Protocol Title: PET imaging of phosphodiesterase-4 (PDE4) in brain and peripheral organs of McCune-Albright syndrome
Abbreviated Title: PDE4 in McCune-Albright syndrome
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Principal Investigator

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Human Research Protections Program Investigator and Staff Training:

For this protocol, the following "Just in time" human subjects protection training courses are required for investigators and staff:

- NIAID GCP course or CITI GCP modules
- Unanticipated Problems and Reporting Requirements in Biomedical Research

Total requested accrual *(separately specify planned accrual for each subject group)*
(20) Patients
(15) Healthy Volunteers

Project Uses Ionizing Radiation: ☐ No ☑ Yes (*attach RSC/RDRC documentation)*
☐ Medically-indicated only
☑ Research-related only
☐ Both

IND/IDE ☐ No ☑Yes *(attach FDA documentation)*
Drug/Device/# [^{11}C](*R*)-rolipram/IND #73,149
Sponsor: Robert B. Innis, MD, PhD

Durable Power of Attorney ☑ No ☐ Yes

Multi-institutional Project ☑ No ☐ Yes

Data and Safety Monitoring Board ☑ No ☐ Yes

Technology Transfer Agreement ☑ No ☐ Yes

Samples are being stored ☑ No ☐ Yes

Flesch-Kincaid reading level of consent form: 8.4 MAS
(exclude boilerplate in assessing reading level) 8.4 HV

Précis:

Objective: McCune-Albright syndrome (MAS) is a mosaic disease arising from early embryonic somatic activating mutations of *GNAS,* which encodes the 3′, 5′-cyclic adenosine monophosphate (cAMP) pathway-associated G-protein, $G_s\alpha$. Constitutive activation of $G_s\alpha$ leads to increased cAMP signaling in brain, as well as in peripheral organs, particularly bones. Although subjects with MAS show psychiatric and neurological symptoms, few studies have attempted to assess brain changes in these individuals. This protocol seeks to study changes in the cAMP cascade both in brain and peripheral organs of individuals with MAS using [^{11}C](*R*)-rolipram PET, which binds to phosphodiesterase 4 (PDE4) and reflects cAMP cascade activity.

Study population: Participants will include 20 subjects with MAS and 15 healthy subjects group-matched to MAS subjects for age and gender. Both MAS subjects and healthy controls will have one or two PET scans: one whole body and one brain scan. We expect ~10 brain and ~10 whole body scan to be performed in each group. In addition, subjects who have undergone a baseline whole body scan will be given the option to have a second blocked whole body scan after administration of the PDE4 inhibitor roflumilast.

Design: Subjects with MAS will be recruited from participants in 98-D-0145 "Screening and Natural History of Patients with Polyostotic Fibrous Dysplasia and the McCune-Albright Syndrome" (PI: Alison Boyce, MD). Only participants in protocol (98-D-0145) who provided self-consent without a legally authorized representative will be recruited. Brain PET scans will be performed by measuring metabolite-corrected arterial input function. Venous blood sampling will be performed for whole body scans. Subjects willing to undergo a second whole body scan will take 500 µg roflumilast P.O. prior to the scan. Blood sampling will be performed for the second whole body scan.

Outcome measures: The primary outcome measure will be obtained in brain scans as the amount of radioligand binding quantified as distribution volume (V_T). Calculated from both brain and plasma data, V_T reflects rolipram binding to PDE4, corrected for any individual differences in metabolism of the radioligand or regional blood flow in brain. The secondary outcome measure will be obtained in whole body scans as area under the curve (AUC) of radioactivity expressed as standard uptake value (SUV). SUV is calculated by normalizing radioactivity in PET images to injection activity and body weight. V_T in brain will be compared between subjects with MAS and healthy controls. AUC will be compared within-subjects with MAS between areas of craniofacial fibrous dysplasia and adjacent unaffected bone. AUC of whole body scans will also be compared between subjects with MAS and healthy controls. We hypothesize that subjects with MAS will show greater rolipram binding than healthy controls in brain regions, as well as greater rolipram uptake in bones affected by fibrous dysplasia than in unaffected bones. The third outcome measure will be the difference in SUV between the baseline whole body scan and the blocked (after roflumilast) whole body scan. This will help determine the amount of specific [^{11}C](*R*)-rolipram binding in the periphery. We hypothesize that the [^{11}C](*R*)-rolipram signal in the blocked scan will be greatly reduced, indicating that a majority of the signal in the baseline scan is specific

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List of Abbreviations

AUC	area under the curve
cAMP	3′, 5′-cyclic adenosine monophosphate
MAS	McCune-Albright syndrome
MDD	major depressive disorder
MRI	magnetic resonance imaging
PDE4	phosphodiesterase type 4
PET	positron emission tomography

SUV	standard uptake value
V_T	total (specific plus non-displaceable) distribution volume

1. Introduction

McCune-Albright syndrome and the cAMP cascade

McCune-Albright syndrome (MAS) is a mosaic disease arising from early embryonic somatic activating mutations of *GNAS,* which encodes the 3′, 5′-cyclic adenosine monophosphate (cAMP) pathway-associated G-protein, $G_s\alpha$. Constitutive activation of $G_s\alpha$ leads to ligand-independent cAMP signaling in affected cells. Clinical manifestations of MAS in a given individual are determined by the timing of the *GNAS* mutation during embryogenesis, the tissues involved, and the role of $G_s\alpha$ in affected tissues. Affected tissues can include those derived from ectoderm, mesoderm, or endoderm, and commonly include skin, skeleton, and endocrine organs. Café-au-lait skin macules result from localized hyperpigmentation, and occur in a characteristic mosaic distribution. Fibrous dysplasia of bone arises from proliferation of undifferentiated skeletal progenitor cells leading to abnormal bone formation. Bone lesions may involve any part and combination of the skeleton, ranging from an isolated monostotic lesion to severe polyostotic disease with progressive scoliosis, facial deformity, and functional impairment. Hyperfunctioning endocrinopathies include any combination of hyperthyroidism, autonomous sex steroid production, growth hormone excess, FGF23-mediated hypophosphatemia, and hypercortisolism.

The mechanism by which $G_s\alpha$ mutations result in the manifestations of MAS is thought to be related to dysregulation of the cAMP signaling cascade. Numerous experimental systems have observed increased cAMP production as a downstream effect of activating $G_s\alpha$ mutations (2, 3). In an *in vitro* model of fibrous dysplasia, skeletal progenitor cells stably transfected with the MAS-causing $G_s\alpha$ mutation were shown to selectively upregulate specific isoforms of phosphodiesterase, an enzyme superfamily that catalyzes hydrolysis of cAMP (1)(Fig. 1). Among these was phosphodiesterase 4 (PDE4), an isoform previously shown to play a role in modulating the differentiation of skeletal progenitor cells into bone-forming osteoblasts (4). This supports a model in which skeletal progenitor cell proliferation in fibrous dysplasia may be due in part to upregulation of PDE4 in response to $G_s\alpha$-dependent cAMP production.

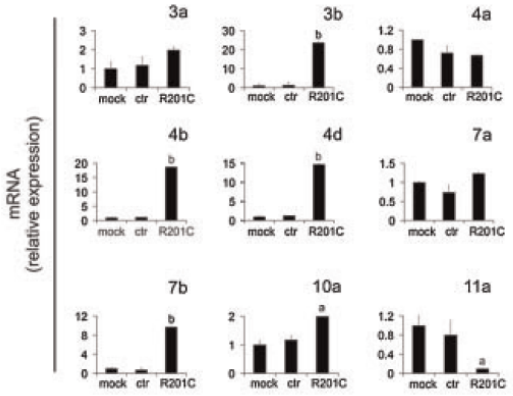


Fig.1. qPCR analysis of PDE isoform expression in skeletal progenitor cells stably transfected with the MAS-causing R201C mutation. Significantly increased expression was detected for PDE-3b, -4b, -4d, and -7b isoforms in in comparison with mock- and vector-treated cells. From (1).

