Treatment of pituitary Cushing disease with a selective CDK inhibitor, R-roscovitine

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Section 1.0 General Information

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Section 2.0 Background information

A1. Clinical features of pituitary corticotroph tumors (Cushing disease) Pituitary adenomas comprise ~15 % of intracranial neoplasms with an overall incidence at 15 to 20 per million per year. Although pituitary tumors are generally benign, tumor compression of critical structures can lead to headaches, visual disorders, cranial nerve dysfunction and failure of pituitary hormone production. Pituitary adenomas also result in clinical features based on their specific cell type. Adrenocorticotropin (ACTH) -producing tumors of the corticotroph lineage (Cushing disease, CD) comprise 10 % of pituitary adenomas.

CD is rare, but it is the most common cause of endogenous hypercortisolism, which predisposes patients to central obesity, diabetes, hypertension, osteoporosis and substantially increases their risk of infection, thrombosis and psychiatric disorders. If inadequately controlled, CD is fatal with mortality rate 4-fold-higher than that of age- and sex-matched controls and *a median survival of 4.6 years*. Cardiovascular disease is the leading cause of death in CD (1).

The standard of care for Cushing disease is multimodal, as no single approach is uniformly effective: although transsphenoidal ACTH-secreting tumor resection yields 30% to 70% surgical cure rates, adenoma recurrence rates are high. Furthermore, over half of all such adenomas are *not visible on MRI*, further challenging a surgical approach. Often, total hypophysectomy, with all its attendant complications, may be blindly performed in an attempt to resolve excess ACTH secretion. Efficacies of other therapeutic modalities, such as pituitary-directed radiation, adrenalectomy, and/or medical suppression of adrenal gland cortisol production, are limited by serous side effects, slow therapeutic responses, development of pituitary insufficiency, and/or uncontrolled pituitary tumor growth (Nelson syndrome) in the setting of adrenal gland resection or inhibition. Currently, there is no safe and effective pharmacotherapy directly targeting corticotroph tumor growth and/or ACTH production(2).

Recently, pasireotide, a somatostatin analogue, has shown benefits as the first therapeutic agent targeted to pituitary corticotrophs in a Phase III trial of patients with CD. However pasireotide has a modest overall disease control rate of 25% (limited to patients with mild disease) and very significant metabolic side effects, including invariable hyperglycemia, and subsequent insulin-requiring diabetes, since the polypeptide is a potent suppressor of insulin synthesis/secretion(3). Peripheral glucocorticoid receptor antagonist is approved for hypercortisolism of CD, but it also has multiple side effects and does not target the pituitary adenoma(4). Other investigational agents, including peroxisome proliferator-activated receptor-γ agonists, retinoic acid, dopamine D_2 receptor angonists and EGFR inhibitors have yet to yield therapeutic efficacy in human CD. Therefore, there is a clearly unmet need for effective and safe pituitary-targeted pharmacotherapy that can normalize ACTH and cortisol levels, control tumor growth, improve clinical signs and symptoms and ultimately leading to reversal of co-morbidities and normalize mortality.

A2. Pituitary corticotroph tumorigenesis and cell cycle regulators Based on our own work, and that of others, cell cycle deregulation is implicated in pituitary tumorigenesis. Pituitary tumors acquire oncogene and tumor suppressor genetic and epigenetic alterations, which

result in unrestrained proliferation, aberrant neuroendocrine regulatory signals and disrupted humoral milieu, mediated directly or indirectly by dysregulated cyclin-dependent kinases (CDKs)(5). Although CDK gene mutations have not been identified in human pituitary tumors, overexpression of cyclins and dysregulation of CDK inhibitors are frequently encountered in pituitary adenomas, indicating the importance of CDK activation for potential therapeutic targeting(6).

Corticotroph tumors exhibit up-regulated levels of cyclin E, which is undetectable in normal cells or tumors arising from other lineages of the pituitary (7). During the normal cell cycle, cyclin E is transcriptionally activated from late G_1 till end of S phase, binds and activates the serine/threonine protein kinase cdk2 to promote G_1-S phase transition. Cyclin E activity and expression are regulated by members of the E2F family of transcription factors and the corepressors pRb and HDACs(8). Cyclin E stimulates its own transcription through a positive feedback mechanism involving E2F activation, and also phosphorylate Cdk2-bound p27 and p21, triggering their degradation by the ubiquitin-proteasome pathway(9, 10). Dysregulated cyclin E activity causes critical disruptions of the G_1-S transition, affecting cell proliferation, differentiation, survival and senescence, and contributing to tumor development (11-14). For pituitary corticotroph tumors, mechanisms underlying lineage-specific cyclin E up-regulation remain to be defined. In mouse corticotroph tumor AtT-20 cells, cyclin E expression is repressed by brahma related-gene 1 (Brg1). Brg1 was implicated in cell cycle control as a repressor of cyclin E, and is mis-expressed in about 30% of human corticotroph tumors(15) (16). We *recently showed that corticotroph overexpression of pituitary tumor transforming gene (PTTG) induces cyclin E, whereas PTTG siRNA suppresses cyclin E expression(17).* As a cell cycle regulator and global transcription factor modulating G1/S and G2/M phase transition, PTTG is overexpressed in more than 90% of all type of pituitary tumors, including corticotroph adenomas (18). PTTG encodes a securin that binds separase in the APC complex, and governs faithful chromosome segregation during mitosis. PTTG was originally isolated from rat pituitary tumor cells(19). Dysequilibrium of intracellular PTTG abundance leads to cell cycle disruption and neoplastic formation, causing chromosomal instability and aneuploidy, and also aberrant G1/S and G2/M transition by transcriptional dysregulation of cyclin expression. On the other hand, PTTG overexpession also activates lineage-specific senescence pathways in pituitary growth hormone (GH)- and gonadotropin (LH, FSH)-expressing tumors, contributing to the benign propensity of pituitary tumors(20, 21). *Our study indicates that corticotroph cyclin E up-regulation may represent another pathway for PTTG-induced pituitary lineage-specific effects, contributing to corticotroph tumor development (17).*

A3. Targeting corticotroph tumors with CDK inhibitor CDK inhibitors have been considered relevant drug candidates for cancer therapy because of their potential role in restoring cell cycle control and a growing number of CDK inhibitors classes are currently in clinical trials for cancer therapy(22). However, clinical development of CDK inhibitors has encountered numerous failures in more than a decade with narrow therapeutic window being one of the major obstacles, complicating the lack of efficacy and increased toxicity issues. CDKs are important for both normal and tumor cells. Although genetic spectrum of tumor-associated mutations and/or their cellular context may dictate specific CDK dependence, it is difficult to predict which CDK inhibitor(s) may be effective against particular tumor types *in vivo*. The key is to identify which regulator(s) is responsible for cell division down stream of a tumorigenic event, so that a proper inhibitor can be selected for a specific tumor type and/or event.

As a rare disease with an annual incidence of 0.7-2.4 per million populations and a US prevalence of 11,000 to 15,000, development of drugs to treat CD presents multiple challenges, leading to sparse basic and translational science research, poorly understood disease

pathophysiology, as well as difficulties in setting up effective clinical evaluations. To overcome these obstacles, animal models that faithfully recapitulate pathological phenotypes of CD are needed. Selection and prioritization of agents for clinical trials is a key challenge in drug development for CD as only a very few agents can be tested in the clinical setting due to small patient numbers, extensive treatment time required, and cost. In the past, a lack of relevant models has precluded preclinical testing.

Through collaborative studies over the past 10 years, *we have developed a transgenic zebrafish Cushing disease model that have utility in the rational preclinical development of drugs for CD(17)*. Combining the CD zebrafish with a transgenic fluorescent reporter line that specifically labels the pituitary proopiomelanocortine (POMC, encoding an ACTH precursor) lineage in live transparent larvae, we tested small-molecule CDK inhibitors, which lead to the identification of a selective CDK inhibitor, R-roscovitine (CYC202 or Seliciclib), directed against neoplastic corticotrophs(17). R-roscovitine is a 2,6,9-trisubstituted purine analogue that competes with ATP for its binding site on CDKs. R-roscovitine is a 2,6,9-trisubstituted purine analogue, which competes with ATP binding site on CDKs, and preferentially inhibits the activity of CDK2/cyclin E complex with an IC50 < 0.1 μM(23). We subsequently confirmed in mouse corticotroph tumor models and in primary *human pituitary corticotroph tumor cell cultures* that down-regulated CDK2/cyclin E activity and dephosphorylation of their natural substrate, pRb (and possibly p27) by R-roscovitine lead to direct cell cycle arrest and apoptosis [(17) and unpublished data].

