needs to be targeted simultaneously to maximize antineoplastic activity. Since Turalio® does not inhibit the mTOR pathway, we added Sirolimus and tested the combination in *in vitro* and *in vivo* models of MPNST, as previously described.

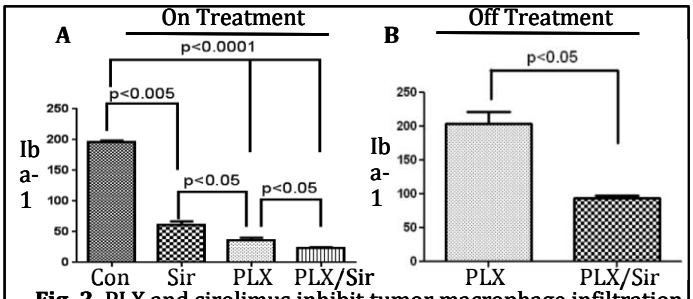

Fig. 3. PLX and sirolimus inhibit tumor macrophage infiltration in Xenograft models. Iba-1 signal on IHC of tumor samples from mice treated with control (Con), sirolimus (Sir), PLX, or the both on-treatment (A) and 1 week off treatment (B). [18]

Our key pre-clinical results indicate that Turalio® and Sirolimus –

- Inhibit MPNST cell viability more potently when compared to imatinib and Sirolimus.
- Inhibit c-KIT, PDGFRβ, AKT, and S6 phosphorylation *in vitro* in addition to c-FMS phosphorylation *in vivo*.
- Result in sustained tumor growth suppression in an MPNST xenograft model.

- Result in sustained inhibition of tumor macrophage infiltration in MPNST xenografts.

Taken collectively, these results provide evidence that combination therapy with Turalio® and Sirolimus has potent activity in MPNST and make testing this combination in patients the next logical step. We propose to translate these positive preclinical findings by testing the combination in a phase I/II clinical study in patients suffering from this disease.

3. INVESTIGATIONAL AGENT

3.1 Pharmacology

Turalio® is an achiral, organic molecule with molecular weight of 418 g/mol and has no functional groups that would be expected to confer high aqueous solubility. The active pharmaceutical ingredient (API) is available as an HCl salt. Turalio® is a selective inhibitor of FMS (CSF1R, the receptor for colony stimulating factor [CSF-1, also known as macrophage-colony stimulating factor, M-CSF], as well as the ligand interleukin 34 [IL-34], KIT (the receptor for stem cell factor, SCF), and oncogenic Flt3 (the receptor for Flt3 ligand) activity intended for oral administration.

3.2 Preclinical Data for Turalio®

Two high-dose GLP 4-week general toxicology studies were conducted with daily oral gavage administration of Turalio® (once daily [QD] in rats and twice daily [BID] in dogs) at doses of 20, 60, and 200 mg/kg/day in rats and 50, 100, and 300 mg/kg/day in dogs, with 16-day (rat) or 14-day (dog) recoveries. Neither a no-effect-level (NOEL) nor a no-adverse-effect-level (NOAEL) of Turalio® could be determined in either species due to toxicity. Significant adverse test article-related observations appear to be related to Turalio®-mediated inhibition of Fms and Kit kinases. Turalio®-related histopathologic observations included testicular spermatagonia reduction, bone marrow hypocellularity, thymic lymphoid reduction, bone hyperostosis and hypertrophy, ovarian follicular degeneration, and liver hepatocellular hypertrophy. All findings were partially or fully reversible.

Two additional GLP toxicology studies at lower dose levels involved daily oral gavage administration of Turalio® HCl for 4 weeks (QD in rats and dogs) at doses of 0.5, 2, and 10 mg/kg/day in rats and 1, 6, and 30 mg/kg/day in dogs, with 8-week recoveries. The NOAELs of Turalio® were determined to be 10 mg/kg/day in rats and 6 mg/kg/day in dogs in these additional studies. All adverse findings were fully reversible, including testicular spermatagonia reduction in dogs.

Two 13-week GLP toxicology studies involved daily oral gavage administration of Turalio® HCl for 13 weeks (QD in rats and dogs), with 8-week recoveries at doses of 0.5, 4 and 20 mg/kg/day in rats and 1, 6, and 30 mg/kg/day in dogs. NOAELs were determined to be 4 mg/kg/day in rats and 6 mg/kg/day in dogs. No new target organ toxicities were seen in either study. In rats, anemia, and bone marrow depletion, and hepatocellular vacuolation associated with increased liver enzymes were seen. In dogs, findings of

reproductive toxicity (spermatogonial reduction) and increased incidence of emesis were seen at the 30 mg/kg dose level, which were reversible.

Potential effects of Turalio® on embryofetal development in rats were assessed at 4, 10, and 40 mg/kg/day. Based on changes in hematology parameters at 40 mg/kg/day in the dams, and fetal external and visceral malformations and skeletal developmental variations (findings primarily related to decreases in ossification) in the fetuses at 40 mg/kg/day, a dose level of 10 mg/kg/day was considered to be the NOAEL.

Turalio® was not mutagenic or clastogenic in the Ames, chromosomal aberration and micronucleus tests, and showed no potential to cause phototoxicity in vitro in the NIH 3T3 fibroblast assay.

3.3 Clinical Data to Date for Turalio®

Turalio® was recently approved by FDA for advanced TGCT. Turalio® can cause hepatic adverse reactions, including liver enzyme abnormalities or mixed and cholestatic hepatotoxicity. Serious and prolonged hepatotoxicity with ductopenia and/or cholestasis has been observed in subjects treated with pexidartinib. In addition, Turalio® is currently being studied in combination with other agents. Additionl details of hepatic adverse reactions, as well as any other adverse reaction are provided in the currently approved Investigators Brochure.

3.4 Other Agent - Sirolimus

Sirolimus is a macrocyclic lactone that binds to FK-506 binding protein 12 and inhibits mammalian target of rapamycin (mTOR) resulting in cell-cycle arrest and apoptosis.

Sirolimus is currently approved as an immunosuppressive agent for organ transplantation and more recently, as a component of cardiac arterial stents because of its potent antiproliferative effects on fibroblasts responsible for restenosis after such a procedure (26) Sirolimus is commonly administered orally on a daily basis, in doses ranging from 2 to 40 mg/day.

Sirolimus has been also evaluated in a phase I clinical trial in advanced cancer patients by Cohen et al. with a similar toxicity profile and pharmacokinetics compared with other mTOR inhibitors (27). In addition, there are several clinical trials evaluating daily Sirolimus in combination with other cancer therapies in advanced solid tumors (28 – 30). Sirolumus at a daily dose of 4 to 6 mg has been utilized in most of these trials with pharmacodynamics suppression of S6K1 observed, with one trial documenting pharmacodynamic efficacy at a daily dose of 5 mg and not at 2mg (30).

4. STUDY DESIGN

4.1 General Design

Phase 1

The objective of this study is to determine an acceptable dose combination of Sirolimus and Turalio®. Patients will be treated with combination therapy at the designated dose levels depicted in Table 1 based on a 28 day cycle. Given that Sirolimus is prescribed up to doses of 6mg daily and dosed by trough levels, and the MTD for Turalio® from clinical studies on solid organ malignancies is 1000mg per day, we will initiate treatment at dose level 2 (2mg of Sirolimus in combination with 800mg of oral Turalio® daily, Table 1). Since these doses are well tolerated individually, we do not anticipate significant toxicities at this combination dose.