While skin, bone, and endocrine disease are the most clearly defined features of MAS, multiple additional tissues may be affected due to the ubiquitous nature of $G_s\alpha$ signaling. Observations from a long-standing NIDCR natural history protocol (98-D-0145, "Screening and Natural History of Patients with Polyostotic Fibrous Dysplasia and the McCune-Albright Syndrome", PI: Alison Boyce,MD) have led to the recognition of a neuropsychiatric phenotype potentially related to effects of the mutated $G_s\alpha$ in the central

nervous system. The clinical features of MAS-associated neuropsychiatric disease are currently being investigated under 98-D-0145 and appear to fall along a spectrum that includes developmental delay, intellectual disability, anxiety, and impairments in attention and activity. Our hypothesis is that similar to the physical manifestations of MAS, these neuropsychiatric features result from $G_s\alpha$ mutation-bearing cells in the central nervous system, and are influenced by the location and function of cAMP signaling in these affected areas of the brain.

PET imaging of the cAMP cascade

PET imaging of phosphodiesterase type 4 (PDE4) using [^{11}C](*R*)-rolipram has been successfully used to study the in vivo activity of the cAMP cascade. PDEs hydrolyze the second messengers cAMP and cyclic guanosine monophosphate (cGMP) to terminate signal transduction. Eleven PDEs exist in the human body. PDE4 is selective to cAMP and is present in both brain and peripheral organs such as heart, lungs, kidney (5), immune cells (6), osteoclast (7), and osteoblast (8). Rolipram is a selective inhibitor of PDE4; PDE4 has four isozymes, and rolipram is not selective among these isozymes.

Our studies—described below—demonstrated that [^{11}C](*R*)-rolipram PET is a valuable method of detecting cAMP cascade activity in living human and animal subjects. Because PDE4 is present in almost all brain regions and no regions without PDE4 are available to use as a reference to measure rolipram binding, we used [^{11}C](*R*)-rolipram concentrations in arterial plasma (i.e., metabolite-corrected arterial input function) to measure rolipram binding in brain (9, 10). We also showed that rolipram binding can be accurately measured using only four arterial samples (9).

Broadly, the cAMP theory of depression posits that depression is caused by low cAMP signaling. The corresponding theory regarding the relevant mechanism of treatment is that chronic, but not acute, administration of antidepressants upregulates cAMP signaling. Although the cAMP theory of depression has limited supporting data, evidence for the mechanism of antidepressants has repeatedly been confirmed in animal studies. Chronic, but not acute, administration of all classes of antidepressants, as well as electroconvulsive therapy, upregulates several components of the cAMP pathway, including PDE4.

PDE4, an important component of the cAMP cascade, selectively metabolizes cAMP in the brain to the inactive monophosphate. Rolipram is a reversible inhibitor of PDE4, and binding of [^{11}C](*R*)-rolipram provides a measure of the activity of this enzyme in brain. Due to a feedback mechanism, *in vivo* binding of [^{11}C](*R*)-rolipram reflects the activity of the cAMP cascade; essentially, increased cAMP stimulates protein kinase A (PKA), which phosphorylates PDE4 that, in turn, increases rolipram binding.

We confirmed in animals that increased [^{11}C](*R*)-rolipram binding reflects the phosphorylated / active state of PDE4. Using this radioligand, we found that PDE4 binding is decreased in unmedicated patients with MDD, consistent with the cAMP theory of depression. Finally, we found that two months of treatment with an SSRI increased (normalized) PDE4 binding, consistent with the cAMP theory of the mechanism of antidepressants.

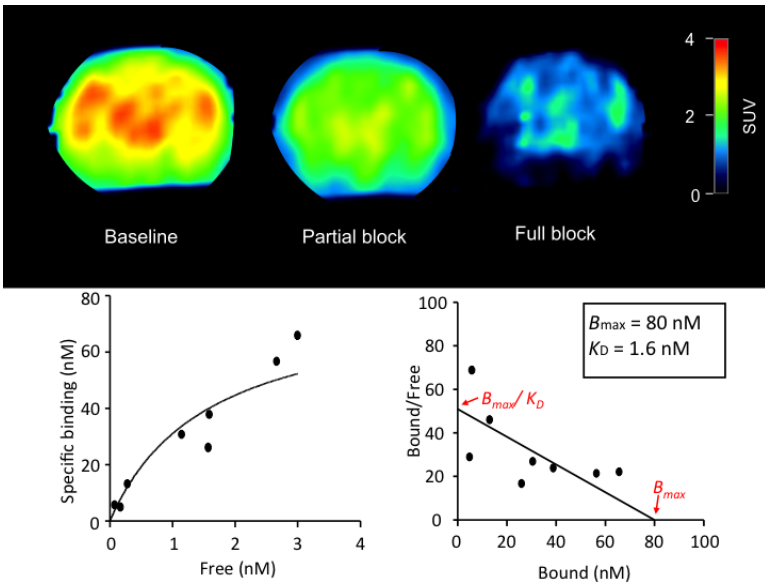


Fig 2. In vivo Scatchard plot of PDE4 binding using [^{11}C](*R*)-rolipram in awake rats. PET scans were performed with increasing concentrations of radioligand in order to saturate binding to PDE4 (bottom left), and analyzed as an in vivo Scatchard plot (bottom right). The brains were subsequently harvested for in vitro Scatchard analysis. Binding density (Bmax) was unchanged, but binding affinity was decreased 5-6 fold after death compared to the awake condition. The decreased affinity after death is consistent with the rapid dephosphorylation (within minutes) of PDE4. The phosphorylation state of PDE4 would not be possible in human postmortem tissue; PET imaging is the only technique currently available to measure the phosphorylated and active state of this enzyme.

In Vivo Density and Affinity. Our initial experiments sought to measure *in vivo* both binding site density and radioligand affinity of [^{11}C](*R*)-rolipram in the rat brain (11). We also studied two critical factors in small-animal PET scans: the influence of anesthesia and the difference in binding under *in vivo* and *in vitro* conditions. Binding site density and radioligand affinity of conscious rats were significantly greater than those of anesthetized rats, by 29% and 59%, respectively. In addition, *in vitro* affinity was five-fold greater than *in vivo* affinity, although density was similar in both conditions (Fig 2). The findings were consistent with rapid dephosphorylation of PDE4, and established that [^{11}C](*R*)-rolipram binding *in vivo* reflects cAMP cascade activity in rats.

In Vivo Phosphorylation Status. We further examined the effects of PKA modulators in conscious rats on [^{11}C](*R*)-rolipram binding compared to the much less active enantiomer [^{11}C](*S*)-rolipram (Fig 3) (12). Two drugs were studied. db-cAMP, which is a cAMP analogue, was used to activate PDE4, thus increasing activity of the cAMP-dependent PKA. PKA then phosphorylated the PDE4 enzyme and increased PDE4 activity. We also studied Rp-cAMP, a PKA inhibitor that directly inhibited PKA function, thereby decreasing PDE4 activity. Unilateral injection of the PKA activator (db-cAMP) and the PKA inhibitor (Rp-cAMP) into the striatum significantly increased and decreased [^{11}C](*R*)-rolipram binding, respectively. These effects were not caused by changes in blood flow or delivery of radioligand to brain, because these agents did not affect [^{11}C](*S*)-rolipram binding. These results supported the importance of measuring *in*

vivo [^{11}C](*R*)-rolipram binding in brain to assess response to physiological or pharmacological challenges to the cAMP second messenger system.