Unexpectedly, R-roscovitine also induces *a rapid suppression of POMC gene expression and ACTH production independent of its cell cycle inhibitory effect*, suggesting a previously unappreciated negative regulatory role of a selective CDKI on POMC gene that encodes the precursor hormone of ACTH in corticotrophs. The POMC gene encodes the precursor hormone of ACTH in corticotrophs. Pituitary POMC gene expression depends on the combination of tissue- and cell-restricted transcription activators such as Pitx1, NeuroD1 and Tpit/Tbx19(24). These factors act on the proximal POMC promoter and integrate inputs from different signaling pathways to regulate POMC and ACTH production. Suppressed POMC gene expression by R-roscovitine may result from inhibition of CDK2/cyclinE that maintains pRB in an inactive hypophosphorylated state, thereby affecting downstream transcriptional pathways. If this is true, amplified cyclin E expression in corticotroph tumors serves as a therapeutic target for the *dual inhibition of both tumor cell growth and ACTH expression*. On the other hand, in addition to targeting the cell cycle CDKs (CDK1 and CDK2), R-roscovitine can also inhibit transcriptional CDKs (CDK7, 8 and 9) that activate mRNA transcription by phosphorylating the C-terminal domain (CTD) of RNA polymerase II, which leads to complex changes in mRNA levels rather than blocking global mRNA transcription(25).

R-roscovitine is currently undergoing phase I and II clinical trials for several malignancies, and is a proven safe oral molecule in humans [IND No. 71,011-CYC202(R-roscovitine or Seliciclib)]. Here, we propose to perform a *phase II clinical trial* on the safety and efficacy of R-roscovitine for treatment of patients with CD.

B. Preliminary Data:
R-Roscovitine inhibits ACTH production from human corticotroph tumor cells by targeting hPOMC gene regulatory sequences

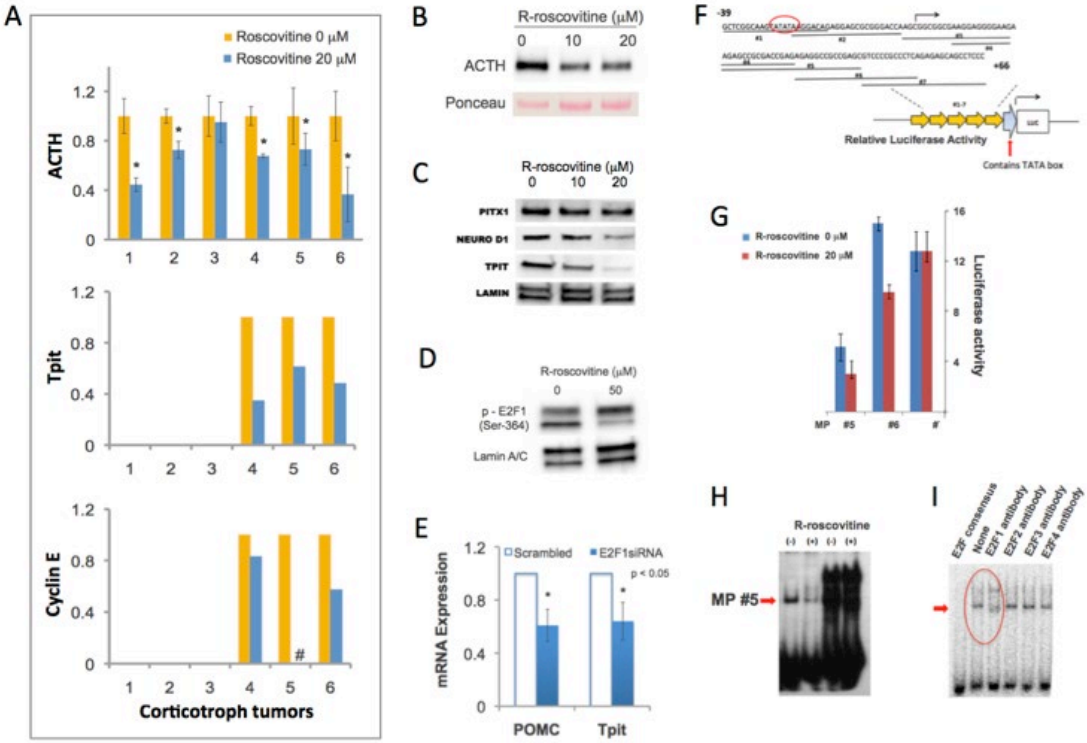

Figure 1. Inhibition of ACTH production by R-roscovitine. (A) Primary culture of human corticotroph tumor cells treated with vehicle or R-roscovitine. Top, ACTH concentration in primary culture medium measured by radioimmunoassay (n=6 tumors, mean ± SE; *P < 0.05). Middle and Bottom, Tpit and cyclin E expression measured by RT-PCR of cDNA derived from tumors No. 4-6. No sufficient cDNA were available from tumors No.1-3. #, level of PCR product was too low to detect. (B) Western blot analysis of ACTH expression in human corticotroph tumor primary culture cells treated with vehicle or R-roscovitine. (C) Western blot analysis of protein extract derived from AtT20 cells treated with vehicle or R-roscovitine. (D) R-roscovitine treatment of human ACTH producing DMS79 cells result in E2F1 dephosphorylation. (E) E2F1 siRNA overexpression in AtT20 cells suppresses POMC and Tpit expression. (F) Schematic representation of hPOMC 5' proximal promoter DNA sequence from -39 to +66. 7 mini-promoter fragments (#1 - #7) were generated for luciferase reporter assay and EMSA. (G) Luciferase activity of mini-promoter #5 and #6 are inhibited by R-roscovitine treatment. (H and I) Protein-DNA mini-promoter #5 complex (red arrow) is interrupted by R-roscovitine (H) and E2F1 antibody (I) treatment.

To further explore the therapeutic potential of R-roscovitine directly targeting human ACTH production, we treated 6 consecutive human pituitary corticotroph tumors in primary cultures with R-roscovitine for 48 hours, which led to significant reduction of ACTH levels in the culture medium (Fig 1A). Among the 6 corticotroph tumors, tumors No. 4 – 6 had sufficient yield of cells in primary culture that allowed further RT-PCR analysis, demonstrating suppressed Tpit/Tbx19 expression (Fig 1A), as well as reduced cyclin E (Fig 1A). Western blot analysis of protein extracts derived from R-roscovitine–treated human corticotroph tumor cells also showed decreased ACTH protein expression (Fig 1B). In mouse AtT20 cells, R-roscovitine inhibited expression of NeuroD1 and Tpit protein in a dose-dependent pattern, without affecting that of Pitx1 (Fig 1C), suggesting that R-roscovitine may target unique corticotroph lineage-specific transcription factors leading to inhibition of POMC expression. As R-roscovitine suppresses corticotroph cyclin E expression and dephosphorylates pRb(17), which maintains E2F in an inactive state thereby affecting down stream transcriptional pathways, we also assessed the role of E2F1 in POMC gene regulation. R-roscovitine treatment of human ACTH-producing DMS 79 cells caused E2F1 dephophorylation (Fig 1 D), and suppressing E2F1 expression with siRNA

resulted in decreased Tpit and POMC expression in AtT20 cells (Fig 1E). Further hPOMC gene promoter driven luciferase reporter assays revealed a R-roscovitine-responsive element within the 5' *hPOMC* promoter (Fig 1 F and G). Electrophoretic mobility shift assay (EMSA) demonstrated that the protein-DNA complex with mini-promoter #5 (+22 - +34) of the hPOMC gene is *disrupted* by either R-roscovitine or specific E2F1 antibody treatment (Fig 1H and I). In summary, R-roscovitine suppresses corticotroph cyclin E expression and dephosphorylates pRb, thereby inhibiting E2F1 activity, which both directly and indirectly down-regulates POMC gene transcription. These preliminary results, combined with our published preclinical data provide a strong rationale to translate our laboratory research to the proposed clinical study of pharmacotherapy directly targeting corticotroph tumors.

Summary:

Taking advantage of a zebrafish transgenic model with early-onset, characteristic pathology of human Cushing disease including corticotroph expansion and partial Gc resistance, combined with pituitary POMC lineage-specific expression of a fluorescent reporter in live transparent larvae, we tested small-molecule CDK inhibitors. Our study identified R-roscovitine as being effective against PTTG-overexpressing corticotroph tumors. Inhibitory effects of R-roscovitine on corticotroph tumor cell expansion and ACTH production were subsequently validated in an *in vivo* and *in vitro* mouse model. Our most recent data on in vitro treatment of human corticotroph tumor cells with R-roscovitine also show consistent suppression of ACTH production. Further study demonstrated that R-roscovitine inhibits human corticotroph ACTH production by targeting E2F1 regulated hPOMC gene activation, providing a strong rationale for use of R-roscovitine as an effective pituitary-targeted therapy for Cushing disease.

Section 3.0 Trial objectives and purpose

Aim: Evaluate the efficacy and safety of R-roscovitine for treatment of pituitary corticotroph tumors (Cushing disease).