Dose Level	**Sirolimus Dose**	Turalio®**Dose**
1	2mg	600mg
2	2mg	800mg
3	2mg	1000mg
4	4mg	1000mg
5	6mg	1000mg

Table 1: Cohort dose levels

Treatment will continue until evidence of unacceptable toxicity, disease progression, or death. The maximum tolerated dose (MTD) combination is defined as the dose combination associated with a target probability of dose limiting toxicity of 0.25. Dose-limiting toxicity (DLT) is defined in section 5.2. The MTD will be estimated using the time to event continual reassessment method (TITE-CRM). The TITE-CRM has the same advantages as the continual reassessment method (CRM) compared to conventional rule-based designs. First, they have been shown to have better performance than the 3+3 design and to treat fewer patients at suboptimal doses. Second, they allow for the specification of a fixed sample size for the trial. Third, they can assign doses after the outcome of every patient is observed using all prior toxicity information in contrast to limiting the toxicity information from the previous assignments. In addition to these advantages, the TITE-CRM compared to the CRM can use the patient's partial information before a complete follow-up is achieved while allowing for a longer toxicity evaluation window beyond the first cycle to account for late onset toxicities. Thus, we can conduct the trial in a continuous fashion without having patients being turned away due to waiting time. While we expect the majority of toxicities to occur in the first cycle of treatment, there is the possibility of toxicities in the second cycle of treatment. Thus, the toxicity evaluation period is 8 weeks. In addition, because the MTD is estimated from partial data, final data may show that patients have been treated with a dose exceeding the MTD. To reduce the potential for

these events, we impose a minimum of 2 weeks of observation between consecutive patients. Patients who discontinue treatment because of progression of disease or death unrelated to treatment without experiencing a DLT will be censored at the time of their last DLT assessment and included up to the point of their last assessment. If the last assessment occurs prior to the 4 week DLT assessment, that is, prior to the end of the first cycle of treatment, the patient will be replenished. To the contrary, if the last assessment occurs 4 weeks or after, that is, after the end of the first cycle of treatment assessment and the patient received at least 80% of the planned doses of Sirolimus and Turalio® the patient will not be replenished. Upon Daiichi Sankyo's request, CUMC will provide updates for the total number of patients enrolled in the study.

The TITE-CRM with an empiric dose-toxicity model, a linear weight function and a normal prior distribution on the parameter with mean 0 and variance of 1.34 is used. The dose-toxicity model is calibrated such that the method will eventually select a dose that yields between 18% and 32% DLT (31, 32). The design does not allow for dose skipping or dose escalation immediately after a DLT is observed (33). The performance of the TITE-CRM is assessed under five potential scenarios with scenarios 3 and 4 being the most likely. The operating characteristics of our design given 2000 simulations are displayed in Table 2. With 18 patients, the design selects the correct MTD with probabilities over 53% for all scenarios. The scenarios were selected to have neighboring doses with DLT rates within 15% of the MTD rate. If the neighboring doses have DLT rates significantly different from the target of 25%, the probability of correct selection will be improved. Likewise, if the neighboring doses have DLT rates very similar to the target rate, the probability of correct selection will worsen.

Doses of Sirolimus Doses of Turalio® Dose Level	2mg 600mg 1	2mg 800mg 2	2mg 1000mg 3	4mg 1000mg 4	6mg 1000mg 5
Scenario 1					
DLT rate	**25%**	**40%**	55%	65%	80%
P(Selection)	**74%**	24%	2%	0%	0%
Scenario 2					
DLT rate	10%	**25%**	40%	55%	65%
P(Selection)	21%	**55%**	22%	2%	0%
Scenario 3					
DLT rate	5%	10%	**25%**	40%	55%
P(Selection)	1%	23%	**53%**	21%	2%
Scenario 4					
DLT rate	2%	5%	10%	**25%**	40%
P(Selection)	0%	2%	26%	**53%**	19%
Scenario 5					

DLT rate	1%	2%	5%	10%	**25%**
P(Selection)	0%	0%	3%	30%	**66%**

Table 2: Probabilities of Selection under 5 different scenarios

Figure 4 below summarizes the observations from a simulated trial using the TITE-CRM method. Trial simulation uses toxicity scenario 4 of Table 2, where the true MTD is dose level 4. The x-axis represents the study time in weeks, the y-axis indicates the dose level assigned to each patient. Each number represents a patient in chronological order. Numbers represent patients enrolled at particular dose level and circled number indicates the toxicity time (if observed) for a particular patient. For example patients 1 and 2 were assigned to dose level 2 (the initial dose) and showed no toxicity in the first 4 weeks. Patients 3 and 4 entered on weeks 6 and 8, and were assigned to dose level 3 and showed no toxicity by study week 10. Due to the lack of DLTs at previous dose levels, patients 5 and 6 started on weeks 10 and 12 and were assigned dose level 4. None of the patients showed toxicity by study week 14 and patient 7 was assigned to dose level 5. Between weeks 14 and 16 one DLT was observed from patient 5. Thus, on week 16 the dose was de-escalated to dose level 3, which was assigned to patient 8. From weeks 18 to 24, Patients 9 to 12 were assigned to dose level 4. Given no new DLTs, the dose was escalated to dose level 5 for patients 13 and 14 enrolled at week 26 and 28. Patient 13 had a DLT by week 30 when patient 15 was enrolled. Thus, patients 15 to 18 enrolled in weeks 30 to 36 were assigned to dose level 4. Patient 14 who was assigned to dose level 5 had a DLT during that period, but the dose was not lowered. By the end of the trial, DLTs were reported from three patients: one at dose level 4 (out of 10), and two (out of 3) at dose level 5. The recommended MTD for this simulated trial was dose level 4. The posterior DLT probability and the 90% probability interval associated with dose level 4 were of 18.6% (6%, 37%).

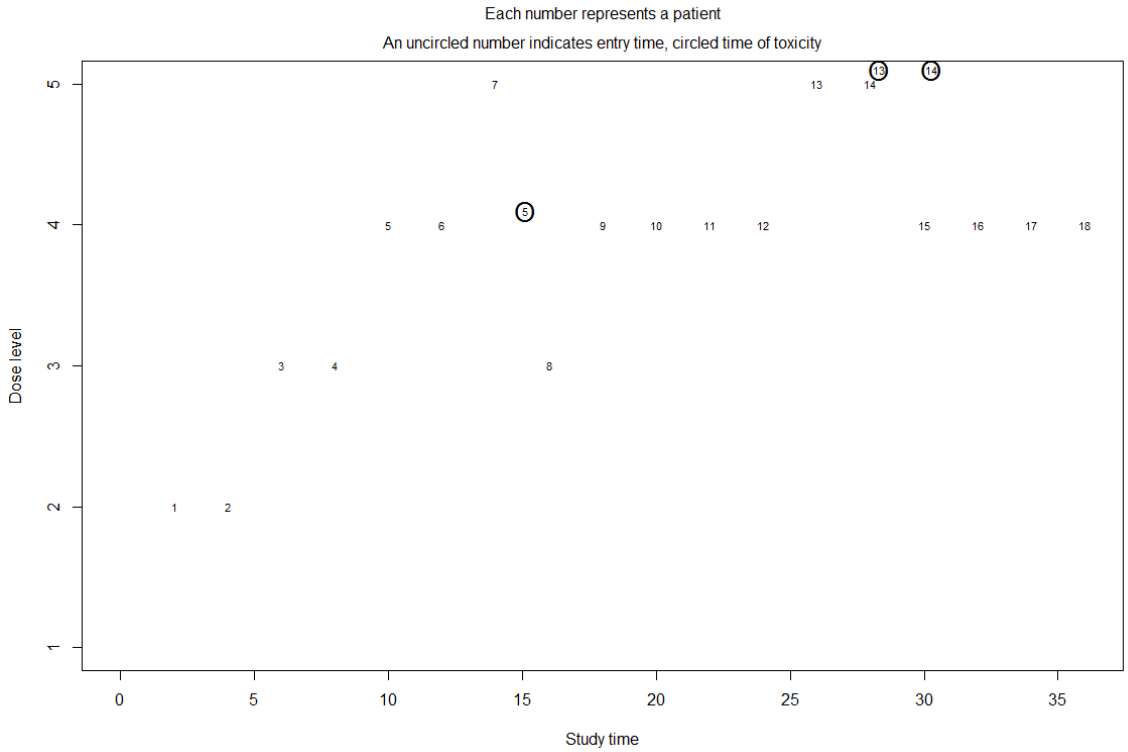

Figure 4: Simulated Trial Using Time to Event Continuous Reassessment Method. Each number represents an enrolled patient while encircled number depicts time at which toxicity experienced by that patient.