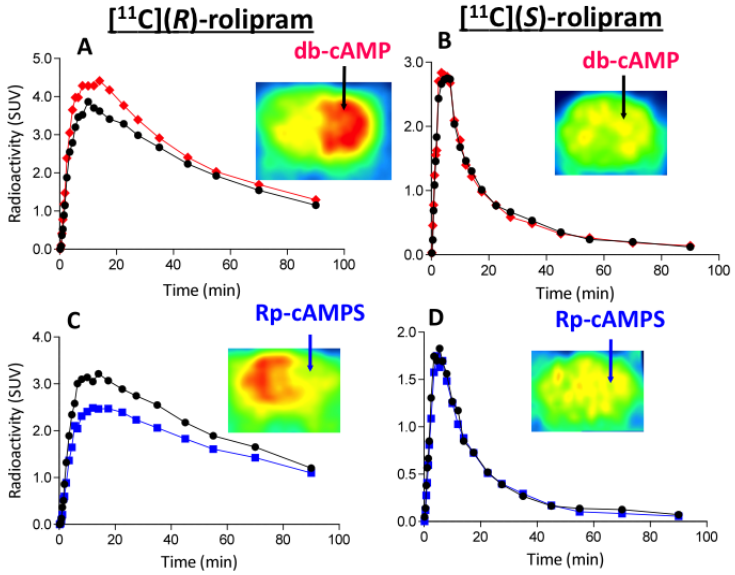


Fig 3. [^{11}C](*R*)-rolipram binding in rat brain depends on the phosphorylation state of PDE4. Rats were unilaterally injected in striatum with either a PKA activator (db-cAMP, upper panels) or a PKA inhibitor (Rp-cAMPS, lower panels). PKA-mediated phosphorylation of PDE4 increased both enzyme activity and the affinity of [^{11}C](*R*)-rolipram binding about 10-fold. Both the PKA activator and inhibitor had the expected effect on radioligand uptake in striatum injected with drug compared to the side injected with saline. These effects were not merely caused by altered blood flow, given that they were not found using the inactive enantiomer [^{11}C](*S*)-rolipram (right panels).

PDE4 Binding in MDD. Based on these preclinical findings, we hypothesized that [^{11}C](*R*)-rolipram PET in humans would provide a unique *in vivo* measure of PDE4 density and affinity not possible in postmortem tissue. Expanding our work, we sought to quantify the binding of [^{11}C](*R*)-rolipram as an indirect measure of this enzyme's activity in the brain of individuals with MDD compared with healthy controls (13). This is particularly important because animal studies had suggested that upregulation of the cAMP cascade, including PDE4, was a mechanism of action common to several antidepressant treatments. To avoid the misleading results that can be obtained from small sample sizes, we have now scanned a total of 43 unmedicated, moderately depressed patients with MDD and 25 age- and gender-matched healthy controls, which is about twice the size of most PET studies in psychiatry. Notably, about half the patients were treatment-naïve. [^{11}C](*R*)-rolipram binding in brain was measured using arterial [^{11}C](*R*)-rolipram levels to correct for the influence of cerebral blood flow. MDD subjects showed a widespread, 18% reduction in [^{11}C](*R*)-rolipram binding ($p=0.0001$) that was not caused by different gray matter volumes (Fig 4). Decreased rolipram binding of similar magnitude was observed in most brain areas. Rolipram binding did not correlate with the severity of depressive or anxiety symptoms. These results were the first to demonstrate that brain levels of PDE4, a critical enzyme that regulates cAMP, are decreased in unmedicated individuals with MDD *in vivo*. Furthermore, the results are in line with human postmortem and rodent studies demonstrating downregulation of the cAMP cascade in MDD and support the hypothesis that PDE4 inhibitors—which increase cAMP cascade activity—may have antidepressant effects.

The Effect of Chronic Antidepressants on PDE4 Binding Building on this work, we sought to determine if antidepressant treatment upregulates PDE4 in humans as it does in animals. In addition to the rolipram PET scans without medication reported above, 22 of the 43 unmedicated MDD patients had a follow up rolipram scan after starting treatment with SSRIs. In this ongoing study, our pre-determined sample size is 25 patients to be scanned after SSRI treatment. These patients showed an increase of 13 ± 36% in rolipram binding after SSRI treatment across all brain regions ($p = 0.001$ when age was used as a covariate). In contrast, 11 healthy controls who had a repeat scan without SSRI showed similar binding on repeat scans with changes of only -2 ± 13%. The change in rolipram binding after SSRI varied markedly among patients as indicated by the large standard deviation of 36%. Those patients with lower rolipram binding before SSRI showed significantly greater increases after therapy ($p < 0.007$ in all regions) indicating normalization of rolipram binding by treatment. Older patients also showed greater increases in rolipram binding after SSRI ($p \leq 0.001$) while young patients with higher baseline rolipram binding tended to show decreased rolipram binding after SSRI. According to the interim analysis, the changes in rolipram binding did not correlate with symptom improvement in all patients. Nevertheless, posthoc analyses after the full sample size has been accrued (i.e., 25 patients) may show correlations in subgroups of patients and/or specific regions of brain.

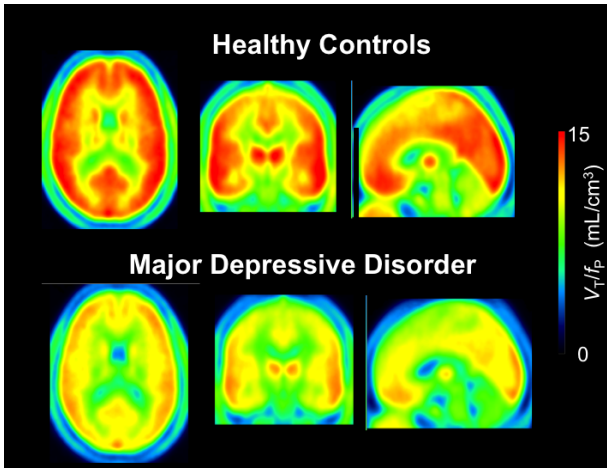


Fig 4. [^{11}C](R)-rolipram binding was globally decreased by 18% in brains of unmedicated MDD patients during a major depressive episode (n = 43) compared to matched controls (n = 35; $p < 0.001$). These mean parametric images of the two groups represent the "absolute" quantitation of radioligand binding calculated on an individual voxel level and using the concentration of radioligand in serial arterial blood samples.

Taken together, these results elucidate two important and related points. First, the cAMP cascade, as indirectly measured with PDE4 binding, was downregulated in unmedicated patients with MDD. Second, antidepressant treatment normalized this [^{11}C](R)-rolipram downregulation. These studies suggest that PDE4 inhibition, perhaps via subtype selective agents, might again be assessed for efficacy in MDD; the results also broadly support the cAMP theory of depression and of antidepressant action.

PDE4 inhibitor roflumilast

There are currently two FDA approved PDE4 inhibitors on the market: roflumilast (Daliresp) and apremilast (Otezla). Both are marketed as anti-inflammatory, but the former is indicated for chronic obstructive pulmonary disease, while the latter is used to treat psoriasis/ psoriatic arthritis. The side effect profiles for both drugs are similar in that the primary complaint is gastrointestinal (Table 1). While both drugs could

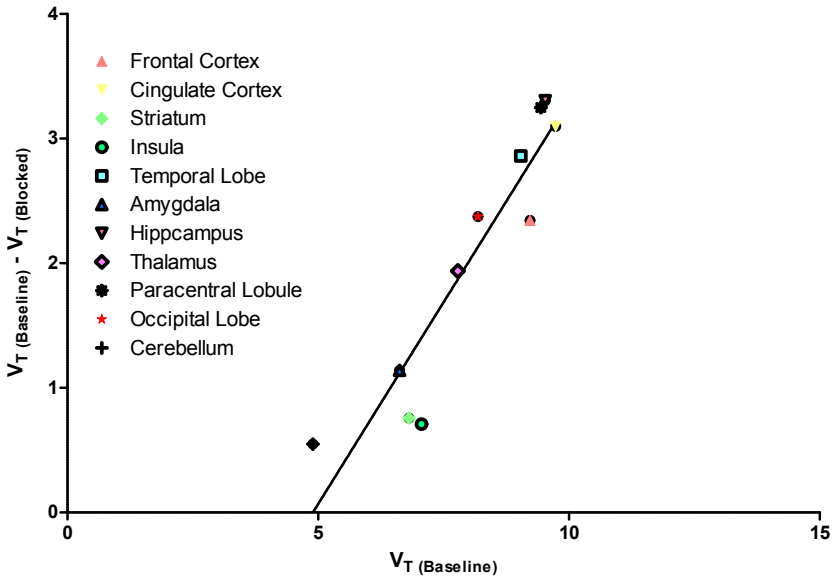
be suitable for this study, roflumilast has been investigated more thoroughly in rodents and humans than apremilast. Therefore, we can better estimate an appropriate dose of roflumilast for this study based on previous experiments and clinical trials. A study conducted in non-human primates found that 200 µg roflumilast resulted in a plasma concentration similar to peak concentration in humans (approximately 5 – 9 ng/ml) (14). This study hypothesized that a 500µg dose in humans would result in a PDE4 occupancy of 30-40%. The recommended dose of roflumilast is 500 µg per day, with or without food. Maximum plasma concentrations occur approximately 1-2 hours after dosing depending on food consumption. The half-life of roflumilast and its primary metabolite, roflumilast N-oxide, are approximately 17 and 30 hours, respectively.