Hypothesis: We hypothesize that R-roscovitine will suppress pituitary corticotroph tumor ACTH production and normalize urinary free cortisol levels in patients with Cushing disease. To date, R-roscovitine has been evaluated in several Phase I and II studies and has shown early signs of anti-cancer activity in approximately 240 patients. Studies included a Phase I study in which single agent seliciclib was administered to patients with advanced non-small cell lung cancer (NSCLC) and two Phase IIa studies in which seliciclib was administered in combination with gemcitabine and cisplatin as first-line treatment and with docetaxel as second-line treatment in NSCLC(26). Seliciclib was also evaluated in a Phase I study in patients with nasopharyngeal cancer (NPC) with evidence of tumor shrinkage and concomitant reduction in copy counts of the EBV virus that is causally associated with the pathogenesis of NPC [IND No. 71,011-CYC202(R-roscovitine or Seliciclib)](27). Results from APPRAISE, a randomized discontinuation, double-blinded, placebo-controlled, Phase IIb study of oral seliciclib capsules as a monotherapy in heavily pretreated patients with NSCLC, demonstrated no difference between the seliciclib and placebo arms in progression free survival but a substantial increase in overall survival was observed (388 versus 218 days respectively (Cyclacel press release Dec 21, 2010). Here, we propose an exploratory, proof of concept clinical trial to determine if seliciclib can safely normalize urinary free cortisol levels by reducing pituitary corticotroph tumor ACTH production in patients with Cushing disease.

Section 4.0 Trial Design

Study Type:

- Interventional

Study Design:

- Phase II, proof-of-concept, open-label and single arm study to assess the safety and efficacy of seliciclib in patients with Cushing disease
- Rationale for study design: due to the rarity of Cushing disease in the general population, it is difficult to do a randomized placebo controlled trial with sufficient sample size numbers to achieve adequate power. By using the subjects as their own controls, we alleviate the issues with recruitment. This is a safety and activity trial to look for a sufficient effect of the drug on normalizing urinary free cortisol before proceeding to a larger, randomized placebo controlled trial.

Study population:

- Patients with *de novo*, persistent, or recurrent Cushing disease will be recruited from the Pituitary Center at Cedars-Sinai Medical Center.

Intervention:

- R-roscovitine 400 mg oral administration twice daily for 4 days every week for total of 4 weeks. The dose of R-roscovitine could be reduced by 25% at any time if patients are unable to tolerate the protocol-specified dosage, and/or investigator believes that an ongoing drug-related adverse effect is present. Cyclacel Pharmaceuticals, Inc. (UK) will provide the drug in 200 mg strength capsules.

- *Rationale for dose and regimen selection:* transsphenoidal pituitary tumor resection is the primary therapy for Cushing disease, and in the best hands, yields 30-70% surgical cure rates. Patients with recurrent or residual tumors, and/or those not deemed surgical candidates typically undergo pituitary-directed radiation, adrenalectomy, treated with pasireotide, and/or medical suppression of adrenal gland cortisol production(2). In this study, we will treat patients with *de novo*, persistent, or recurrent pituitary ACTH-dependent hypercortisolism with R-roscovitine for 4 weeks before the standard care of pituitary surgery, radiation, surgical or medical adrenalectomy are offered. The dose selected is based on data from the CYC202-07-15 study in patients with nasopharyngeal cancer [IND No. 71,011-CYC202(R-roscovitine or Seliciclib)]. As the pre-op evaluation and preparation time at our center usually takes about 4 weeks, patients can be treated with R-roscovitine during this period without compromising standard of care. As Cushing disease is a chronic longstanding disorder, the risks of experimental treatment prior to surgery or other standard management are deemed to be acceptable by our IRB. After completion of the study, subjects will be ready to start standard care. If a given patient responds to the study drug and chooses to stay on the drug after cessation of the trial, clinical data will continue to be collected for further analysis of drug response and safety monitoring.

Primary objectives:

- To evaluate the efficacy of seliciclib 400 mg oral administration twice daily for 4 days every week for total of 4 weeks on normalizing 24 hour urinary free cortisol (24 h UFC) levels in CD patients.

Secondary objectives:

- To evaluate changes in levels of pituitary hormones and metabolic abnormality; changes in clinical signs and symptoms; changes in tumor size; and safety of seliciclib 400 mg oral administration twice daily for 4 days every week for total of 4 weeks in CD patients.

Primary endpoint:

- A normalized urinary free cortisol level at week 4

Secondary endpoint:

- A urinary free cortisol level above the upper limit of the normal range but reduced by ≥50% from baseline at week 4
- Plasma ACTH, hour urinary free cortisol, serum and salivary cortisol levels over time
- Hemoglobin A1C (HbA1C), fasting blood glucose and blood electrolytes, e.g. K^+
- Cushing's syndrome clinical signs and symptoms, e.g. weight, body mass index, blood pressure
- Changes in tumor size
- Safety end points: hematologic and blood chemistry including total sodium, potassium, urea, serum creatinine, creatinine clearance, total protein; LFT (ALT, AST, total bilirubin, albumin, ALP, tal cholesterol (TC), LDL-cholesterol, triglycerides: coagulation (PT, INR) and ECG

Section 5.0. Selection and Withdrawal of Subjects

Estimated enrollment:

- 16 subjects

Inclusion criteria:

- Male and female patients at least 18 years old
- Patients with confirmed pituitary origin of excess adrenocorticotropic hormone (ACTH) production:
    - Persistent hypercortisolemia established by two consecutive 24 hr UFC levels at least 1.5x the upper limit of normal
    - Normal or elevated ACTH levels
    - Pituitary macroadenoma (>1 cm) on MRI OR
    - Inferior Petrosal Sinus Sampling (IPSS) central to peripheral ACTH gradient >2 at baseline and >3 after CRH stimulation
    - Recurrent or persistent Cushing disease is defined as pathologically confirmed resected pituitary ACTH-secreting tumor, and 24 hour UFC above the upper limit of normal reference range beyond post-surgical week 6
    - Patients on medical treatment for Cushing's disease the following washout periods must be completed before screening assessments are performed :
        - Inhibitors of steroidogenesis (metyrapone, ketoconazole): 2 weeks
        - Somatostatin analogs (pasireotide): 2 weeks
        - Progesterone receptor antagonist (mifepristone): 2 weeks

- Dopamine agonists (cabergoline): 4 weeks
- CYP3A4 strong inducers or inhibitors: varies between drugs; minimum 5-6 times the half-life of drug

Exclusion criteria:

- Patients with compromised visual fields, and not stable for at least 6 months
- Patients with abutment or compression of the optic chiasm on MRI and normal visual fields
- Patients with Cushing's syndrome due to non-pituitary ACTH secretion
- Patients with hypercortisolism secondary to adrenal tumors or nodular (primary) bilateral adrenal hyperplasia
- Patients who have a known inherited syndrome as the cause for hormone over secretion (i.e. Carney Complex, McCune-Albright syndrome, MEN-1)
- Patients with a diagnosis of glucocorticoid-remedial aldosteronism (GRA)
- Patients with cyclic Cushing's syndrome defined by any measurement of UFC over the previous 1 months within normal range
- Patients with pseudo-Cushing's syndrome, i.e. non-autonomous hypercortisolism due to overactivation of the HPA axis in uncontrolled depression, anxiety, obsessive compulsive disorder, morbid obesity, alcoholism, and uncontrolled diabetes mellitus
- Patients who have undergone major surgery within 1 month prior to screening
- Patients with serum $K^+ < 3.5$ while on replacement treatment
- Diabetic patients whose blood glucose is poorly controlled as evidenced by HbA1C >8%
- Patients who have clinically significant impairment in cardiovascular function or are at risk thereof, as evidenced by
    - Congestive heart failure (NYHA Class III or IV), unstable angina, sustained ventricular tachycardia, clinically significant bradycardia, high grade AV block, history of acute MI less than one year prior to study entry
- Patients with liver disease or history of liver disease such as cirrhosis, chronic active hepatitis B and C, or chronic persistent hepatitis, or patients with ALT or AST more than 1.5 x ULN, serum total bilirubin more than ULN, serum albumin less than 0.67 x LLN at screening
- Serum creatinine $\geq$ 2 x ULN
- Patients not biochemically euthyroid
- Patients who have any current or prior medical condition that can interfere with the conduct of the study or the evaluation of its results, such as
    - History of immunocompromise, including a positive HIV test result (Elisa and Western blot). An HIV test will not be required, however, previous medical history will be reviewed
    - Presence of active or suspected acute or chronic uncontrolled infection

  - History of, or current alcohol misuse/abuse in the 12 month period prior to screening