Phase 2

The primary outcome measure is progression free survival (PFS) in patients with MPNST at the recommended dose determined from the Phase 1 study, Dose level 3 (1000mg of Turalio® orally total daily in combination with 2mg of Sirolimus orally once daily). Progression free survival is defined as the time from the start of treatment until disease progression or death from any cause. Patients who have not progressed will be censored at the date of their last follow-up. Patients will be evaluated weekly for the first three weeks, at least every other week during the first two cycles and then at the beginning of every subsequent cycle. Tumor response will be assessed by CT scans performed every six weeks for the first six months, every eight weeks for the following six months, and then every 12 weeks thereafter using Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Assuming a median PFS of 6 weeks in patient treated with the standard of care (23), with a sample size of 25, we have 90% power to detect a difference of 12 weeks in median PFS (6 weeks versus 18 weeks) given a 2-sided test with an alpha of 0.05.

Patients will be required to obtain research-specific biopsies as follows:

1) Baseline biopsy of a target lesion
2) Repeat biopsy during last week of cycle 1 for pharmacodynamic analysis
3) Optional biopsies at time of progression

All biopsies will attempt to obtain 4-5 cores. Two of the cores will be processed for formalin fixation and 2-3 will be frozen in liquid nitrogen for exploratory analyses.

4.2 Dose Limiting Toxicities for Phase I

All patients who receive at least one dose of both study drugs will be evaluable for toxicity.

In order for a patient to be evaluable for DLT assessments, the patient must either incur a DLT during the DLT evaluation period or have received at least 80% of the planned doses of Sirolimus and Turalio®. Patients who do not fulfill one of these criteria should be considered non-evaluable for DLT assessment purposes, and be replaced.

A DLT is defined as an AE assessed as being possibly, probably, or definitely related to study drug administration that is:

- Not due to the underlying malignancy;
- Has no clear evidence of an alternative etiology; and
- Meets one of the following CTCAE v.4.03 criteria during the first 56 days of Turalio® and Sirolimus administration:
    - Any Grade ≥ 4 hematologic toxicity except grade 4 lymphopenia
    - Grade 3 neutropenia with fever
    - Grade 3 thrombocytopenia with clinically significant bleeding
    - Any circumstance that results in dose reduction.
    - Any Grade ≥ 3 non-hematologic toxicity except:
        - Nausea, vomiting, and/or diarrhea of Grade 3 severity that resolves within 3 days with optimal prophylaxis and/or treatment.
        - Grade 3 fatigue that resolves to Grade ≤ 2 within 2 days.
        - Grade ≥3 alkaline phosphatase that is related to underlying malignancy (eg, bone metastasis)
        - For hypercholesterolemia, hypertriglyceridemia, and hyperglycemia if unresolved after appropriate treatment with standard of care.

Handling of product specific AESIs (Adverse Events of Special Interest)
All AESIs defined in the study protocol and noted below should be collected and be exchanged between the Parties within the process and timeframes outlined in the Section - Exchange of Safety Information from Investigator/Institution to DSI (please see below):

- AESI
  - Hepatic events (both serious and non-serious) which meet the potential Hy's Law criteria defined as an elevated (ALT and/or AST) ≥3 x ULN with simultaneous total bilirubin ≥2 x ULN, regardless if it is due to disease progression per Investigator assessment, that may occur at different time points during the study conduct, should always be reported to DSI.

4.3 Number of Patients

Approximately 43 evaluable patients are planned for enrollment.

Phase I: 18 patients
Phase II: 25 patients

5. SUBJECT SELECTION AND WITHDRAWAL

5.1 Inclusion Criteria

5.1.1. Disease site/type with pathologic confirmation of diagnosis at participating cancer site.

Note: If recurrence occurred greater than two years after resection, a biopsy to confirm recurrence should be performed and used for confirmation of diagnosis at the participating site.

Phase 1: Advanced, unresectable sarcoma (any subtype, except for patients with pigmented villonodular synovitis for which metastatic disease is required).
Phase 2: Advanced, unresectable malignant peripheral nerve sheath tumors (MPNSTs)

5.1.2. Extent of disease
Unresectable

5.1.3. Allowable prior therapy
Phase 1: Progressed on standard of care therapy, if one is available.

Phase 2: MPNST with 0-3 prior cytotoxic systemic therapies (no prior radiotherapy is necessary).

5.1.4. ECOG performance status
0, 1, or 2

5.1.5. Age greater or equal to 18 years. Because no dosing or adverse event data are currently available on the use of Turalio® in combination with Sirolimus in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

5.1.6. Presence of measurable lesions by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1

5.1.7. Allowable laboratory values with date range

- Labs should be done within 14 days of starting Cycle 1 Day 1 treatment
- ANC $\geq$1.5 X 10^9/L, Hgb >10 g/dL, and platelet count $\geq$100 X 10^9/L
- AST/ALT/ALP/GGT $\leq$ upper limit of normal (ULN)
- Total bilirubin and direct bilirubin $\leq$ ULN
- Patients with hyperbilirubinemia clinically consistent with an inherited disorder of bilirubin metabolism (e.g., Gilbert syndrome) will be eligible at the discretion of the principal investigator if total bilirubin is $\leq$ 1.5 X ULN
- Albumin $\geq$ 3.0g/dL.
- Creatinine $\leq$ 1.5 X ULN or calculated creatinine clearance (CrCl) > 60 mL/min using the Cockcroft-Gault formula less than eight days prior to start of treatment.

5.1.8. Women of child-bearing potential must have a negative serum pregnancy test at screening and must agree to use an effective form of contraception from the time of the negative pregnancy test and for a minimum of 3 months after the last dose of study drug. Effective forms of contraception include abstinence, hormonal contraceptive (injectable or implantable) in conjunction with a barrier method, a double barrier method, diaphragm or cerival cap with a cream or gel that kills sperm, bilartreal tubal occlusion, vasectomy, or an intrauterine device (IUD). Women of non-child-bearing potential must have been postmenopausal for $\geq$ 1 year or surgically sterile. The effects of Turalio® and Sirolimus on the developing human fetus are unknown. For this reason women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation and 3 months after completion of Turalio® and Sirolimus administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, sshe should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to

the study, for the duration of study participation, and 3 months after completion of Turalio® and Sirolimus administration.

5.1.9. Fertile men must agree to use an effective method of birth control during the study and for up to 3 months after the last dose of study drug.

5.1.10. Willingness and ability to provide written informed consent prior to any study-related procedures and to comply with all study requirements.

5.1.11. For phase 2 specifically, agree to pre- and on-treatment tumor biopsies. If subject is unable to undergo paired research biopsies safely, subject may enroll onto the study without a research biopsy(ies) pending number of evaluable paired biopsies available at the discretion of the Principal Investigator.