Table 1. Adverse reactions reported in ≥2% of patients

	Roflumilast (500 mcg daily over 6 – 12 months in N=4438)	Placebo	**Apremilast** (30 mg BID over 1 -5 days in N=497).	Placebo
Diarrhea	9.5%	2.7%	9.3%	1.2%
Weight decreased	7.5%	2.1%	NR	NR
Nausea	4.7%	1.4%	7.4%	1.4%
Headache	4.4%	2.1%	4.8%	1.8%
Back pain	3.2%	2.2%	NR	NR
Influenza	2.8%	2.7%	0.6%	0.6%
Dizziness	2.1%	1.1%	NR	NR
Decreased appetite	2.1%	0.4%	NR	NR
Vomiting	NR	NR	0.8%	0.4%

BID, twice a day; NR, not reported
Table modified from (15) and (16)

The purpose of this study is to assess whether uptake in dysplastic bone reflects parent radioligand binding to the enzyme or merely accumulation of radiometabolites, which are expected to have no affinity for the enzyme. For this purpose, we need not administer a receptor saturating dose of roflumilast, as the Lassen occupancy plot has great sensitivity to measure the percentage occupancy and then to extrapolate to 100% occupancy (Cunningham et al., *J. Cereb. Blood Flow & Metab.,* 30: 46-50, 2010). The sensitivity derives from the fact that it is a within subject comparison of multiple regions (in brain or periphery) at baseline and after blockade. We have used the Lassen plot numerous times, including to measure the occupancy of PDE4D subtype by the therapeutic candidate BPN14470. The figure below shows that that the occupancy (i.e., slope) is ~65% and the nondisplaceable uptake (V_{ND} or x-intercept) is ~4.9 mL $\cdot$cm^{-3}.



2. Study Objectives

This study will investigate cAMP signaling in the brains of individuals with MAS using [^{11}C](*R*)-rolipram PET imaging.

a. Primary objectives

We will study PDE4 levels in patients with MAS by comparing [^{11}C](*R*)-rolipram binding in various brain regions between patients and age-matched healthy controls. In addition, in whole body imaging of patients with MAS, we will compare [^{11}C](*R*)-rolipram uptake between areas of fibrous dysplasia and adjacent unaffected bones. In whole body scans, we will also compare [^{11}C](*R*)-rolipram uptake in peripheral organs between subjects with MAS and healthy controls. The reason for doing the latter comparison is that some tissues of MAS may contain the *GNAS* mutations even if there are no obvious structural changes.

b. Secondary objective

In order to quantify the amount of [^{11}C](*R*)-rolipram binding in patient whole body scans, we will perform a blocking scan with 500 µg roflumilast, a FDA approved PDE4 inhibitor. Because of potential differences in metabolism of [^{11}C](*R*)-rolipram in in the periphery of healthy controls and patients, it is difficult to interpret whether or not the [^{11}C](*R*)-rolipram uptake is specific. By performing a blocking scan, we can quantify the amount of specific [^{11}C](*R*)-rolipram binding to determine if there is a difference between healthy controls and patients.

3. Subjects

a. Description of study populations

This study will have two populations: subjects with MAS and healthy control subjects. MAS subjects will be recruited from participants in 98-D-0145 “Screening and Natural History of Patients with Polyostotic Fibrous Dysplasia and the McCune-Albright

Syndrome" (PI: Alison Boyce, MD). Participation in our protocol will not delay or interfere with the patient's treatment. For all patients, the diagnosis of MAS will have been made under 98-D-0145.

Healthy subjects will be recruited and screened by the Molecular Imaging Branch (MIB) under a separate protocol (01-M-0254 or 17-M-0181), and group-matched for age to the MAS subjects.

Our accrual number will be 20 for subjects with MAS and 15 for healthy controls. Assuming a withdrawal / dropout rate of ~20%, our target is to complete the study with 16 MAS subjects and 12 healthy controls. Both MAS and healthy subjects have one or two PET scans: one whole body and one brain scan. We expect that healthy subjects are more likely to have both brain and whole body scans and MAS subjects are less likely to do so. We expect ~10 brain and ~10 whole body scan to be performed in each group.

b. Inclusion criteria

Subjects with MAS

1. At least 18 years of age
2. Able to provide self-consent
3. Diagnosed with MAS under 98-D-0145.
4. Have craniofacial fibrous dysplasia

Healthy Subjects

1. At least 18 years of age.
2. Healthy based on medical history and physical examination.

c. Exclusion criteria

Subjects with MAS

1. Serious medical conditions, which may interfere with study procedures. Such conditions include but not limited to significant bone abnormalities in wrist areas of both arms, which makes it difficult to place a radial arterial line, clinically marked dysfunction of liver or kidney, which may delay clearance of [^{11}C](*R*)-rolipram.
2. Clinically significant laboratory abnormalities not linked to endocrine abnormalities but that may interfere with the PET measurement or affect safety of the participant during this study.
3. Positive HIV test.
4. Head trauma resulting in a period of unconsciousness lasting longer than one hour.
5. Metallic foreign bodies that would be affected by the magnetic resonance imaging (MRI) magnet, or fear of enclosed spaces likely to make the subject unable to undergo an MRI scan.
6. Recent research-related exposure to radiation (i.e., PET from other research) that, when combined with this study, would be above the allowable limits.
7. Inability to lie flat on camera bed for about two and a half hours.
8. Pregnancy or breastfeeding.
9. NIMH employees/staff and their immediate family members will be excluded from the study per NIMH policy.

10. Currently taking a PDE4 inhibitor (such as roflumilast or apremilast), a drug that inhibits or induces cytochrome P450 (such as rifampicin or erythromycin), or oral contraceptives containing gestodene and ethinyl estradiol (such as Minulet and Femodene).

Healthy Subjects

1. Serious medical conditions, which may interfere with study procedures. Such conditions include but not limited to clinically marked dysfunction of liver or kidney, which may delay clearance of [^{11}C](*R*)-rolipram.
2. Clinically significant laboratory abnormalities that may interfere with the PET measurement or affect safety of the participant during this study.
3. Personal history of any DSM Axis I disorder.
4. Positive HIV test.
5. Head trauma resulting in a period of unconsciousness lasting longer than one hour.
6. Metallic foreign bodies that would be affected by the MRI magnet, or fear of enclosed spaces likely to make the subject unable to undergo an MRI scan.
7. Recent research-related exposure to radiation (i.e., PET from other research) that, when combined with this study, would be above the allowable limits.
8. Inability to lie flat on camera bed for about two and a half hours.
9. Pregnancy or breastfeeding.
10. Current substance use disorder based on DSM.
11. NIMH employees/staff and their immediate family members will be excluded from the study per NIMH policy.
12. Currently taking a PDE4 inhibitor (such as roflumilast or apremilast), a drug that inhibits or induces cytochrome P450 (such as rifampicin or erythromycin), or oral contraceptives containing gestodene and ethinyl estradiol (such as Minulet and Femodene).

4. Study Design and Methods

a. Study overview

This study will have two populations: subjects with MAS and healthy control subjects. Both MAS subjects and healthy controls will have up to three PET scans. All subjects will be given the option to have the following: one brain scan, one baseline whole-body scan, and one blocked whole-body scan after roflumilast administration. The two whole body scans can be performed on the same day or on separate days. The blocked whole body scan will be performed approximately 1-2 hours after the administration of 500 µg roflumilast P.O.