- Female patients who are pregnant or lactating, or are of childbearing potential and not practicing a medically acceptable method of birth control. If a woman is participating in the trial then one form of contraception is sufficient (pill or diaphragm) and the partner should use a condom. If oral contraception is used in addition to condoms, the patient must have been practicing this method for at least two months prior to screening and must agree to continue the oral contraceptive throughout the course of the study and for 3 months after the study has ended. Male patients who are sexually active are required to use condoms during the study and for three month afterwards as a precautionary measure (available data do not suggest any increased reproductive risk with the study drugs)
- Patients who have participated in any clinical investigation with an investigational drug within 1 month prior to screening or patients who have previously been treated with seliciclib
- Patients with any ongoing or likely to require additional concomitant medical treatment to seliciclib for the tumor
- Patients with concomitant treatment of strong CYP3A4 inducers or inhibitors.
- Patients who were receiving mitotane and/or long-acting somatostatin analogs (octreotide LAR or lanreotide)
- Patients who were receiving pasireotide or ketoconazole before study entry must complete a 2 week washout period prior to receiving seliciclib
- Patients who have received pituitary irradiation within the last 5 years prior to the baseline visit
- Patients who have been treated with radionuclide at any time prior to study entry
- Patients with known hypersensitivity to seliciclib
- Patients with a history of non-compliance to medical regimens or who are considered potentially unreliable or will be unable to complete the entire study
- Patients with presence of Hepatitis B surface antigen (HbsAg)
- Patients with presence of Hepatitis C antibody test (anti-HCV)

Withdrawal of Subjects:

- Monitoring for adverse effects: Patients will be evaluated for adverse effects weekly with physical examinations, chemistry and hematology panels. Adverse events will be graded by the NCI-CTCAE and include: fatigue, fever, nausea, vomiting, anorexia, hepatic dysfunction (increased ALT/SGPT, AST/SGOT and AP), hypokalemia, hyponatremia and insomnia [IND No. 71,011-CYC202(R-roscovitine or Seliciclib)]. Seliciclib will be discontinued in those who developed worsening of visual fields, and those with toxicity greater than or equal to Grade 4 on the NCI-CTCAE. Demonstration of compromised visual fields at any time will lead to withdrawal from the study and surgical referral.

Section 6.0 Treatment of Subjects

Transsphenoidal pituitary tumor resection is the primary therapy for Cushing disease, and yields 30-70% surgical cure rates. Patients with recurrent or residual tumors, and/or are not surgical candidates typically undergo pituitary-directed radiation, adrenalectomy, treated with pasireotide, and/or medical suppression of adrenal gland cortisol production. In this study, we will treat patients with *de novo*, persistent, or recurrent Cushing disease with seliciclib during the period of evaluation and preparation for standard care, which usually takes 4 weeks at our center. We are excluding any subjects who need immediate surgical intervention, i.e. unstable visual field deficit, tumor compression of optic chiasm, or sever metabolic complications. We only will recruit those whom we would recommend for routine evaluation process for standard care. After completion of their participation in the study, subjects will be ready to start standard care. If a given patient responds to the study drug and chooses to stay on the drug after cessation of the trial, clinical data will continue to be collected for further analysis of drug response and monitoring safety.

Study calendar: baseline physical exam, vital signs, weight and laboratory evaluations will be conducted within 7 days prior to the start of therapy. Pituitary imaging and ECG will be performed within 60 days prior to the start of therapy. All procedures and evaluations may be performed within 3 calendar days of that specified in the protocol to accommodate patient convenience and scheduling. Patient informed consent will be obtained prior to any protocol-specific procedures. The schedule of study events is provided in Table 1.

Patients will be administered a dose of oral seliciclib 400 mg twice daily for 4 days every week for total of 4 weeks. Patients will be seen on days 1, 8, 15, 22 and 29 for repeat laboratory testing, assessment of treatment compliance, and toxicity according to National Cancer Institute Common Toxicity Criteria version 3.

The following tests will be performed:

Screening visit: Day -60 to -1

- History and physical exam
- Dexamethasone dispensed
    - Dexamethasone will be dispensed during the screening visit. Study doctor will explain when the subject should take it. During an overnight dexamethasone suppression test, 8mg of dexamethasone will be taken orally the night before at 11:00 PM. Subjects will go to the local Quest laboratory the next morning and will have their blood drawn between 8:00 AM - 9:00 AM to test their serum cortisol levels. Dexamethasone, which acts like a cortisol, decreases the amount of ACTH released by the pituitary gland, which then decreases the amount of cortisol released by the adrenal glands. More than 50% suppression of morning cortisol level after dexamethasone administration will confirm the diagnosis of Cushing's disease. Patients who had previously undergone dexamethasone suppression test at Cedars-Sinai Medical Center or if the patient has been confirmed with diagnosis such as a previous surgery or IPSS a repeat exam is not required.
- 24 hour urinary free cortisol x 2
    - Subjects will be given 2 containers to collect 2 - 24 hour UFC
    - The 2 - 24 hour UFC can be collected anytime between one week after taking dexamethasone and the baseline visit
    - Subjects will drop off the 2 - 24 hour UFC at their local Quest laboratory

- ECG
- Visual fields
- Pituitary MRI
- HbA1C
- CBC with differential, CMP (including fasting blood glucose), LFTs
- Hormone profile: PRL, TSH, thyroid panel, free T4, IGF-1, LH, FSH, estradiol for females, testosterone (total and free) for males
- PT/INR
- Serum Pregnancy Test (for females of childbearing potential)
- Serum cortisol level at 8:00-9:00 AM on the day of screening visit, then at 8:00-9:00 AM the next day after an overnight 8 mg dexamethasone

Baseline visit 1: Day -7

- CBC with differential, CMP (including fasting blood glucose), PT/INR

Fasting lipid profile, HbA1C, Hormone profile: PRL, TSH, adjusted T4, free T4, IGF-1, LH, FSH, estradiol for females, testosterone (total and free) for males,

- Plasma ACTH and serum cortisol levels (every three hours from 9:00 – 15:00)
    - Blood draws will be done via intravenous catheter every 3 hours from 9AM to 3PM.
        - In the case that an intravenous catheter cannot be placed, one blood draw will be performed at every 3 hour interval.
- Serum Pregnancy Test (for females of childbearing potential)
- ECG
- Visual fields
    - 2 x late night salivary cortisol collection at 23:00-24:00 PM on any nights between baseline visit 1 and visit 2.The subject will rinse their mouth thoroughly with water and discard (do not swallow). The subject will hold the Salivette(R) at the rim of the suspended insert and remove the stopper and remove the swab. The swab will be placed under the tongue until well saturated (approximately one minute). The subject will then return the saturated swab to the suspended insert and close the Salivette(R) firmly with the stopper.
- 24 hour urinary free cortisol x 3
    - Subjects will be given 3 containers to collect 3 - 24 hour UFC
    - The 3 - 24 hour UFC can be collected anytime between the baseline visit 1 and visit 2.
    - Subjects will drop off the 3 - 24 hour UFC at their local Quest laboratory

Visit 2: Day #1

- History and physical exam
- Serum Pregnancy Test (for females of childbearing potential)
- Plasma ACTH and serum cortisol levels (every three hours from 9:00 – 15:00)
    - Blood draws will be done via intravenous catheter every 3 hours from 9AM to 3PM.
        - In the case that an intravenous catheter cannot be placed, one blood draw will be performed at every 3 hour interval.
- 24 hour urinary free cortisol any time between days 3-7
    - Subjects will collect 3 - 24 hour UFC any time between days 3-7 prior to coming in for study visit 3

- 2 - late night salivary cortisol at 23:00-24:00 between days 3-7

Visit 3: Day #8
- History and physical exam
- Adverse events assessment
- CBC with differential, CMP (including fasting blood glucose), PT/INR
- Thyroid panel: TSH, adjusted T4, free T4
- Serum Pregnancy Test (for females of childbearing potential)
- Plasma ACTH and serum cortisol levels (every three hours from 9:00 – 15:00)
    - Blood draw will be done via intravenous catheter every 3 hours from 9AM to 3PM.
        - In the case that an intravenous catheter cannot be placed, one blood draw will be performed at every 3 hour interval.
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- ECG
- 24 hour urinary free cortisol any time between days 10-14
    - Subjects will collect 3 - 24 hour UFC any time between days 10-14 prior to coming in for study visit 4
- 2 - late night salivary cortisol at 23:00-24:00 between days 10-14
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Visit 4: Day #15
- History and physical exam
- Adverse events assessment
- CBC with differential, CMP (including fasting blood glucose), PT/INR
- Thyroid panel: TSH, adjusted T4, free T4
- Serum Pregnancy Test (for females of childbearing potential)
- Plasma ACTH and serum cortisol levels (every three hours from 9:00 – 15:00)
    - Blood draw will be done via intravenous catheter every 3 hours from 9AM to 3PM.
        - In the case that an intravenous catheter cannot be placed, one blood draw will be performed at every 3 hour interval.
- ECG
- 24 hour urinary free cortisol any time between days 17-21
    - Subjects will collect 3 - 24 hour UFC any time between days 17-21 prior to coming in for study visit 5
- 2 - late night salivary cortisol at 23:00-24:00 between days 17-21

Visit 5: Day #22
- History and physical exam
- Adverse events assessment
- CBC with differential, CMP (including fasting blood glucose), PT/INR
- Thyroid panel: TSH, adjusted T4, free T4
- Serum Pregnancy Test (for females of childbearing potential)
- Plasma ACTH and serum cortisol levels (every three hours from 9:00 – 15:00)
    - Blood draw will be done via intravenous catheter every 3 hours from 9AM to 3PM.
        - In the case that an intravenous catheter cannot be placed, one blood draw will be performed at every 3 hour interval.