5.1.12. Prior treatment-related AEs must be ≤ grade 1 (CTCAE v4.0), except alopecia, at time of initiating study drug.

5.2 Exclusion Criteria

5.2.1 Prior treatments –
i. Patients who have had systemic cytotoxic therapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) or targeted therapy with small molecule inhibitors within 2 weeks prior to entering the study.
ii. Patients who have not recovered from adverse events (≤ grade 1; except alopecia) due to agents administered more than 4 weeks earlier.
iii. Patients need to be free of adverse effects (≤ grade 1; except alopecia) from prior treatments for at least 14 days from cycle 1 day 1 of Turalio® and Sirolimus.

5.2.2 Patients who are receiving any other investigational agents concurrently.

5.2.3 Concomitant treatment with other anti-neoplastic agents (hormonal therapy acceptable).

5.2.4 Patients with symptomatic brain metastases. Subjects with untreated brain metastasis ≤ 1 cm can be considered eligible if deemed asymptomatic by the investigator upon consultation with the medical monitor and do not require immediate radiation or steroids. Subjects with brain metastasis that is treated and stable for 1 month may be considered eligible if they are asymptomatic and on stable dose of steroids or if they do not require steroids following successful local therapy.

5.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Turalio® or Sirolimus.

5.2.6 For Phase 2 - Prior exposure to a receptor tyrosine kinase or mammalian target of Rapamycin inhibitor.

5.2.7 Pregnant women are excluded from this study because Turalio® and Sirolimus are agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Turalio® and Sirolimus, breastfeeding should be discontinued if the mother is treated with Turalio® and Sirolimus.

5.2.8 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, active liver disease, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

5.2.9 Active secondary malignancy unless the malignancy is not expected to interfere with the evaluation of safety and is approved by the Sponsor. Examples of the latter include basal or squamous cell carcinoma of the skin, in-situ carcinoma of the cervix, and isolated elevation of prostate-specific antigen. Subjects with a completely treated prior malignancy and no evidence of disease for ≥ 2 years are eligible.

5.2.10 Major surgical procedure or significant traumatic injury within 14 days of initiating study drug or anticipation of the need for major surgery during the study.

5.2.11 Previous radiotherapy to 25% or more of the bone marrow and/or radiation therapy within 28 days prior to study entry.

5.2.12 Inability to swallow capsules, or refractory nausea and vomiting, malabsorption, an external biliary shunt, or significant bowel resection that would preclude adequate absorption.

5.2.13 Congestive heart failure (CHF) New York (NY) Heart Association class III or IV; unstable coronary artery disease (myocardial infarction [MI] more than 6 months prior to study entry is permitted); or serious cardiac arrhythmia.

5.2.14 Baseline QTcF ≥ 450 ms (males) or ≥ 470 ms (females)

5.2.15 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with PLX3997. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Similarly, patients with chronic or acute hepatitis C virus (HCV) or hepatitis B virus (HBV) infection are also ineligible. Prior hepatitis infection that has been treated with highly effective therapy with

no evidence of residual infection and with normal liver function (ALT, AST, total and direct bilirubin ≤ ULN) is allowed.

5.2.16 Of the five major CYP isoforms, 3A4 (BFC) may be involved in Phase I metabolism of Turalio®, with possibly CYP1A2 playing a minor role. Until information regarding exposure toxicity and exposure-response relationships are available with Turalio®, concomitant strong CYP3A4 inhibitors and inducers are not permitted in the event they alter the systemic exposure to Turalio® (see Attachment 1 for a list of common CYP3A4 inhibitors and inducers). These include anticonvulsants, mycin antimicrobials, and antiretrovirals. Some common examples include inhibitors such as erythromycin, fluoxetine, gemfibrozil, and inducers such as rifampicin, carbamazepine, phenytoin, efavirenz, and nevirapine.

If concomitant use with a strong CYP3A inhibitor, UGT inhibitor, or acid reducing agent cannot be avoided, see below for guidance taken from the approved USPI.

Avoid concomitant use of Turalio®with strong CYP3A inhibitors or UGT inhibitors during treatment with Turalio®. If concomitant use with a strong CYP3A inhibitor or UGT inhibitor cannot be avoided, reduce the Turalio® dose according to the recommendations in table below. If concomitant use of a strong CYP3A inhibitor or UGT inhibitor is discontinued, increase the Turalio® dose (after 3 plasma half-lives of the strong CYP3A inhibitor or UGT inhibitor) to the dose that was used before starting the inhibitor [see Clinical Pharmacology (12.3)].

Recommended Dosage Reductions for Turalio®for Concomitant Use of Strong CYP3A Inhibitors or UGT Inhibitors		
Planned Total Daily Dose	**Modified Total Daily Dose**	**Administration of Modified Total Daily Dose**
800 mg	400 mg	200 mg twice daily
600 mg	400 mg	200 mg twice daily
400 mg	200 mg	200 mg once daily

* Planned total daily dose refers to recommended dose reductions for Turalio® for adverse reactions based on dosing recommendations in Section 8.2 Dose Modificiations

Avoid the concomitant use of proton pump inhibitors (PPI) while taking Turalio®. As an alternative to a PPI, administer Turalio® 2 hours before or 2 hours after taking a locally-acting antacid, or if using a histamine 2 (H2)-

receptor antagonist, administer Turalio® at least 2 hours before or 10 hours after taking an H2-receptor antagonist.

Concomitant treatment is permitted if the medication is not expected to interfere with the evaluation of safety or efficacy of the study drug. During the study, if the use of any concomitant treatment becomes necessary (e.g., for treatment of an adverse event), the treatment must be recorded on the eCRF, including the reason for treatment, generic name of the drug, dosage, route, and start and stop dates of administration.

Sirolimus undergoes extensive hepatic and intestinal metabolism via CYP3A4 and CYP3A5, as well as excretion by P-glycoprotein. Strong CYP3A inhibitors such as ketoconazole or grapefruit juice are not permitted. Patients should be monitored for supratherapeutic toxic levels of Sirolimus and Turalio®. As bone marrow suppression including anemia, neutropenia, and thrombocytopenia have been reported in patients receiving Sirolimus monotherapy, these adverse effects may be exacerbated in combination with Turalio® for which patients will be closely monitored.

5.2.17. Any patients on warfarin therapy.

5.2.18. Hepatobiliary diseases including biliary tract diseases, autoimmune hepatitis, inflammation, fibrosis, cirrhosis of liver caused by viral, alcohol, or genetic reasons. Gilbert's disease is allowed if TBil is $\leq 1.5 \times$ ULN.

If concomitant use with a strong CYP3A inhibitor, UGT inhibitor, or acid reducing agent cannot be avoided, see below for guidance taken from the approved USPI

Recommended Dosage Reductions for Turalio®for Concomitant Use of Strong CYP3A Inhibitors or UGT Inhibitors		
Planned Total Daily Dose	**Modified Total Daily Dose**	**Administration of Modified Total Daily Dose**
800 mg	400 mg	200 mg twice daily
600 mg	400 mg	200 mg twice daily
400 mg	200 mg	200 mg once daily

* Planned total daily dose refers to recommended dose reductions for Turalio® for adverse reactions based on dosing recommendations in Section 8.2 Dose Modificiations

Avoid the concomitant use of proton pump inhibitors (PPI) while taking Turalio®. As an alternative to a PPI, administer Turalio® 2 hours before or 2 hours after taking a locally-acting antacid, or if using a histamine 2 (H2)-receptor antagonist, administer Turalio® at least 2 hours before or 10 hours after taking an H2-receptor antagonist.