All MAS subjects will also have a brain MRI scan and will administered the WAIS and SCID neuropsychiatric evaluations. Each of these scans requires one outpatient visit lasting up to several hours; therefore, subjects in this study will have between one and five study visits depending on the number of scans they participate in. In addition, under

this protocol MAS subjects will have their blood tested to rule out serious medical conditions. Women of child-bearing potential will be tested for pregnancy within 24 hours before each PET scan. Resting EKG will also be obtained.

All PET and MRI scans will be performed at the NIH Clinical Center.

b. Recruitment

MAS patients will be recruited from the existing cohort of subjects enrolled in protocol 98-D-0145. Referrals will be made by a study team member of 98-D-0145. Criteria will be based on the inclusion and exclusion criteria of this PET protocol. Eligible subjects will be contacted by a member of the study team of this PET protocol who has not been directly involved in their clinical care under protocol 98-D-0145.

Healthy volunteers will be recruited by the MIB and undergo screening under a separate protocol (01-M-0254 or 17-M-0181). We expect to recruit approximately five MAS subjects and five healthy controls per year.

c. Screening

Written, informed consent will be obtained before any study procedures, including screening procedures, are done. For MAS subjects, eligibility will be determined based on a previous diagnosis of MAS as documented in 98-D-0145. The procedures to determine eligibility will not be repeated under this PET protocol if the same information has already been obtained under 98-D-0145 within six months. Tests done solely for the purpose of diagnosis of MAS will not be repeated even if not done within the last six months. With participant permission, identifiable data will be shared between 98-D-0145 in order to confirm the diagnosis of MAS. If the information on medical history or examination is more than six months old, the updated information will be obtained under the current PET protocol. If some of laboratory testing described below in subsection ii is more than six months old or missing, the laboratory testing will be performed under this PET protocol.

Healthy volunteers will be screened under a separate protocol (01-M-0254 or 17-M-0181) to determine eligibility for the study procedures in this protocol. For healthy subjects, SCID-NP will be performed as a part of the screening under 01-M-0254 or 17-M-0181 to determine eligibility.

Patients with MAS who have completed a baseline whole body scan and return to undergo a blocked whole body scan will be re-screened if it has been more than 6 months since their initial screening. Healthy volunteers who are returning to do a blocked whole body scan will be rescreened if it has been more than 12 months since their initial screening.

i. Medical history and examination (about one hour)

Under this PET protocol, MAS subjects will undergo a comprehensive medical history and examination by a clinically credentialed study investigator if those procedures were not done within 6 months under 98-D-0145. The purpose of the medical history and examination is to rule out serious medical conditions that may be contraindications to safe protocol participation. If H&P was done within 6 months under 98-D-0145, H&P

will not be repeated in this PET protocol. A copy of the medical evaluation will be placed in the subject's NIH chart.

For healthy subjects, H&P will be done under 01-M-0254 or 17-M-0181 but not in this PET protocol.

ii. Blood draw for laboratory testing

Under this PET protocol, for MAS subjects, blood testing will be performed to rule out serious medical conditions. Clinical laboratory testing will include complete blood count, PT/PTT, chemistry panel, liver function tests, lipid panel, HIV status, hepatitis serologies, and urine drug screen. Resting EKG will also be obtained. If any of these lab tests have been done in the last 6 months, they will not be repeated. With participant permission, identifiable data will be shared between 98-D-0145 in order to prevent unnecessary repeat of blood tests.

For healthy subjects, laboratory testing including urine drug screen will be done under 01-M-0254 or 17-M-0181 but not in this PET protocol.

iii Pregnancy testing: Women of child-bearing potential will be tested for pregnancy with a urine test at the time of screening.

d. Study procedures

Study procedures, including the PET scan, are for research purposes only.

The entire study procedures are summarized below.

Subjects with MAS:

1. Neuropsychiatric testing: Three hours.
2. Brain MRI: One hour.
3. Brain PET scan: Five hours.
4. Baseline whole body PET: Four hours.
5. Roflumilast administration and blocked whole body PET: Five hours

As long as the schedule of the participant allows, some of these procedures are combined in a single visit. MRI, brain and whole body PET scans do not need to be performed in a specific order.

6. Pregnancy tests: For women able to become pregnant, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. PET and MRI will not be done if the pregnancy test is positive.

Healthy controls:

1. Brain MRI: One hour.
2. Brain PET scan: Five hours.
3. Baseline whole-body PET: Four hours.
4. Roflumilast administration and blocked whole body PET: Two hours
5. Pregnancy tests: For women able to become pregnant, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. PET and MRI will not be done if the pregnancy test is positive.

As long as the schedule of the participant allows, some of these procedures are combined in a single visit. The MRI, brain scan, and whole-body PET scans do not need to be performed in a specific order, however if the subject agrees to doing both whole body scans, the baseline scan will be performed before the blocked scan. The subject cannot do a blocked whole body scan without doing a baseline scan first. The baseline and blocked whole body scans can be performed in the same day, or on separate days. Subjects who have previously had a baseline whole body scan will be given the option to return for the blocked whole-body scan. For MAS patients, the goal will be to perform the blocked whole body scan within 6 months of the baseline whole body scan. If, however this is not possible due to scheduling restraints, screening procedures will be repeated to ensure that there has not been significant disease progression. Please note that MAS is a relatively stable condition in adulthood, and little change is expected over 6 months. For healthy controls, the time limit between the baseline and blocked whole body scans is 1 year.

All subjects with MAS will be asked to have up to three [^{11}C]*(R)*-rolipram PET scans: one brain, one baseline whole body, and a blocked (with roflumilast) whole body scan. The subject can choose whether to undergo a brain scan or a baseline whole body scan, or both, but the subject is only eligible for the blocked whole body scan if they have the baseline whole body scan first. For brain scans, one arterial line will be placed for blood sampling, and one venous line will be placed for radioligand injection. For whole body scans, two venous lines will be placed, one for radioligand injection and another to measure [^{11}C]*(R)*-rolipram concentrations. In the case that a patient is only willing to do one of the scans, either a brain or a whole body scan will be performed based on patient preference. If the patient has no preference, one whole body scan will be performed. If patients are willing to have two PET scans (one whole body and one brain), the scans will not be performed in any particular order. All subjects with MAS will undergo the WAIS- or KBIT-2, Edinburgh Handedness Inventory, and Structured Clinical Interview for Non-Patient (SCID-NP).

Healthy controls will similarly have the option to undergo either one brain scan and/or a baseline whole body scan. The blocked whole body scan will be offered only if the subject has agreed to the baseline whole body scan.

i. Brain [^{11}C](R)-rolipram PET

Healthy controls will undergo PET scanning as outpatients, while MAS patients may be either inpatient or outpatient. An arterial line will be placed for blood sampling. A venous line will be placed for radioligand injection. To minimize discomfort, an anesthesiologist will place the arterial catheter in the subject's wrist after numbing the area with a local anesthetic prior to the placement.

PET scanning will be performed in the NIH Clinical Center and will begin with a transmission scan by either CT, ^{68}Ge- or ^{137}Cs-source to correct for attenuation in the subsequent emission images. The PET radioligand [^{11}C](*R*)-rolipram (maximum activity of 20 mCi) will be injected intravenously over about one minute. The subject will be scanned over approximately two hours. To quantify the density of PDE4 in the body, we will obtain arterial blood samples to correct for individual differences in clearance of the

radioligand. The volume of blood drawn will not exceed 200 mL per scan. Following the PET scan, the arterial and venous catheters will be removed.

ii. Whole body [^{11}C](R)-rolipram PET

Healthy controls will undergo PET scanning as outpatients, while MAS patients may be either inpatient or outpatient. Two intravenous catheters will be placed, one for radioligand injection and another to measure [^{11}C]*(R)*-rolipram concentrations. Total volume of blood sampling is about 30 mL.

PET scanning will be performed in the Clinical Center and will begin with a transmission scan by either CT or ^{68}Ge or ^{137}Cs source to correct for attenuation in the subsequent emission images. The PET radioligand [^{11}C](*R*)-rolipram (maximum activity of 20 mCi) will be injected intravenously over about one minute. The subject will be scanned over approximately two hours. Following the PET scan, the venous catheter will be removed.