- ECG
- 24 hour urinary free cortisol any time between days 24-28
    - Subjects will collect 3 - 24 hour UFC any time between days 24-28 prior to coming in for study visit 6
- 2 - late night salivary cortisol at 23:00-24:00 between days 24-28

Visit 6: Day #29

- History and physical exam
- Adverse events assessment
- CBC with differential, CMP (including fasting blood glucose), , PT/INR
- Fasting lipid profile, HbA1C
- Hormone profile: PRL, TSH, adjusted T4, free T4, IGF-1, LH, FSH, estradiol for females, testosterone (total and free) for males
- Serum Pregnancy Test (for females of childbearing potential)
- Plasma ACTH and serum cortisol levels (every three hours from 9:00 – 15:00)
    - Blood draw will be done via intravenous catheter every 3 hours from 9AM to 3PM.
        - In the case that an intravenous catheter cannot be placed, one blood draw will be performed at every 3 hour interval.
- ECG
- Visual Fields
- Pituitary MRI
- 24 hour urinary free cortisol on days any time between 30-35
    - Subjects will collect 3 - 24 hour UFC any time between days 30-35 prior to coming in for study completion visit
- 2 - late night salivary cortisol at 23:00-24:00 between days 30-35

Study Completion Visit: Day 36

- History and physical exam
- Adverse events assessment

Table 1. Study procedure flow sheet

Procedures	Screening Visit -60 to -1	Visit 1 Baseline	Visit 2 Day 1	Visit 3 Day 8	Visit 4 Day 15	Visit 5 Day 22	Visit 6 Day 29	Study Completion Visit
Standard of Care Procedures: Items, drugs and services that are part of regular care and would be done even if you did not take part in this research study. These will be billed to you and/or your insurance company.								
Visual Field Exam	X[1]							
MRI of the Pituitary	X[2]							
Research Related Procedures: Items, drugs and services done for research purposes only. These will be covered by the sponsor of the study and will NOT be billed to your insurance company.								
Review of medical records	X							
History and Physical Exam	X	X	X	X	X	X	X	X
Serum Pregnancy Test (for female of childbearing potential)	X	X	X	X	X	X	X	X
Blood Draw for blood count with differential, comprehensive metabolic panel (CMP), and prothrombin time/INR	X	X		X	X	X	X	
Thyroid panel (TSH, adjusted T4, free T4)				X	X	X		
Hormone profile (Prolactin, FSH, LH, estradiol for female, total and free testosterone for male, IGF-1, TSH, adjusted T4, free T4)	X	X					X	
Fasting lipid profile		X					X	
HbA1C	X	X					X	
Dexamethasone dispensed[3]	X							
Electrocardiogram (ECG)	X	X		X	X	X	X	
24 hour Urine Collection for free cortisol	X[4]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	
Visual Field Exam		X					X	
Insertion of IV catheter peripherally for repeated blood draws		X	X	X	X	X	X	
Plasma ACTH and serum cortisol (every 3 hours from 9AM to 3PM)	X[6]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	
MRI of the Pituitary							X	
Saliva sample for salivary cortisol		X	X	X	X	X	X	
Study drug dispensed[8]			X	X	X	X		
Adverse events assessment			X	X	X	X	X	X

1. If previously performed greater than 28 days from start of therapy, repeat exam will be RES.
2. If previously performed greater than 28 days from start of therapy, repeat exam will be RES.
3. Dexamethasone is taken one time the night of the screening visit. If previously performed at Cedars-Sinai Medical Center, or if the subject has been confirmed with diagnosis such as previous surgery or IPSS repeat exam is not required.

4. 2 - 24 hour UFC can be collected anytime between one week after taking dexamethasone and the baseline visit.
5. 3 - 24 hour UFC will be collected for 3 days prior to coming in for study visit.
6. Blood draws will be done only for cortisol level between 8:00-9:00 AM on the day of screening visit, and 8:00-9:00 AM the next day after 8 mg dexamethasone
7. Blood draws will be done via intravenous catheter every 3 hours from 9AM to 3PM. In the case that an intravenous catheter cannot be placed, one blood draw will be performed at every 3 hour interval.
8. Study drug is taken orally twice per day for 4 days each week.

Section 7.0 Assessment of Efficacy

The primary efficacy population will include patients who had baseline mean UFC levels 1.5x above the upper normal range, and who completed the 4-cycle, 4-week treatment period with no more than 2 weeks of interrupted drug doses. Baseline UFC levels are defined as the mean of three 24-hour urine specimens collected within one week of baseline visit.

Primary outcome:

- The primary efficacy outcome will be normalization of mean UFC levels after 4-weeks treatment. This will be measured as the mean of three 24-hour urine specimens collected at baseline and within 5 days before completion of the study. Patients who achieve a mean post-treatment UFC level within the normal range are considered responders.

Secondary outcome:

- Plasma ACTH, serum cortisol levels will be measured at baseline, day 1, 8, 15, 22 and 29 every 3 hours from 9:00–15:00. The areas under the concentration-time curve (AUC_{0-8h}) for ACTH and cortisol will be calculated using a trapezoidal method from 0–6 h (the last sampling time point) by WinNonlin software (Pharsight, Mountain View, CA). Visual field will be measured at baseline and end of the study.

Section 8.0 Assessment of Safety

Cross reference: [IND No. 71,011-CYC202(R-roscovitine or Seliciclib)].

Monitoring for adverse effects (further detailed below):

- Patients will be seen in clinic every week and evaluated for adverse effects with physical exam, ECG, chemistry, hematology panels, as listed in Table 1. Adverse events will be graded according to NCI Common Terminology Criteria (NCI-CTCAE). Known common adverse events of R-roscovitine at 400mg oral dose twice daily include fatigue, fever, nausea, vomiting, anorexia, hepatic dysfunction (increased ALT/SGPT, AST/SGOT and AP), hypokalemia, hyponatremia, anemia and insomnia (cross reference: IND No. 71,011-CYC202(R-roscovitine or Seliciclib)).
-
- If at any time a patient demonstrates a worsening of their visual fields or tumor growth on MRI, they will be withdrawn from the study and referred for surgery.
- All patients will be followed regularly until the resolution or stabilization of drug-related toxicity. A post-treatment follow-up visit will be conducted within 4 weeks after the last dose of the study drug or prior to the initiation of new treatment. This post-treatment evaluation may be conducted over the telephone if the patient is unable to return to the clinic.
- In all cases where the subject is withdrawn due to unusual or unusually severe adverse event considered related to R-roscovitine, the withdrawal will be reported as an SAE and on the CRFs.

Dose delays and dose reductions:

- Dose delay or reduction for drug-related toxicity is permitted; however the DSMB will be consulted prior to implementing any change in dosing. Dose delay or reduction will only be implemented when all supportive care measures have been exhausted without an improvement of patient status.
- Those subjects with toxicity less than or equal to NCI-CTCAE Grade 2 will continue their full dose, unless the investigator, based on clinical judgment, feels it is necessary to have their dose reduced or held.
- Patients experiencing unacceptable toxicity (Grade 3 or higher) that the investigator considers directly attributable to R-roscovitine should have their dose held, reduced, or should be withdrawn from the study (**Table 2 and 3**).
- Treatment may be delayed, up to 2 weeks, until the patient recovers completely or the adverse event reverts to Grade 1 or to baseline grade.
- A maximum two dose reductions are permitted per subject and subjects will not be re-challenged to a higher dose level.
- The Investigator will consult the DSMB prior to continuing therapy for any subject requiring a delay of more than 2 weeks for unresolved toxicity, but in general, such subjects should be withdrawn from the study.