7.3 Duration of Therapy

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

- Disease progression
- Therapy may be permitted beyond progression if patient is deriving clinical benefit after initial tumor growth or ambiguity regarding the existence of disease progression in consultation with the principle investigator, sponsor, and CUMC IRB. If subsequent imaging confirms tumor growth, progression will be back dated to the prior scan. If patient agrees to treatment beyond progression, the patient will be required to sign a “Treatment Beyond Progression” informed consent form.
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse events(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
- Major protocol violation

7.4 Duration of Follow Up

Patients will be followed after completion or removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Thereafter, patients will be contacted by phone every 3 months during year 1 and every 6 months thereafter to obtain information about subsequent treatment(s) and survival status.

7.5 Criteria for Removal from Study

The reasons a patient may discontinue or be withdrawn from the study include, but are not limited to, adverse event, clinically significant disease progression, patient request, investigator decision, protocol violation, patient noncompliance, and study termination by the Sponsor or institutional review board (IRB)/independent ethics committee (IEC). When a patient discontinues or is withdrawn, the investigator will notify the Sponsor and should perform the procedures indicated in the End of Study column in the Schedule of Events within 28 days after discontinuation of study drug and before initiation of any new anti-cancer therapy. Follow-up information will be obtained for patients who discontinue their participation in or are withdrawn from the study.

Patients withdrawn from the study for reasons other than toxicity or clinically significant disease progression (e.g., protocol violation or noncompliance) may be replaced at the discretion of the medical monitor and the investigator. Study drug administration may be discontinued for an adverse event or at the discretion of the investigator.

The consequence of withdrawal of all consent by a patient will be that no new information will be collected from that patient and added to the existing data or any database. However, every effort will be made to follow all patients for safety.

8. DOSING DELAYS/DOSE MODIFICATIONS

8.1 Phase 1 Dose Modification

All patients may have dose modifications at any time. During Cycle 1 in the phase 1 portion, however, the DLT must be recorded for safety purposes prior to dose reduction. Patients may have drug held for any reason for up to 14 days before discontinuing study treatment. Refer to Tables 4 - 7 below for specific dose modification instructions.

Patients will undergo therapeutic monitoring with trough levels of Sirolimus on day 2 of cycle 1 before receiving daily dose of Sirolimus on day 2 (+2 day window)**,** day 8, day 15, and day 22 of Cycle 1, on day 1 of each subsequent cycle, unless clinically indicated, and during the End-of-Study visit. Trough levels may be measured at other time points as clinically indicated. As levels >18.0μg/L are more likely to be associated with an adverse events, dosing of Sirolimus will be held and/or reduced in the case of trough levels >18.0μg/L as indicated in the dose modification table (Table 4). Deviation of this dose modification can be done at the discretion of the treating investigator in discussion with the study principal investigator.

Sirolimus Monitoring and Dose Modification	
Trough level > 18µg/L	First Event Hold Sirolimus for three days and re-measure trough level on fourth day (+2 day window): • If ≤ 18µg/L, reinstitute drug at original dose • If > 18µg/L hold drug for additional three days and re-measure trough level on fourth day (+2 day window). Repeat dose interruption for additional three days and re-measure trough level the following day until trough level ≤ 18µg/L, at which time re-institute Sirolimus at next lower dose. Dose reduction constitutes a DLT. Second Event Hold Sirolimus for three days and re-measure trough level on fourth day (+2 day window): • If ≤ 18µg/L, reinstitute Sirolimus at next lower dose. Dose reduction constitutes a DLT. • If > 18µg/L hold Sirolimus for additional three days and re-measure trough level on fourth day (+2 day window). Repeat dose interruption for additional three days and re-measure trough level the following day until trough level ≤ 18µg/L, at which time re-institute Sirolimus at next lower dose. Dose reduction constitutes a DLT.

Table 4. Sirolimus level dose modification recommendations

8.1.1 Dose Modification for Hepatotoxicity, Nausea/Emesis, and Hyperglycemia

8.1.1.1 **Hepatotoxicity –** Turalio® and Sirolimus should be discontinued if hepatotoxicity is observed as detailed in Table 5.

Reductions or interruptions of the Turalio® dose for toxicity may take place at any time during the study according to the guidelines in Tables 5 and 6. Dose reduction/interruption guidelines for hematologic and nonhematologic treatment-related TEAEs are based on severity. Dose interruptions can be implemented at the discretion of the treating Investigator to manage intolerable or clinically significant toxicity. If a dose interruption is required, study assessments should be performed as scheduled, irrespective of the study drug delay, with the exception of PK assessments, which should be deferred until treatment is resumed. Interruptions due to toxicity lasting >14 d require treatment discontinuation unless the medical monitor approves continuation.

Table 6: Additional Liver Evaluation

Evaluation	Comments
Increase frequency of testing liver chemistries to twice per wk, including INR, PT, total and indirect bilirubin, and albumin and continue until liver chemistries have stabilized, and then reduce to weekly until liver chemistries return to normal or baseline.	Turalio® may be started after liver function tests recover to Grade 0 to 1 or baseline level, and in consultation with the Principal Investigator.
Detailed history focusing on medications in a weekly clinic visit and substances used: alcohol, change in medication dosages, new medications added, attention to use of acetaminophen, OTC medication use, and recreational drug use. Check for change in diet or use of dietary supplements, with particular attention to dose and duration of any herbal product.	Suspect medications will be discontinued or substituted for if possible.
Detailed medical history and physical examination seeking new abnormalities in a weekly clinic visits. Frequency of visits as detailed in Table 5.	Evaluate abnormalities found.
Full serological evaluation for hepatitis A, B, C, and E (IgG and IgM). Check for autoimmune hepatitis with serological laboratory studies.	If viral hepatitis or autoimmune hepatitis suggested, have patient evaluated by hepatologist.
Liver ultrasound performed to evaluate liver and biliary tree.	Evaluate any abnormalities found.
Check history for exposure to chemical agents.	Remove chemical exposure and have patient seen by hepatologist.
Obtain hepatology consult if liver function continues to rise beyond 14 d.	)Daiichi Sankyo Safety Lead, Jason Jiang at jjiang@dsi.com
We request that cases be discussed with the Principal Investigator as defined in the protocol whenever investigational product is being held for liver function test abnormality.	

Ig = Immunoglobulin; INR = international normalized ratio; OTC = over-the-counter.

For suspected cases of cholestatic liver injury (eg, aminotransferase increase concurrent with hyperbilirubinemia, or liver biopsy suggesting cholestasis and/or ductopenia), patients will be followed to assess long-term outcome. Additional diagnostic and follow-up procedures might be implemented as appropriate to fully assess the event.

8.1.1.2 **Nausea and Vomiting** – Turalio® may be associated with nausea and/or emesis that can arise early in the course of therapy. Sirolimus may also contribute to nausea and emesis and may be held as well at the discretion of the treating physician.

Nausea and Vomiting	
Grade 3	Hold Turalio® dosing until improved to grade 1 or baseline. If delay <48 hours from start of maximal supportive treatment, then restart at cohort with next lower dose for Turalio®. If delay for ≥48 hours despite maximal supportive treatment, discontinue study treatment.
Grade 4	If any grade 4 toxicity despite maximal supportive treatment, discontinue study treatment.

Table 7. Nausea and emesis dose modification recommendations

8.1.1.3 **Hyperglycemia** – immunosuppressive agents, including Sirolimus have been associated with hyperglycemia that responds to treatment.