If the brain or whole body PET scan is cancelled or incomplete for any reason—eg, synthesis failure of [^{11}C](*R*)-rolipram, camera issues, or other problems—and if the study participant did not receive any radioactive material, the scan will be repeated on the same or on another day as long as the participant is willing. The transmission scan may be repeated as well.

iii. Blocked Whole body scan [^{11}C](R)-rolipram PET with roflumilast

Healthy controls will undergo PET scanning as outpatients, while MAS patients may be either inpatient or outpatient. Two intravenous catheters will be placed, one for radioligand injection and another to measure [^{11}C](R)-rolipram concentrations. Total volume of blood sampling is about 30 mL per scan.

PET scanning will be performed in the Clinical Center and will begin with a transmission scan by either CT or ^{68}Ge or ^{137}Cs source to correct for attenuation in the subsequent emission images. The PET radioligand [^{11}C](R)-rolipram (maximum activity of 20 mCi) will be injected intravenously over about one minute at the start of each scan. The subject will be scanned over approximately two hours. Subjects will be administered 500 µg roflumilast P.O. approximately 1-2 hours prior to [^{11}C](R)-rolipram administration. Because food consumption can affect the absorption of roflumilast, subjects will be asked to have a light breakfast (and light lunch if scan occurs in the afternoon) prior to the scan. Following the PET scan, the venous catheter will be removed.

iv. Brain MRI

All subjects, including those who receive only a whole body scan, will undergo a dedicated MRI of the brain for co-registration of PET images for analysis and to estimate brain morphology. Portions of the whole body PET scans image the brain. However, both volunteers and patients need not repeat the MRI scan if they had one of appropriate sequence within the prior year.

The MRI will be performed on a 3 Tesla magnet without the use of gadolinium contrast. Subjects will undergo safety screening prior to the MRI to rule out contraindications. The MRI will take place at the NIH Clinical Center and requires about

thirty to forty minutes. If subjects become anxious during a scan, lorazepam (0.5–1 mg) or another benzodiazepine may be administered orally, if not clinically contraindicated. Subjects can also request that the operator stop the scan at any time. Women of child-bearing potential without a history of sterilization will be tested for pregnancy with a urine test prior to the MRI.

If the scan is cancelled or incomplete due to a problem with the scanner, the scan will be repeated on the same or on another day if the participant is willing.

v. Neuropsychiatric testing

Subjects with MAS will have the following examinations:

1. Wechsler Adult Intelligence Scale, Fourth Edition (WAIS-IV) or the Kaufman Brief Intelligence Test, Second Edition (KBIT-2) (17, 18)
2. Structured Clinical Interview for Non-Patient (SCID-NP) or SCID Patient Edition (SCID-P)
3. Edinburgh Handedness Inventory

Both the WAIS-IV and KBIT-2 provide an overall assessment of intelligence. SCID-NP characterizes neuropsychiatric symptoms. The Edinburgh Handedness Inventory assesses the dominance of a person's right or left hand. A decision to perform either the SCID-NP or SCID-P will be based on the patient's psychiatric history.

The WAIS-IV, KBIT-2, or the Edinburgh Handedness Inventory will not be repeated if the subject has already undergone these assessments under protocol 98-D-0145. The SCID-NP (or SCID-P) will not be repeated if performed within the past month.

e. End of participation

Study participation will end with the last PET or MRI scan the participant agreed to do. Patients will return for follow up under 98-D-0145. No follow up care will be needed for healthy volunteers.

5. Management of Data and Samples

a. Storage

Demographic and clinical data will be archived on a password-protected server. Imaging and blood data will be offloaded from the scanner/blood sampling device to a password-protected server. Clinical Safety Monitoring data will be archived together with other data. Laboratory test results will be stored on CRIS. Only study investigators will have access to stored data.

Blood samples for measurement of radioligand will be discarded after analysis, which will be performed on the day of the PET scan.

b. Data (*if applicable*: including genomic data) and sample sharing plan

Data may be shared. Data may be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data from this protocol may be open-access or restricted access.

Data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

6. Additional Considerations

a. *Research with investigational drugs or devices*

[^{11}C](*R*)-rolipram will be administered under IND #73,149, with Robert Innis, MD as the Sponsor and Investigator. The radioligand will be synthesized by the NIMH radiochemistry laboratory directed by Victor Pike, PhD.

Roflumilast is an FDA approved drug and meets the criteria of an IND exempt drug:

1. The drug product is lawfully marketed in the United States.
2. The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug.
3. In the case of a prescription drug, the investigation is not intended to support a significant change in the advertising for the drug.
4. The investigation does not involve a route of administration, dose, patient population or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product (21 CFR 312.2 (b)(1)(iii).
5. The investigation is conducted in compliance with the requirements for review by an IRB (21CFR 56) and with the requirements for informed consent (21 CFR part 50).
6. The investigation is conducted in compliance with the requirements of 312.7 (i.e. the investigation is not intended to promote or commercialize the drug product).

b. *Gene therapy*

None.

7. Risks and Discomforts

Potential risks from this study include those associated with: a) laboratory testing including urine toxicology testing, b) placement of an arterial and venous line, c) arterial and venous blood sampling, d) MRI, possibly including benzodiazepine drug administration, e) radiation exposure from PET scans, and f) PET scanning.

a. Blood Draw and laboratory testing

Laboratory testing is associated with minimal risks. We will first explain and familiarize subjects with laboratory testing to minimize discomfort, if any, during testing. During the screening it will be necessary to test urine for illegal drugs (urine toxicology). Subjects will be informed that if the drug test is positive, they will be told promptly. The results of the drug testing will be noted in their NIH medical record. There is minimal medical risk to urine collection for pregnancy testing.

b. Arterial/Venous line placement

Arterial catheterization has been shown to be a generally safe and reliable method of obtaining arterial blood samples (19). Placing a radial arterial catheter may cause bruising or infection. There is also a risk of occlusion and microemboli. In the past, over 3,000 arterial catheters have been placed at NIH Clinical Center. Of these, two complications requiring physician's care were reported. In the first case, a small radial artery aneurysm developed several months later, which was successfully repaired surgically. In the second case, a radial artery thrombosis developed 28 days later, which was also successfully repaired surgically. The arterial line will be placed by a member of the anesthesiology staff after confirming normal double circulation (both radial and ulnar arteries).

Venous catheter insertion, which is less invasive than arterial catheterization, can be associated with bruising, infection, or clot formation. Using proper placement techniques will minimize these risks.

c. Arterial and venous sampling

Blood draw volumes will not exceed the limits allowed by the NIH Clinical Center (Medical Administrative Policy M95-9: Guidelines for Blood Drawn for Research Purposes in the Clinical Center); in this study, the volume of blood drawn will not exceed 230 mL per scan. Blood sampling may lead to the formation of a small subcutaneous hematomas caused by blood leaking from a punctured blood vessel. Such hematomas cause only minor discomfort. They are not dangerous and require no treatment other than reassuring the patient. There is also a small risk of infection at the site of the needle puncture, which can be readily treated with antibiotic therapy. Subjects will be asked not to donate blood for a period of eight weeks after participation is completed.

d. MRI

Three-Tesla MRI is widely used as a clinical imaging tool. Subjects will be screened and excluded for the presence of any metallic prostheses both at the time of recruitment and just prior to the MRI. Subjects will wear earplugs to minimize exposure to excessively loud noises. Occasionally subjects become anxious during the scan. In that case, lorazepam (0.5–1 mg) or a comparable benzodiazepine anti-anxiety agent may be

given orally before the MRI as requested by subjects, and if not clinically contraindicated. Subjects can also request that the operator stop the scan at any time. Side effects of anti-anxiety medications may include prolonged sedation (usually less than eight hours), lightheadedness, lassitude, motor incoordination, and difficulties with balance. These effects are transient and go away after several hours. Subjects will be instructed to not drive, handle machinery, or drink alcohol until the next day. Subjects who receive a sedative will be observed in clinic until the sedation wears off and will be required to bring a driver or will be provided a taxi home.