Table 2. Guideline for Dose Modifications

Patients	Adverse event	Action
All	Grade ≤ 2 (mild to moderate)	No drug dose adjustments*
	Grade 3 (severe)	Hold the dose for a maximum of 14 days until the patient recovers completely or the adverse event reverts to ≤ Grade 1 or to baseline grade. Resume at one dose level lower (**Table 3**). If AE recurs at grade 3, reduce dose by 200 mg q.d. again and maintain at lower dose. If AE does not improve to grade ≤ 2 within 1 week on 200 mg b.i.d., patient is to discontinue treatment.
	Grade 4 (life-threatening)	Discontinue study drug

*Unless the investigator, based on clinical judgment, feels it is necessary to have dose reduced or held.
Note: for hepatic safety management follow ***Liver Chemistry Stopping and Follow up Criteria***

Table 3. Dose Reduction Table

Dose Level	Weekly R-roscovitine Dose and schedule
-2	200mg, two times daily x 4 days
-1	200mg, three times daily x 4 days
0	400mg, twice daily x 4 days

- If treatment is delayed for reasons other than toxicity (i.e., unplanned travel or vacation, or lack of transportation to the site) and the subject has insufficient investigational

product available, the subject should resume the usual dosing schedule once drug supply has been made available. However, if the subject has been off therapy for more than 2 weeks, the Investigator will consult the DSMB prior to continuing therapy.

- If a patient permanently discontinues study treatment, the patients will be considered as prematurely discontinued from the study. Patients will have to perform a study phase completion evaluation on the day of the last study drug administration.

Hepatic adverse events
Hepatobiliary events have been seen in subjects taking seliciclib. As a precaution, the following will be reported as an SAE:

- ALT or AST >3 × ULN and total bilirubin >2.0 × ULNOther hepatic events should be documented as an AE or an SAE as appropriate.

Liver chemistry stopping and follow up criteria have been designed to assure subject safety and evaluate liver event etiology. All subjects who meet liver chemistry criteria requiring permanent discontinuation of investigational product (IP) must continue to be followed for the study assessments and procedures as defined in **Table 4**.

Table 4. Liver Chemistry Stopping Criteria

Hepatic AE	Management/Next Dose for R-roscovitine
• ALT or AST >3 × ULN *and* <5 × ULN **and** total bilirubin ≤2 × ULN, without signs or symptoms of hepatitis or hypersensitivity, **and** who can be monitored weekly	• Hold IP for maximum 2 weeks, repeat liver chemistry testing weekly • Discuss with DSMB for possible re-challenge with IP and define dose reduction • If the treatment is exhibiting efficacy *and* the subject wants to continue after being informed of the liver chemistry results, then the IP may be re-started at the DSMB defined dose reduction. • Liver chemistries and aforementioned signs and symptoms should be monitored at a minimum of every week until resolution, stabilization, or a return to baseline values, at which point monitoring should be continued per protocol.
• ALT or AST >8 × ULN; *or* • ALT or AST >5 × ULN persisting for ≥2 weeks; *or* • ALT or AST >3 × ULN *and* (total bilirubin >2.0 × ULN *or* INR >1.5); *or* • ALT or AST>3 × ULN with signs or symptoms of hepatitis or hypersensitivity*	• Immediately and permanently discontinue IP; • Complete the SAE data collection tool, the liver event CRF, and the liver imaging and/or liver biopsy CRFs, if these tests are performed; • In addition to the liver event follow up assessments defined below, the following are suggested: specialist or hepatology consultation; anti-nuclear antibody, anti-smooth muscle antibody, and Type 1 anti-liver kidney microsomal antibodies; and liver imaging and/or liver biopsy to evaluate liver disease; • Promptly report the event to Cyclacel within 24 hours of learning its occurrence • Monitor every week until liver chemistries resolve, stabilize or return to within baseline values; • **Do not re-challenge with investigational product.**

Hepatic AE	Management/Next Dose for R-roscovitine
• If ALT or AST>3 and < 5 × ULN for > 4 weeks	Discontinue study drug

*Includes: worsening of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia

Liver Chemistry Follow up Criteria

For all subjects who meet any of the liver chemistry criteria described above, make every attempt to carry out the liver event follow up assessments described below:

- Viral hepatitis serology including:
    - Hepatitis A IgM antibody;
    - Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM);
    - Hepatitis C RNA;
    - Cytomegalovirus IgM antibody;
    - Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing);
    - Hepatitis E IgM antibody (if subject resides or has traveled outside USA or Canada in past 3 months);
- Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH);
- Complete blood count with differential to assess eosinophilia;
- Record the appearance or worsening of clinical symptoms of hepatitis, or hypersensitivity, fatigue, decreased appetite, nausea, vomiting, abdominal pain, jaundice, fever, or rash as relevant on an AE report form;
- Record use of concomitant medications, acetaminophen, herbal remedies, other over the counter medications, or putative hepatotoxins, on the concomitant medications report form;
- Record alcohol use on the liver event alcohol intake case report form.

Adrenal adverse events

If at any time this subject meets any of the adrenal suppression stopping criteria, then proceed as described below.

Table 5. Adrenal Suppression Stopping Criteria

Adrenal AE	Management/Next Dose for R-roscovitine
24 hr UFC < LLN with signs or symptoms of adrenal insufficiency (fatigue, nausea, vomiting, abdominal pain and hypotension)	• Hold IP for maximum 2 weeks • Repeat 24 hr UFC in 1 week • Discuss with DSMB the possibility of re-challenge with IP
24 hr UFC <LLN without signs or symptoms of adrenal insufficiency, **and** who can be monitored weekly	• Continue IP • Monitor weekly until 24 hr UFC stabilize, then monitor 24 hr UFC as per protocol assessment schedule

If the subject wants to continue for potential benefit of R-roscovitine therapy after being informed of the results of 24 hr UFC testing, then the IP may be re-started at the reduced dose agreed upon by the investigator. 24 hr UFC and aforementioned signs and symptoms

should be monitored at a minimum of every week until resolution, stabilization, or a return to baseline values, at which point monitoring should be continued per protocol.

Glucose metabolism adverse events
The principal investigator is to evaluate the risks for hyperglycemia in all patients, following established guidelines by the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD). The principal investigator is to educate the patient on the signs and symptoms of hyperglycemia.

Patients with a prior history or new diagnosis of impaired fasting glucose, impaired glucose tolerance or diabetes mellitus, or at risk of developing these conditions should monitor their blood glucose by fingerstick twice daily (fasting and 2-hours post-prandial). The patients will be encouraged to keep a diary for their blood glucose for appropriate management of the disease throughout the trial and present the collected data to their physician/diabetes specialist for evaluation.

Any patient showing a fasting plasma glucose > 130 mg/dL (7.2 mmol/L), or 2-hour post-prandial capillary glucose (PPG) ≥ 180 mg/dl (10 mmol/L) on two consecutive measurements that are approximately 14 days apart, and/or HbA1c > 7% is evaluated by a diabetes specialist for appropriate treatment. In addition, these patients are given information regarding diabetes disease management. Initiation or adjustment of hypoglycemic treatment is considered as early as possible at the discretion of the diabetes specialist. The above instructions regarding glucose monitoring by fingerstick and collection of blood level in a diary are given to the patients.

Patients who develop symptoms of diabetes mellitus out of control and/or fasting blood glucose values consistently as high as or above 240 mg/dL in spite of appropriate therapeutic interventions are discontinued from the study, as are those patients whose HbA_{1c} is greater than or equal to 8% in spite of appropriate therapeutic intervention.

Electrolyte metabolism adverse events
High levels of cortisol also exert mineralcorticoid (aldosterone) activity, stimulating absorption of sodium and excretion of potassium at the renal collecting tubules. Hypokalemia is most often associated with Cushing's syndrome due to ectopic ACTH production. Patients with pituitary corticotroph tumors may present with mild hypokalemia (3.0-3.5 mmol/L). Hypokalemia associated with seliciclib appeared to be dose dependent, occurring only in doses higher than 1200 mg b.i.d. 3 days per every 2 weeks. Patients who develop serum potassium values consistently <3.0 mmol/L in spite of appropriate therapeutic interventions are discontinued from the study.

Dermatologic (skin) adverse events
Significant skin adverse events (Grade 3 or more) resulting from seliciclib are rare (<1%). For NCI-CTCAE v3.0 Grade 4 rash manifested as toxic epidermal necrolysis (i.e. Stevens-Johnson's Syndrome etc) seliciclib must be permanently discontinued. Subjects with poorly tolerated skin adverse events may be successfully managed by providing a brief (up to 14 days) therapy interruption; the daily dose of seliciclib should then be reinstated. However, the rash may improve without the need for interrupting therapy with seliciclib. A variety of agents can be used to manage skin rashes. These include mild-to-moderate strength steroid creams, topical or systemic antibiotics, topical or systemic antihistamines, and occasionally, retinoid creams. There is no standard, known, or established treatment proven effective for drug-related skin rashes or changes due to seliciclib. The need for oral or topical antibiotics will be a clinical decision of the investigator and should be preceded by a culture of affected areas and, if

indicated, a dermatology consultation. Oral steroids will be strongly discouraged. Other options for treatment of significant rashes may be determined upon consultation with dermatologist.

Gastrointestinal adverse events
If GI adverse events are not appropriately managed, they may be associated with the development of dehydration. Management of gastrointestinal adverse events is discussed in detail in below.