Hyperglycemia – Assessed by Fasting Blood Glucose and NOT CTCAE grades	
Grade 1 = 140-199 mg/dL	Maintain Turalio® and Sirolimus dosing and institute diet modifications. Address any underlying contributing factors (infection, steroids) and consider initiating blood glucose lowering therapy.
Grade 2 = 200-249 mg/dL	Maintain Turalio® and Sirolimus dosing and initiate blood glucose lowering therapy.
Grade 3 = 250-399 mg/dL	Hold only Sirolimus dosing until improved to ≤ Grade 1. • If ≤7 days, dose reduction is at discretion of treating physician. • If >7 days, dose reduce to cohort with next lower dose of Sirolimus.
Grade 4 = 400 mg/dL or higher OR Any hyperglycemia leading to diabetic ketoacidosis, hyperosmolar nonketotic	Discontinue from study drug treatment.

coma, or requiring IV insulin infusion.	

Table 8. Hyperglycemia dose modification recommendations

Toxicities that occur outside the DLT window will be taken into consideration when determining the RP2D. Reduction/interruption of dosing for AEs may take place at any time. Below are guidelines for dosage modification for Turalio®–related toxicities as well as guidelines for their management. Dose reductions should occur in increments of 200 mg/day, depending on the toxicity grade, as noted in Table 9. These parameters are only a guide and are not intended to supersede the clinical judgment of the treating physician. All adjustments should be made in consultation with the principal investigator. Dosing interruptions longer than three weeks for any reason should generally result in discontinuation from the study, unless the patient has demonstrated a clinical benefit from therapy and would like to continue dosing with study drug after discussion between the investigator and the Sponsor.

Toxicity Grade (CTCAE v4)	**Turalio® and Sirolimus dose changes during current treatment period**	**Dose adjustments for resumption of treatment**
Hematologic Toxicity		
Any Grade 4, or Grade 3 neutropenia with fever, or thrombocytopenia with bleeding and except for Grade 4 lymphopenia		
1st Appearance	Interrupt until ANC recovers to ≥ $1X10^9$/L; growth factor support permitted	Dose reduce one dose level. If dose level 1 at time of AE, discontinue both drugs.
2nd Appearance	Interrupt until ANC recovers to ≥ $1X10^9$/L; provide growth factor support	Dose reduce one additional dose level. If dose level 1 at time of AE, discontinue both drugs.
3rd Appearance	Interrupt until ANC recovers to ≥ $1X10^9$/L; provide growth factor support	Dose reduce one additional dose level. If dose level 1 at time of AE, discontinue both drugs.
4th Appearance	Discontinue Permanently	N/A

Non-Hematologic Related Grade 3 or Grade 4 not related to abnormal liver function (start symptomatic treatment when possible).		
1st Appearance	Interrupt until recovered (grade 0-1) and provide supportive management.	Dose reduce one dose level. If dose level 1 at time of AE, discontinue both drugs.
2nd Appearance	Interrupt until recovered (grade 0-1) and provide supportive management	Dose reduce one additional dose level. If dose level 1 at time of AE, discontinue both drugs.
3rd Appearance	Interrupt until recovered (grade 0-1) and provide supportive management	Dose reduce one additional dose level. If dose level 1 at time of AE, discontinue both drugs.
4th Appearance	Discontinue Permanently	N/A

Table 9: Recommended Turalio® and Sirolimus Dose Modifications

Dose interruptions for Grade 2 non-hematologic toxicity for up to 1 week can be implemented at the discretion of the treating physician to manage intolerable or clinically significant toxicity. No dose reduction is required when resuming treatment.

8.2 Phase 2 Dose Modification

Patients may have dose modifications at any time. Patients may have either drug held for any reason for up to 21 days before discontinuing study treatment. Refer to Tables 10 – 14 below for specific dose modification instructions.

8.2.1 Dose Modification for Sirolimus

Patients will undergo therapeutic monitoring with trough levels of Sirolimus on day 2 of cycle 1 before receiving daily dose of Sirolimus on day 2 (+2 day window)**,** day 8, day 15, and day 22 of Cycle 1, on day 1 of each subsequent cycle, unless clinically indicated, and during the End-of-Study visit. Trough levels may be measured at other time points as clinically indicated. As levels >18.0μg/L are more likely to be associated with an adverse event, dosing of Sirolimus will be held and/or reduced in the case of trough levels >18.0μg/L as indicated in the dose modification table (Table 10). Deviation of this dose modification can be done at the discretion of the treating investigator in discussion with the study principal investigator.

Sirolimus Monitoring and Dose Modification	
Trough level > 18µg/L	**First Event** Hold Sirolimus for three days and re-measure trough level on fourth day (+2 day window): • If ≤ 18µg/L, reinstitute drug at 2 mg orally daily. • If > 18µg/L hold drug for additional three days and re-measure trough level on fourth day (+2 day window). Continue dose hold for up to 21 days total, re-measuring trough levels every fourth day (+2 day window) until trough level ≤ 18µg/L, at which time re-institute Sirolimus at 1mg orally daily. **Second Event** Hold Sirolimus for three days and re-measure trough level on fourth day (+2 day window): • If ≤ 18µg/L, reinstitute Sirolimus at 1mg orally daily. • If > 18µg/L hold Sirolimus for additional three days and re-measure trough level on fourth day (+2 day window). Continue dose hold for up to 21 days total, re-measuring trough levels every fourth day (+2 day window) until trough level ≤ 18µg/L, at which time re-institute Sirolimus at 0.5mg daily. **For Third Event or higher,** hold Sirolimus for three days and re-measure trough level on fourth day (+2 day window). If ⩽ 18µg/L, reinstitute Sirolimus at 0.5mg daily. **If Sirolimus trough level is > 18µg/L at the 0.5mg orally daily dose, the patient should discontinue study treatment.**

Table 10. Sirolimus level dose modification recommendations

8.2.2 Dose Modification for Hepatotoxicity, Nausea/Emesis, and Hyperglycemia

Hepatotoxicity – Turalio® and Sirolimus should be discontinued if hepatotoxicity is observed as detailed in Tables 5 and 6.

Reductions or interruptions of the Turalio® dose for toxicity may take place at any time during the study according to the guidelines in Tables 5 and 6. Dose reduction/interruption guidelines for hematologic and nonhematologic treatment-related TEAEs are based on severity. Dose interruptions can be implemented at the discretion of the treating Investigator to manage intolerable or clinically significant toxicity. Interruptions due to toxicity lasting > 21 d require treatment discontinuation unless the medical monitor approves continuation.

Nausea and Vomiting – Turalio® may be associated with nausea and/or emesis that can arise early in the course of therapy. Sirolimus may also contribute to nausea and emesis and may be held as well at the discretion of the treating physician.

Nausea and Vomiting	
Grade 3	Hold Turalio® and Sirolimus dosing until improved to grade 1 or baseline. If improvement of symptoms in less than 48 hours from start of maximal supportive treatment, then restart at 400mg orally in AM and 400mg orally in PM daily. If symptoms do not improve within 48 hours despite maximal supportive treatment, hold Turalio® and Sirolimus, and consult with Principal Investigator.
Grade 4	If grade 4 toxicity despite maximal supportive treatment, hold Turalio® and Sirolimus, and consult with Principal Investigator.

Table 12. Nausea and emesis dose modification recommendations

Hyperglycemia – immunosuppressive agents, including Sirolimus have been associated with hyperglycemia that responds to treatment.

Hyperglycemia – Assessed by Fasting Blood Glucose	
Grade 1 = > ULN - 160 mg/dL	Maintain Turalio® and Sirolimus dosing and institute diet modifications. Address any underlying contributing factors (infection, steroids) and consider initiating blood glucose lowering therapy.
Grade 2 = > 160 - 250 mg/dL	Maintain Turalio® and Sirolimus dosing and initiate blood glucose lowering therapy.
Grade 3 = > 250-500 mg/dL OR Hospitalization indicated.	Hold only Sirolimus and initiate blood glucose lowering therapy. Reinstitute Sirolimus if ≤ Grade 2. • If ≤7 days, dose reduction is at discretion of treating physician. • If >7 days, dose reduce to 1mg orally daily if study related.
Grade 4 = > 500 mg/dL OR Life threatening consequences.	Discontinue from study drug treatment if AE considered study related.