Severely claustrophobic subjects find it difficult to undergo MRI scans; thus, such subjects will be excluded at the time of recruitment.

e. Radiation exposure

Radiation exposure in this protocol will be from [^{11}C](*R*)-rolipram and transmission scans for PET using ^{68}Ge or ^{137}Cs source or CT transmission for PET/CT. Among these three types of transmission scans, CT transmission causes the highest radiation as calculated by Craig Barker, PhD, CC PET Dept., July 22 2015; according to ICRP103. Therefore, the radiation exposure from CT transmission is used to calculate total radiation exposure in 88-23(a). Radiation dose estimates for [^{11}C](*R*)-rolipram are based upon biodistribution data in healthy human subjects performed by the MIB, NIMH under protocol 06-M-0002 "PET whole body biodistribution and test retest brain imaging studies using a phosphodiesterase 4 inhibitor (*R*)-[^{11}C]rolipram" (PI: M Fujita, MD, PhD). That study showed that the highest effective dose in this protocol is about four rem for patients with MAS and healthy controls who would undergo one brain scan and two whole-body scans with two CT transmission scans for each brain and whole-body scan, respectively. Although CT transmission scans have higher radiation exposure than ^{68}Ge or ^{137}Cs transmission scans, CT transmission scans are far more commonly performed in both clinical and research settings because they are the only choice for PET/CT scanners. This amount is within NIH RSC guidelines. Detailed results of this dosimetry study are included in the attached NIH 88-23(a). In case the subject moves between the transmission scan and the injection of [^{11}C](*R*)-rolipram, the MIB routinely includes two transmission scans for each PET scan to allow a repeat of transmission. Note: the designation of radiation dose of "about four rem" was recently approved by the Radiation Safety Committee as an alternative way to present risk to patients using rounded numbers, which better reflects the actual dose to an individual subject.

All subjects will be asked about any prior research participation involving radiation exposure so that the total exposure, in combination with the present study, will not exceed an effective dose of five rem per 12 months. Examples may include mammograms, x-rays taken in radiology departments, cardiac catheterization, and fluoroscopy as well as nuclear medicine scans in which radioactive materials were injected into your body.

f. PET scan

PET scanning, which detects injected radioactivity within the body, has been used as a clinical imaging tool. We routinely use a series of procedures to minimize the risk of discomfort during scanning sessions. Namely, the procedures are conducted in the presence of trained health professionals to whom subjects will have ready access should

they experience any problems. Subjects can communicate with the trained health professionals while in the scanner and can be removed from the scanner or withdraw from the study at any time if they wish to do so.

Occasionally subjects become anxious during the scan. In that case, subjects can request that the operator stop the PET scan.

g. Roflumilast administration

Roflumilast is an FDA approved PDE4 inhibitor, used commonly to treat COPD. In this study, subjects may receive a single dose of 500 µg roflumilast P.O. For treatment of COPD, roflumilast is normally given at a dose of 500 µg over a period of weeks or months (15). Therefore, we feel that the risks of serious reactions are low given that we are administering only a single dose. Adverse reactions to roflumilast administration reported by ≥ 2% of patients (n = 4438) include: diarrhea, weight loss, nausea, headache, back pain, influenza, dizziness, and decreased appetite. Psychiatric disorders (anxiety, depression) occurred at a frequency of 1 to 2% greater than placebo. We will contact all patients who receive roflumilast 24-48 hours after its administration to confirm they are feeling well.

8. Subject Safety Monitoring

Participants will be carefully monitored by clinical staff throughout the study. Participants will be asked about the presence of adverse events throughout the procedure. To confirm the absence of the pharmacological effects of the PET ligand, for each PET scan, a clinically credentialed investigator will monitor vital signs within three hours before tracer injection, and again at approximately 30 and 90 minutes after radioligand injection. The site of the arterial and venous catheter insertion will be carefully monitored for signs of bleeding during the PET scans and also after removal of the catheter.

All adverse events (AEs) will be recorded on the case report form. All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be evaluated until satisfactory resolution. After removal of venous lines, the site of the catheter insertion will be carefully monitored for signs of bleeding.

Toxicity tables/criteria to be used

Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v.4.03 http://evs.nci.nih.gov/ftp1/CTCAE/About.html.

Criteria for individual subject withdrawal

We will stop study procedures for any subject with any Grade 2, Grade 3, or greater non-laboratory toxicity that is intolerable, persistent, or uncorrectable for more than several minutes. In addition, the Medical Advisory Investigator or a clinically credentialed investigator may terminate study procedures based on clinical judgments. Likely causes are pain at the site of catheter insertion and difficulties to stay in MRI and PET scanners, and the judgement of withdrawal by study investigators will be done as described above. In addition, study participants can voluntarily withdraw at any time for any reason.

9. Outcome Measures

a. Primary outcome measures

The primary outcome measure will be obtained in brain scans as the amount of radioligand binding quantified as distribution volume (V_T). Calculated from both brain and plasma data, V_T reflects the density of PDE4, corrected for any individual differences in metabolism of the radioligand or regional blood flow in brain.

b. Secondary outcome measures

The secondary outcome measure will be obtained in whole body scans as area under the curve (AUC) of radioactivity expressed as standard uptake value (SUV). SUVs are calculated by normalizing radioactivity in PET images to injection activity and body weight. Although AUC of SUV is not as fully quantitative as V_T, the AUC is widely used in clinical studies where arterial data are not obtained. If patients and healthy controls have markedly different clearance of [^{11}C](*R*)-rolipram, group comparison using AUC of SUV may include errors. To correct group difference in clearance, in whole body imaging, venous concentrations of [^{11}C](*R*)-rolipram will be measured at several time points. MRI measures of brain morphology including gray matter, white matter and CSF volume and folding will be estimated from the MRI images.

c. Tertiary outcome measure

The third outcome measure will be the difference in SUV between the baseline and the blocked whole-body scans. SUV is calculated as stated above.

10. Statistical Analysis

a. Analysis of data/ study outcomes

PET and MRI images will be co-registered for anatomic definition of brain regions. Brain and plasma data will be analyzed with compartmental and graphical modeling to calculate V_T, which is proportional to the density of receptor or enzyme. V_T will be calculated both for regions of the brain and on a voxel basis. PET whole body images will be analyzed by drawing organs and by calculating AUC of SUV. For comparisons between subjects with MAS and healthy controls in brain and peripheral organs, an unpaired test, such as t- and Mann-Whitney tests will be used. In addition, in peripheral organs of MAS, for comparisons of PET data between affected and unaffected organs, a paired test will be applied if the contralateral side is unaffected. If contralateral side is also affected or not available, an unpaired test will be applied to compare the same tissue/organ between patients and healthy controls. For the statistical analyses, SPSS, PMOD, SPM, Freesurfer or equivalent programs will be used.

b. Power analysis

This is an exploratory study focusing on PET imaging. Clinical data obtained under 98-D-0145 may be used to interpret the relationship between PET findings and clinical manifestations of the disease. Although constitutive activation of $G_s\alpha$ is expected to increase cAMP signaling, which increases PDE4 levels, PDE4 levels in MAS are not known in postmortem tissue either in brain or in peripheral organs such as bone. Therefore, we cannot do a realistic power calculation.

To achieve the goals of this exploratory study, we request a total of 35 subjects: ~10 MAS subjects to undergo brain PET scan, ~10 MAS subjects to undergo whole body PET, and 15 healthy controls to undergo a brain scan and/or a whole body PET scan. By limiting subjects with MAS to those who have craniofacial fibrous dysplasia and neuropsychological symptoms, we expect to increase the likelihood of detecting changes in PET in this small sample size. To limit the number of study participants, all MAS and healthy subjects will be asked if they would be willing to have both brain and whole body PET scans. A smaller number of 15 healthy subjects are requested because healthy subjects are more likely to have both brain and whole body scans. Our previous study of [^{11}C](*R*)-rolipram brain scans in healthy subjects found that the coefficient of variation (SD/mean) of VT was 19%. Based on the activation of $G_s\alpha$, we hypothesize that MAS patients will show increased rolipram binding for whichever one-sided test is appropriate. Assuming a 20% dropout from 10 subjects (thus, eight subjects per group) plus a 19% coefficient of variation (SD/mean) will provide a power of 0.80 to detect a 19% increase.