Nausea, vomiting, or both
In subjects who have emesis and are unable to retain seliciclib, every attempt should be made to obtain control of nausea and vomiting. A dose may be repeated if tablets can be visually found after the vomiting episode.

PHARMACEUTICAL INFORMATION

Seliciclib *(*NSC # *)*

Chemical Name: 2-(R)-(1-Ethyl-2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine

Other Names: CYC202

Mode of Action: Selective CDK inhibitor

How Supplied: 200 mg/capsule

Storage: Cross reference [IND No. 71,011-CYC202(R-roscovitine or Seliciclib)].

Stability: Cross reference [IND No. 71,011-CYC202(R-roscovitine or Seliciclib)].

Route of Administration: Oral

REGULATORY AND REPORTING REQUIREMENTS

It is the responsibility of the investigator to document all adverse events, which occur during the investigation. An adverse event is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of the medicinal product, whether or not considered related to the medicinal product. *An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.* Anticipated day-to-day fluctuations of the disease under study that do not represent a clinically significant exacerbation or worsening need not be considered an adverse event.

All adverse events occurring from the first dose of investigational product until five days after the last dose will be recorded **REGARDLESS OF WHETHER OR NOT THEY ARE CONSIDERED DRUG RELATED.** In addition, any SAEs, which occur as a result of protocol specific diagnostic procedures or interventions will also be reported.

Assessment of Causality
Every effort will be made by the investigator to explain each adverse event and assess its relationship, if any, to study drug treatment. Causality will be assessed using the following categories: no (not related), or yes (reasonable possibility).

The degree of certainty with which an adverse experience is attributed to drug treatment (or alternative causes, e.g. natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the experience can be understood in terms of the following:

- Known pharmacology of the drug
- Reaction of similar nature being previously observed with this drug or class of drug
- The event having often been reported in literature for similar drugs as drug related (e.g. skin rashes, blood dyscrasia)
- The event being related by time to drug administration terminating with drug withdrawal (dechallenge) or reproduced on rechallenge.

The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE form. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Following-up of Adverse Events
Investigators should follow-up subjects with adverse events until the event has subsided (disappeared) or until the condition has stabilized.

Definition of Serious Adverse Events:
A serious adverse experience is any event, which is fatal, life threatening, disabling or incapacitating or results in hospitalization, prolongs a hospital stay or is associated with congenital abnormality. In addition, any experience which the investigator regards as serious or which would suggest any significant hazard, contraindication, side effect or precaution that may be associated with the use of the drug should be reported as a serious adverse event. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Abnormal laboratory findings (e.g., clinical chemistry, hematology) or other abnormal assessments (e.g., x-rays, scans, vital signs, etc.) that are judged by the investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definition of an AE or SAE.

Life threatening definition:
An adverse event is life threatening if the patient was at immediate risk of death from the event as it occurred (i.e. it does not include a reaction that if it had occurred in a more serious form might have caused death). For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening even though drug-induced hepatitis could be fatal.

Disability/incapacitating definition:
An adverse experience is incapacitating or disabling if the experience results in a substantial and/or permanent disruption of the patient's ability to carry out normal life functions.

Hospitalization definition:
Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

Additional SAE definitions
- All Grade 4 laboratory abnormalities
- Hepatobiliary events have been seen in subjects taking seliciclib. As a precaution, the following will be reported as an SAE:

  - ALT >3 × ULN and total bilirubin >2.0 × ULN (>35% direct; bilirubin fractionation required).
  - Other hepatic events should be documented as an AE or an SAE as appropriate.SAEs, pregnancies, and liver function abnormalities meeting pre-defined stopping criteria will be reported promptly to Cedars-Sinai Office of Research Compliance as described in the following table once the investigator determines that the event meets the protocol definition for that event.

Reporting Serious Adverse Events
Any serious adverse events, which occur during the clinical study or within 5 days of receiving the last dose of study medication, whether or not related to the study drug, will be reported by the investigator. In addition, any SAEs, which occur as a result of protocol specific diagnostic procedures or interventions will also be reported.

Lack of Efficacy
"Lack of efficacy" *per se* will not be reported as an AE. The signs and symptoms or clinical sequelae resulting from lack of efficacy will be reported if they fulfill the AE or SAE definition.

All serious adverse events will be reported to the FDA, Cedars-Sinai Office of Research Compliance and Cyclacel Pharmaceutical, Inc. by the investigator.
The SAE report will comprise a full written summary, detailing relevant aspects of the adverse events in question. Where applicable, information from relevant hospital case records and autopsy reports will be included. Follow-up information will also be forwarded within 24 hours.

SAEs brought to the attention of the investigator at any time after cessation of seliciclib and considered by the investigator to be related or possibly related to seliciclib will be reported if and when they occur. Additionally, SAEs that are related to study participation (e.g., procedures, invasive tests, change from existing therapy) or are related to a concurrent medication will be collected and recorded from the time the subject consents to participate in the study until he/she is discharged.

Pregnancy
Patients who become pregnant during the study should discontinue the study immediately. Patients should be instructed to notify the investigator if it is determined after completion of the study that they become pregnant either during the treatment phase of the study or within five days after the treatment period. Whenever possible a pregnancy should be followed to term, any premature termination reported, and the status of the mother and child after delivery reported.

Section 9.0 Statistics

Due to the rarity of Cushing disease in the general population, it is difficult to do a randomized placebo controlled trial with sufficient sample size numbers to achieve adequate power. By using subjects as their own controls, we measure pre- and post-treatment changes on the same population, therefore alleviate the issues with recruitment.

We used a Simon exact single-stage phase II design (8) to determine the sample size for 80% power and a one-sided 0.05 significance level. The trial tests the null hypothesis that the seliciclib (response rate) ≤ 5% (unacceptable response rate) versus the alternative hypothesis that RR ≥ 25% (promising response rate). We enroll 16 patients. If at least 3 of the 16 respond, we conclude that seliciclib is promising in the treatment of ACTH-producing pituitary tumors.
A regression model will be used to investigate the relationship between end-of-study UFC and baseline UFC. The comparison between serum cortisol or plasma ACTH in patients who responded to seliciclib and in those who did not respond will be performed by a Student's *t* test. Categorical variables, i.e. normalization of 24 hour UFC, development of new pituitary deficits, and visual field deficits will be compared in the pre- and post- treatment time points using fisher's exact test.

Section 10.0 Quality Control and Quality Assurance

Only samples and data collected with appropriate consent will be used for the proposed analysis. Specimens and data collected under the proof of concept clinical trial of seliciclib will be coded in such a way so that other members of the research team will be unable to ascertain the identity of specific patients. All of our data will be maintained confidentially. Data will be kept in locked file cabinets and in password protected files on computer systems.

Section 11.0 Ethical considerations relating to the trial

Subjects will be recruited from Cedars-Sinai Pituitary Center. They will be patients followed clinically. Patients will be approached regarding participation in the study and given consent forms to review at home. They will be reassured that their clinical care will not be affected if they choose not to participate. Patients will be given opportunities to ask questions and review the consent process. Once they consent and enroll in the study, their data will be de-identified for the duration of the study. All patients entering the trial will be given the study drug. There is no placebo arm. The risks of the study are related to the side effects of the study drug and patients will be monitored for this and informed on the effects. The benefits of the study are normalization of urinary free cortisol. There are potential conflicts of interest between the patient's need for surgical referral and participation in the research study. However, by limiting the study period to 4 weeks, which is the average pre-op preparation time for our pituitary surgery patients, we will minimize the conflict of interest.

Section 12.0 Data Handling and Recordkeeping

Data will be maintained in separate research charts in a locked cabinet in a locked office. The data will be entered as well into an electronic database on a password- protected computer. Only study staff and the investigators will have access to these records.

Section 13.0 Financing and Insurance

Funding for this study will be through a Cedars-Sinai institutional grant. Patients will only be charged for the initial MRI and visual field test.

Section 14.0 Publication Policy

We intend to submit our research findings to relevant peer-reviewed journals for publication. We will submit data for presentation at international meetings. We will adhere to the NIH Grants

Policy Statement on Sharing of Biomedical Research Resources, including the “Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources: Final Notice” (64FR 72090, December 23, 1999; and described at http://ott.od.nih.gov/NewPages/RTguide/final.html.

References

1. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. The Journal of clinical endocrinology and metabolism. 2008;93(5):1526-40. Epub 2008/03/13. doi: 10.1210/jc.2008-0125. PubMed PMID: 18334580; PubMed Central PMCID: PMC2386281.