Table 13. Hyperglycemia dose modification recommendations

8.2.3 Dose Modification for Non-Hematologic Toxicity

Reduction/interruption of dosing for other AEs may take place at any time. Below are guidelines for dosage modification for Turalio®–related toxicities as well as guidelines for their management. Dose reductions should depend on the toxicity grade, as noted in Table 14. These parameters are only a guide and are not intended to supersede the clinical judgment of the treating physician. All adjustments should be made in consultation with the principal investigator. **Dosing holds longer than 21 days for any reason should generally result in discontinuation from the study**, unless the patient has demonstrated a clinical benefit from therapy and would like to continue dosing with study drug after discussion between the investigator and the Principal investigator.

Toxicity Grade (CTCAE v4)	**Turalio® and Sirolimus dose changes during current treatment period**	**Dose adjustments for resumption of treatment**
Hematologic Toxicity		
Any Grade 4, or Grade 3, neutropenia with fever, or thrombocytopenia with bleeding, except for Grade 4 lymphopenia		
1st Appearance	Interrupt until ANC recovers to ≥ $1X10^9$/L; growth factor support permitted	Dose reduce Turalio® to 400mg orally in AM and 400mg orally in PM daily.
2nd Appearance	Interrupt until ANC recovers to ≥ $1X10^9$/L; provide growth factor support	If Turalio® total daily dose at 800mg orally, dose reduce Turalio® to 200mg in AM and 400mg in PM. If Sirolimus 2.0mg, decreased to 1.0mg.
3rd Appearance	Interrupt until ANC recovers to ≥ $1X10^9$/L; provide growth factor support	Discontinue study treatments unless subject deriving clinical benefit. Consult with Principal investigator and consider decreasing Turalio® to 200mg twice daily and decrease Sirolimus to half the dose unless at 0.5mg.
4th Appearance	Discontinue Permanently	N/A
Non-Hematologic Related Grade 3 or Grade 4 not related to abnormal liver function (start symptomatic treatment when possible).		
1st Appearance	Interrupt until recovered (grade 0-1) and provide supportive management.	Dose reduce Turalio® to 400mg orally in AM and 400mg orally in PM daily and Sirolimus to 1.0mg orally in AM.
2nd Appearance	Interrupt until recovered (grade 0-1) and provide supportive management	Continue Turalio® at 400mg orally in AM and 400mg orally in PM daily and dose reduce Sirolimus to 0.5mg orally in AM.

3rd Appearance	Interrupt until recovered (grade 0-1) and provide supportive management	Discontinue study treatments.

Table 14: Recommended Turalio® and Sirolimus Dose Modifications

Dose interruptions for Grade 2 non-hematologic toxicity for up to 1 week can be implemented at the discretion of the treating physician to manage intolerable or clinically significant toxicity. No dose reduction is required when resuming treatment (unless hepatotoxicity).

9. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

9.1 Adverse Events Most Likely Related to Turalio® and Sirolimus

9.1.1 Turalio®

For a complete adverse event profile, please refer to IB version 12. Side effects that may be seen with Turalio® given by itself or in combination with other anti-cancer treatments.

Common side effects (≥10% by frequency) associated with the use of Turalio® include the following:

- Nausea
- Vomiting
- Constipation
- Abdominal pain
- Abnormal, altered, or loss of sense of taste
- Decreased appetite
- Rash
- Pruritus (itching of skin)
- Hair color changes (to white or gray; original color usually returns after stopping study drug)
- Increases in liver blood tests
- Fatigue or tiredness
- Headache (including migraine)
- Hypertension (high blood pressure)
- Edema (swelling of tissues in limbs or face)
- Eye edema (swelling in or around the eyes)

Less common side effects (less than 10%) associated with the use of Turalio® include the following:

- Hepatotoxicity (liver injury), which may be serious and fatal
- Cognitive disorder (includes memory impairment, amnesia, cognitive disorder, attention deficit hyperactivity disorder, disturbance in attention)
- Dry mouth
- Neuropathy (numbness or weakness)
- Stomatitis (inflammation inside of mouth, usually a small sore or ulcer)
- Alopecia (loss of hair)
- Skin color changes
- Pyrexia (fever)
- Vision changes (including blurred vision, sensitivity to light, double vision, and reduced visual clarity)
- Increase in bilirubin (produced when the liver breaks down red blood cells)
- Decrease in white blood cell counts (which could include one or more of the following: white blood cell counts, lymphocytes, neutrophils, leukocytes; this may lead to increased risk of infection)
- High cholesterol

Turalio® **may cause liver injury, also known as hepatotoxicity. Such an injury can be severe and prolonged despite stopping of study medication. The liver injury may be fatal.** Should your liver tests become abnormal you may be required to have additional monitoring and evaluation, which may include a liver biopsy. Treatment may include blood filtering to remove waste products of the liver. One patient required a liver transplant. One patient died before the liver injury got better. Taking Turalio® together with other medications may increase the risk of severe liver injury.

Turalio® . This type of rash may sometimes include fever and inflammation of internal organs.

Animals treated with Turalio® showed dec rease in the pumping action (contraction) of the heart. There have been two reports of reduced ejection fraction (the amount of blood pumped by your heart with each beat) in patients receiving Turalio® at a dose of 3000 mg/day, a much higher dose than you will receive. The role of Turalio® in these events is undetermined at this time.

The severity of the side effects listed above could range from mild to severe or even life-threatening. In addition, there may be other risks or side effects from Turalio® that are not listed above or unknown at this time. It is also possible to experience a serious allergic reaction, which could become life-threatening or fatal. Symptoms of an allergic reaction include rash, hives, itching, swelling of the mouth, face, lips or tongue, dizziness, low blood pressure, tightness in the chest, or trouble breathing.

Reproductive Risks
If a patient or partner becomes pregnant or fathers a child while in this research study or 90 days following study completion, they must notify the study doctor immediately. The risk of taking this product in pregnant women has not been fully determined from either human studies or animal studies. There is no clinical experience in humans with Turalio® in pregnant or lactating women.

Turalio® can cause fetal defects in rodents and rabbits, and should be considered potentially harmful to the human fetus, with the potential to cause fetal malformations. As a result, Turalio® should not be administered to pregnant women or lactating women who are breastfeeding. Patients and partners should avoid becoming pregnant over the course of this study. Becoming pregnant while participating in the study will expose the patient or partner to a potential loss of the pregnancy, or other unknown effects on an unborn child such as, but not limited to, birth defects and premature delivery. They will be withdrawn from the study if they become pregnant or have a positive pregnancy test. If they become pregnant, they will be followed until the time the pregnancy is completed or terminated in order to collect information about the pregnancy, determine the outcome of the pregnancy, childbirth and the health of the baby (first well-baby visit). If the pregnancy continues to term, the outcome (health of infant) must also be reported. All pregnancy/lactation reports, including pregnancy reports in which the patient or a partner of a patient may have been exposed to the Product(s) via either maternal or paternal exposure, lactation reports, unintended pregnancy, with or without an associated adverse event, where the fetus could have been exposed to the study drug shall be collected.

In animal studies, Turalio® caused a decrease in sperm count and changes in the ovaries which were reversible at the end of the study. Turalio® may therefore affect fertility in both men and women. If you are a male, you may ask your study doctor about sperm banking.