11. Human Subjects Protection

a. Subject selection

The study will be open to all racial and ethnic groups and will not target any particular group. Healthy volunteers will be recruited to match the patient group for age. Those who are HIV positive are excluded because the possible viral effects on the brain could interfere with interpretation of the outcome of the study.

b. Justification for exclusion of children

We will exclude children younger than 18 years of age to avoid radiation exposure to minors because the risks of radiation exposure outweighs the benefits.

c. Justification for inclusion of other vulnerable subjects

No population of vulnerable subjects will be included. Pregnant and breastfeeding females will be excluded because of greater risks of radiation to fetus and babies. Subjects without consent capacity will be excluded because this protocol has more than minimal risks and provides no direct benefits. Protections for NIH employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff.

d. Justification for sensitive procedures

This protocol does not include sensitive procedures such as use of placebo, medication withdrawal, provocative testing, or deception.

e. Safeguards for vulnerable populations and sensitive procedures

Pregnancy testing and interview will be performed for females with childbearing potential to prevent the enrollment of pregnant females. This protocol may enroll NIH employees. Protections for NIH employees and staff participating in this study include 1)

assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff.

When reviewing responses to the SCID, experienced clinical interviewers will supervise interviewers. Although rare, should the study staff identify symptoms of suicidality or suicidal ideations during the interview procedures, all necessary steps will be taken. The on-call clinician will be contacted to provide further evaluation, the subjects' own clinician may be notified after permission is granted by the subject. If not, other referral sources are provided and followed up, and necessary treatment steps (e.g., hospitalization, referral to a care provider, etc.) will be offered. However, NIH does not pay for outside hospitalization if that is required.

12. Anticipated Benefit

This study offers no direct benefit to individual subjects, but will lead to generalizable knowledge about MAS.

13. Classification of Risk

a. For adults

This study has more than minimal risk.

b. For adults without consent capacity

Not applicable.

c. For children

Not applicable.

d. Overall risk and benefit consideration

The risks are reasonable in relation to anticipated benefit.

14. Consent Documents and Process

a. Designation of those obtaining consent

Study investigators designated as able to obtain consent in section 11f above will obtain informed consent. All study investigators obtaining informed consent have completed the NIMH HSPU 'Elements of Successful Informed Consent' training.

b. Consent procedures

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing. NIMH Human Subject Protection Unit (HSPU) will be consulted if there is any question about the consent capacity of an individual.

c. Consent documents

Two separate consent forms have been submitted: one for healthy volunteers, one for subjects with MAS. Both of these groups will be asked if they are willing to have one brain and one whole body PET scans. In addition, there will be two separate consent forms (one for healthy volunteers and one for subjects with MAS) giving subjects who have previously had a baseline whole-body scan the option to return for a blocked whole-body scan with roflumilast administration. All of these consent forms contain all required elements.

15. Data and Safety Monitoring

a. Data and safety monitor

This study will be monitored by the following independent safety monitor: Kenneth Towbin, MD. Dr. Towbin is a Senior Research Physician at NIMH, board-certified in adult psychiatry, and in child and adolescent psychiatry and Chief of Clinical Child and Adolescent Psychiatry in the Emotion and Development Branch. Dr. Towbin has been the primary physician overseeing care for inpatients with major depression treated in the Mood and Brain Disorders Unit. He also assists in overseeing care, as needed, in the Section on Developmental Affective Neuroscience (SDAN).

b. Data and safety monitoring plan

The PI will prepare a report on data and safety parameters for the Independent Monitor for the first ten subjects or annually, whichever comes first. The Independent monitor will provide a written monitoring report to be submitted to the IRB at the time of continuing review.

c. Criteria for stopping the study or suspending enrollment or procedures

The Independent Monitor and IRB will determine if changes are needed for the research to continue or if it will be closed. Any changes required as conditions for resuming the research must be submitted as an amendment and IRB-approved before the changes can be implemented. The PI/Independent Monitor and IRB will determine if changes are needed for the research to continue or if it will be closed. Any changes required as conditions for resuming the research must be submitted as an amendment and IRB-approved before the changes can be implemented.

16. Quality Assurance

a. Quality assurance monitor

Quality assurance will be monitored by the PI, The research team and the NIMH Office of Regulatory Compliance (ORO).

b. Quality assurance plan

ORO monitors intramural research studies to ensure compliance with GCP, organizational policies and regulations. Audit frequency is determined by the ORO SOP based on the study level of risk. Results of ORO audits are provided to the PI, The Clinical Director and the CNS IRB. As an IND study, this protocol will be subject to GCP audits at study initiation and after the first enrolled subject. Timing of subsequent review will be established by the ORO but no less frequent than every other year.

17. Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations
Reportable events for this protocol will be tracked and reported in compliance with Policy 801.

18. Alternatives to Participation

Subjects do not receive any treatment in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

19. Privacy

All research activities will be conducted in as private a setting as possible.

20. Confidentiality

a. For research data and investigator medical records

Data will be stored using codes that we assign. Data will be kept in password-protected computers. Only study investigators will have access to the data. The results of urine drug screen will be in the NIH Medical Record.

This study collects sensitive information on drug and alcohol use. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

b. For stored samples

Samples are not stored.

c. Special precautions

Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data. De-identified results from clinical trials will be posted on cctrials.gov.

Any clinically relevant results will be returned to the participant.

21. Conflict of Interest

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

There are no conflicts of interest to report.

c. Role of a commercial company or sponsor

Commercial company or sponsor is not involved.

22. Technology Transfer

No Technology Transfer is involved in this study.

23. Research and Travel Compensation

Subjects will be compensated for time and research-related inconveniences. Reimbursement is based on NIH standards for time devoted to the research project. This study will also provide travel compensation based on NIH standards. Subjects will be paid for each portion of the study they have completed whether or not they opt for early

withdrawal from participation. MAS patients and controls may receive a maximum of $1190 and $930, respectively. Subjects will be paid for each portion of the study they have completed whether or not they opt for early withdrawal from participation. Payment will be sent after participation has been completed. Employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation.

Schedule of Visits and Remuneration

	Inconvenience Units	Inconvenience Remuneration	Time (hours)	Time Remuneration	Total Remuneration
Visit 1: screening (patient only)*					
Medical history, physical exam, EKG, and lab tests if not obtained in prior six months	2	$20	2	$30	$50
Neuropsych Testing	4	$40	3	$40	$80
Visit 2					
Pregnancy test***	1	$10			$10
MRI	8	$80	2	$30	$110
Visit 3					
Pregnancy test***	1	$10			$10
Brain PET scan	15	$150	5	$60	$210
Venous catheter	3	$30			$30
Arterial catheter	7	$70			$70
Movement restriction	1	$10			$10
Visit 4					
Pregnancy test***	1	$10			$10
Whole body PET scan	15	$150	4	$50	$200
Venous catheter x2	6	$60			$60
Movement restriction	1	$10			$10
Visit 5					
Roflumilast admin	1	$50			$50
Pregnancy test*** †	1	$10			$10
Whole body PET scan	15	$150	4	$50	$200
Venous catheter x2 ††	6	$60			$60
Movement restriction	1	$10			$10
Total	89	$930	20	$260	$1190

*Screening will be repeated for subjects with MAS if no information is available within 6 months under 98-D-0145. Healthy volunteers will be screened under protocol 01-M-0254 or 17-M-0181.

**The order of visits 2, 3, 4 and 5 may change depending on the availability of the scanners.

***Pregnancy testing for women of childbearing potential

†The pregnancy test for the second whole body PET scan will not be conducted if the scan takes place the same day as the baseline whole body scan.

†† Subjects will not receive compensation for placement of catheters if blocked scan occurs on the same day as baseline scan and catheters are already in place.
Note: Some visits may be combined.

24. References

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25. Attachments/ Appendices

- Eligibility checklist
- Case report forms (CRFs)

Consent Forms

- Patient
- Healthy adult volunteer