2. Biller BM, Grossman AB, Stewart PM, Melmed S, Bertagna X, Bertherat J, Buchfelder M, Colao A, Hermus AR, Hofland LJ, Klibanski A, Lacroix A, Lindsay JR, Newell-Price J, Nieman LK, Petersenn S, Sonino N, Stalla GK, Swearingen B, Vance ML, Wass JA, Boscaro M. Treatment of adrenocorticotropin-dependent Cushing's syndrome: a consensus statement. The Journal of clinical endocrinology and metabolism. 2008;93(7):2454-62. Epub 2008/04/17. doi: 10.1210/jc.2007-2734. PubMed PMID: 18413427; PubMed Central PMCID: PMC3214276.

3. Henry RR, Ciaraldi TP, Armstrong D, Burke P, Ligueros-Saylan M, Mudaliar S. Hyperglycemia associated with pasireotide: results from a mechanistic study in healthy volunteers. The Journal of clinical endocrinology and metabolism. 2013;98(8):3446-53. Epub 2013/06/05. doi: 10.1210/jc.2013-1771. PubMed PMID: 23733372.

4. Fleseriu M, Biller BM, Findling JW, Molitch ME, Schteingart DE, Gross C. Mifepristone, a glucocorticoid receptor antagonist, produces clinical and metabolic benefits in patients with Cushing's syndrome. The Journal of clinical endocrinology and metabolism. 2012;97(6):2039-49. Epub 2012/04/03. doi: 10.1210/jc.2011-3350. PubMed PMID: 22466348.

5. Melmed S. Pathogenesis of pituitary tumors. Nature reviews Endocrinology. 2011;7(5):257-66. Epub 2011/03/23. doi: 10.1038/nrendo.2011.40. PubMed PMID: 21423242.

6. Quereda V, Malumbres M. Cell cycle control of pituitary development and disease. Journal of molecular endocrinology. 2009;42(2):75-86. doi: 10.1677/JME-08-0146. PubMed PMID: 18987159.

7. Jordan S, Lidhar K, Korbonits M, Lowe DG, Grossman AB. Cyclin D and cyclin E expression in normal and adenomatous pituitary. European journal of endocrinology / European Federation of Endocrine Societies. 2000;143(1):R1-6. Epub 2000/06/28. PubMed PMID: 10870044.

8. Zhang HS, Gavin M, Dahiya A, Postigo AA, Ma D, Luo RX, Harbour JW, Dean DC. Exit from G1 and S phase of the cell cycle is regulated by repressor complexes containing HDAC-Rb-hSWI/SNF and Rb-hSWI/SNF. Cell. 2000;101(1):79-89. doi: 10.1016/S0092-8674(00)80625-X. PubMed PMID: 10778858.

9. Geng Y, Eaton EN, Picon M, Roberts JM, Lundberg AS, Gifford A, Sardet C, Weinberg RA. Regulation of cyclin E transcription by E2Fs and retinoblastoma protein. Oncogene. 1996;12(6):1173-80. PubMed PMID: 8649818.

10. Sengupta T, Abraham G, Xu Y, Clurman BE, Minella AC. Hypoxia-inducible factor 1 is activated by dysregulated cyclin E during mammary epithelial morphogenesis. Molecular and

cellular biology. 2011;31(18):3885-95. doi: 10.1128/MCB.05089-11. PubMed PMID: 21746877; PubMed Central PMCID: PMC3165725.

11. Minella AC, Loeb KR, Knecht A, Welcker M, Varnum-Finney BJ, Bernstein ID, Roberts JM, Clurman BE. Cyclin E phosphorylation regulates cell proliferation in hematopoietic and epithelial lineages in vivo. Genes & development. 2008;22(12):1677-89. doi: 10.1101/gad.1650208. PubMed PMID: 18559482; PubMed Central PMCID: PMC2428064.

12. Kossatz U, Breuhahn K, Wolf B, Hardtke-Wolenski M, Wilkens L, Steinemann D, Singer S, Brass F, Kubicka S, Schlegelberger B, Schirmacher P, Manns MP, Singer JD, Malek NP. The cyclin E regulator cullin 3 prevents mouse hepatic progenitor cells from becoming tumor-initiating cells. The Journal of clinical investigation. 2010;120(11):3820-33. doi: 10.1172/JCI41959. PubMed PMID: 20978349; PubMed Central PMCID: PMC2964969.

13. Ma Y, Fiering S, Black C, Liu X, Yuan Z, Memoli VA, Robbins DJ, Bentley HA, Tsongalis GJ, Demidenko E, Freemantle SJ, Dmitrovsky E. Transgenic cyclin E triggers dysplasia and multiple pulmonary adenocarcinomas. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(10):4089-94. doi: 10.1073/pnas.0606537104. PubMed PMID: 17360482; PubMed Central PMCID: PMC1820713.

14. Loeb KR, Kostner H, Firpo E, Norwood T, K DT, Clurman BE, Roberts JM. A mouse model for cyclin E-dependent genetic instability and tumorigenesis. Cancer cell. 2005;8(1):35-47. doi: 10.1016/j.ccr.2005.06.010. PubMed PMID: 16023597.

15. Roussel-Gervais A, Bilodeau S, Vallette S, Berthelet F, Lacroix A, Figarella-Branger D, Brue T, Drouin J. Cooperation between cyclin E and p27(Kip1) in pituitary tumorigenesis. Mol Endocrinol. 2010;24(9):1835-45. Epub 2010/07/28. doi: 10.1210/me.2010-0091. PubMed PMID: 20660298.

16. Bilodeau S, Vallette-Kasic S, Gauthier Y, Figarella-Branger D, Brue T, Berthelet F, Lacroix A, Batista D, Stratakis C, Hanson J, Meij B, Drouin J. Role of Brg1 and HDAC2 in GR trans-repression of the pituitary POMC gene and misexpression in Cushing disease. Genes & development. 2006;20(20):2871-86. Epub 2006/10/18. doi: 10.1101/gad.1444606. PubMed PMID: 17043312; PubMed Central PMCID: PMC1619949.

17. Liu NA, Jiang H, Ben-Shlomo A, Wawrowsky K, Fan XM, Lin S, Melmed S. Targeting zebrafish and murine pituitary corticotroph tumors with a cyclin-dependent kinase (CDK) inhibitor. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(20):8414-9. Epub 2011/05/04. doi: 10.1073/pnas.1018091108. PubMed PMID: 21536883; PubMed Central PMCID: PMC3100964.

18. Vlotides G, Eigler T, Melmed S. Pituitary tumor-transforming gene: physiology and implications for tumorigenesis. Endocrine reviews. 2007;28(2):165-86. Epub 2007/02/28. doi: 10.1210/er.2006-0042. PubMed PMID: 17325339.

19. Pei L, Melmed S. Isolation and characterization of a pituitary tumor-transforming gene (PTTG). Mol Endocrinol. 1997;11(4):433-41. Epub 1997/04/01. PubMed PMID: 9092795.

20. Chesnokova V, Zonis S, Kovacs K, Ben-Shlomo A, Wawrowsky K, Bannykh S, Melmed S. p21(Cip1) restrains pituitary tumor growth. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(45):17498-503. Epub 2008/11/05. doi: 10.1073/pnas.0804810105. PubMed PMID: 18981426; PubMed Central PMCID: PMC2577704.

21. Chesnokova V, Zonis S, Zhou C, Ben-Shlomo A, Wawrowsky K, Toledano Y, Tong Y, Kovacs K, Scheithauer B, Melmed S. Lineage-specific restraint of pituitary gonadotroph cell adenoma growth. PloS one. 2011;6(3):e17924. Epub 2011/04/06. doi: 10.1371/journal.pone.0017924. PubMed PMID: 21464964; PubMed Central PMCID: PMC3064664.

22. Lapenna S, Giordano A. Cell cycle kinases as therapeutic targets for cancer. Nature reviews Drug discovery. 2009;8(7):547-66. doi: 10.1038/nrd2907. PubMed PMID: 19568282.

23. Legraverend M, Grierson DS. The purines: potent and versatile small molecule inhibitors and modulators of key biological targets. Bioorganic & medicinal chemistry. 2006;14(12):3987-4006. Epub 2006/03/01. doi: 10.1016/j.bmc.2005.12.060. PubMed PMID: 16503144.

24. Lamolet B, Pulichino AM, Lamonerie T, Gauthier Y, Brue T, Enjalbert A, Drouin J. A pituitary cell-restricted T box factor, Tpit, activates POMC transcription in cooperation with Pitx homeoproteins. Cell. 2001;104(6):849-59. Epub 2001/04/06. PubMed PMID: 11290323.

25. Wesierska-Gadek J, Krystof V. Selective cyclin-dependent kinase inhibitors discriminating between cell cycle and transcriptional kinases: future reality or utopia? Annals of the New York Academy of Sciences. 2009;1171:228-41. doi: 10.1111/j.1749-6632.2009.04726.x. PubMed PMID: 19723060.

26. Siegel-Lakhai Wea. ASCO Proceedings, Abs 2060. 2005.

27. Yeo et al. J Clin Oncol 2009 27-15s (Suppl abstr 6026).