9.1.2 **Sirolimus**

Sirolimus is approved for prophylaxis of organ rejection in patients receiving renal transplants. Known toxicities include risk of opportunistic infections, interstitial lung disease, hyperlipidemia, risk of malignancy (including lymphoma), proteinuria, mucositis, nausea, emesis, diarrhea, edema, elevated liver enzymes, and bone marrow suppression including anemia, neutropenia, and thrombocytopenia.

Side effects that may be seen with Sirolimus given by itself or in combination with other anti-cancer treatments.

Some common side effects (≥30% by frequency) seen with the use of Sirolimus in patients with cancer include the following:

- Peripheral edema
- Hypertriglyceridemia
- Hypercholesterolemia
- Increased creatinine (kidney dysfunction)
- Hypertension (an increase in blood pressure)
- Nausea
- Abdominal pain
- Diarrhea
- Constipation
- Headache
- Fever
- Urinary tract infection

- Anemia
- Thrombocytopenia
- Arthralgia
- Peeling of the skin over large areas of the body
- Swelling just below the surface of the skin, most often around the lips and eyes
- Hypersensitivity

Other side effects that are less common (3% to less than 20%):

- Sepsis
- Pneumonia (lung infection)
- Pyelonephritis (kidney infection)
- Virus infection (herpes zoster, herpes simplex)
- Venous thromboembolism (pulmonary embolism, deep venous thrombosis)
- Tachycardia
- Stomatitis
- Thrombotic thrombocytopenic purpura/hemolytic uremic syndrome
- Hemolytic uremic syndrome (a kidney condition where destroyed red blood cells block kidney filtering abilities.
- Leukopenia
- Abnormal healing
- Increased lactic dehydrogenase (LDH)
- Hypokalemia
- Bone necrosis
- Epistaxis (nose bleed)
- Skin cancers (including melanoma, squamous cell carcinoma, and basal cell carcinoma)

Other side effects that are possible, some may be serious include:

- Hepatotoxicity (liver dysfunction), including fatal hepatic necrosis, with elevated Sirolimus trough concentrations
- Pericardial effusion (fluid around the heart that may require an intervention)
- Progressive multifocal leukoencephalopathy (PML)
- Lung disease (including pulmonary fibrosis and pulmonary hemorrhage)
- Nephrotic syndrome and kidney dysfunction
- Azoospermia (lack of sperm production) which is reversible after discontinuation of Sirolimus

Taking Sirolimus together with other medications that can cause liver function abnormalities may further increase the risk of severe liver injury.

Study Drugs

9.6 Drug Descriptions

9.6.1 Turalio® Description

Turalio®will be supplied in 200mg capsules and should be taken twice daily as split doses approximately 12 hours apart. Each dose should be taken with approximately 8 ounces of water. Subject should fast for at least two hours before and one hour after dosage. Each cycle of treatment will last 28 days. Turalio® should not be taken with medications that may decrease the acid content of the stomach as it may affect drug absorption.

The dose level for the phase 2 portion of the study was determined to be dose level 3 (Turalio® should be taken 400mg orally in AM and 600mg in PM daily in combination with Sirolimus 2mg orally daily in AM in a fasting state).

Sirolimus Description

Sirolimus will be supplied in 0.5mg, 1.0mg, or 2.0mg tablets and should be taken once daily (in AM) approximately 24 hours apart. Each dose should be taken with approximately 8 ounces of water when taking the AM dose of Turalio®. Subject should fast for at least two hours before and one hour after dosage. Each cycle of treatment will last 28 days.

9.7 Treatment Regimen

Turalio® and Sirolimus are oral tablets and are intended for continuous outpatient administration in 28 day cycles until criteria for ending the study have been met. Both drugs are to be taken two hours before or after a meal or snack.

Turalio® is to be taken twice daily. Sirolimus is to be taken once daily. The drugs can be taken at the same time. Dose levels tested in the phase 1 portion of the study are indicated in Table 1, Section 5.1.

The phase 1 study was executed as an open-label, continual reassessment method dosing of Turalio® and Sirolimus which began enrollment with a Turalio® dose of 800 mg per day, i.e., 400 mg PO AM and 400mg PO PM along with Sirolimus 2mg PO daily in AM. Dose escalation groups were as described in Section 4.1.

Review of the phase 1 safety data was assessed by the continual reassessment method and identified dose level 3 (Turalio® 1000mg in combination with Sirolimus 2mg) to be the recommended phase 2 dose. For dose modification guidelines for the phase 2 portion of the study, please refer to section 8.2 and Tables 10 – 14 above.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every six weeks within the first 36 weeks. In addition to a baseline scan, confirmatory scans should also be obtained six weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eur J Ca 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria. Therapy may be permitted beyond progression if patient is deriving clinical benefit after initial tumor growth or ambiguity regarding the existence of disease progression in consultation with the principle investigator, sponsor, and central IRB. If subsequent imaging confirms tumor growth, progression will be back dated to the prior scan. If patient agrees to treatment beyond progression, the patient will be required to sign a "Treatment Beyond Progression" informed consent form.

11.1.1 **Definitions**

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with Turalio® and Sirolimus.

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

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

11.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) 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).

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

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

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

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. Cystic lesions thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters

(longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

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

11.3 Methods for Evaluation of Measurable Disease

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

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

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

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

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

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

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

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

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

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [JNCI 96:487-488, 2004; J Clin Oncol 17, 3461-3467, 1999; J Clin Oncol 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [JNCI 92:1534-1535, 2000].

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

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

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

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

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

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

11.4 Response Criteria

11.4.1 Evaluation of Target Lesions

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

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

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

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

11.4.2 Evaluation of Non-Target Lesions

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

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

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

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.4.3 Evaluation of Best Overall Response

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

Response classification for Patients with Measurable Disease (e.g., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	8 weeks
CR	Non-CR/Non-PD	No	PR	8 weeks
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	8 weeks
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD**	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

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

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.5 Duration of Response

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

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

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

11.6 Progression-Free Survival

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

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

For study design and statistical consideration, please refer to section 5.1.

Phase 1 study Primary aim:
To determine the recommended phase 2 dose (RP2D) and safety for Turalio® when administered orally in combination with Sirolimus in patients with unresectable or metastatic sarcoma. A total of 18 evaluable unresectable or metastatic sarcoma patients were enrolled.

Phase 2 study primary aim:
To determine the preliminary efficacy of Turalio® in combination with Sirolimus in patients with unresectable or metastatic MPNST by determining the median progression free survival.

The primary endpoint is progression free survival (PFS), which is defined as the time from the start of the treatment until disease progression or death from any cause. Assuming a median PFS of 6 weeks in patients treated with standard of care (23) with a sample size of 25, we have 90% power to detect a difference of 12 weeks in median PFS (6 weeks versus 18weeks) given a 2-sided test with an alpha of 0.05. Thus, a total of 25 evaluable unresectable or metastatic MPNST patients will be enrolled.

Phase 2 study Secondary aims:
To investigate the objective response rate (ORR) and overall survival (OS) of Turalio® in combination with Sirolimus in patients with unresectable malignant peripheral nerve sheath tumors (MPNSTs).

13.3 Analysis of Primary and Secondary Endpoints and Evaluation of Tissue

13.3.1 Primary Analysis

Phase I: The MTD will be estimated using the Continual Reassessment Method. The proportion of DLT at each dose level will be reported along with the final estimates of the probability of DLT based on the TTE-CRM.

Phase II: Progression free survival will be estimated using the Kaplan Meier method. Median PFS and 95% confidence intervals will be reported.

Secondary analysis:
Overall survival, defined as the time from the start of treatment until death, will be estimated using the Kaplan Meier method. Median OS and 95% confidence intervals will be reported.

Best response rate at weeks will be reported as a proportion along with the exact 95% confidence interval.

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