INVESTIGATIONAL NEW DRUG APPLICATION

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SECTION 12

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SECTION 12.3

INTRODUCTORY STATEMENT

Positron emission tomography (PET) is a technique for measuring the 3 dimensional distribution of radioactive tracers in the living human. In this IND, we propose to use anti-1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid (anti-[^{18}F]FACBC) to study the time-course, degree of uptake and retention of this Leucine-("L")-type amino acid substrate in brain and systemic tumors.

Amino acids are important nutrients for tumor growth. PET labeled amino acid based tracers have been studied to image cancers (i,ii).

Aminocyclopentane carboxylic acid (ACPC) is an unnatural alicyclic amino acid which was developed in the 1960s as a therapeutic agent for malignant tumors. The mechanism of action of ACPC is competitive inhibition of valine synthesis by tumor cells (iii). In 1978, Washburn reported on development of aminocylcobutane carboxylic acid (ACBC), a synthesized L-leucine analog for imaging labeled with carbon-14, and tested on 4 rats transplanted with Morris 51236 hepatomas (iv). ^{14}C-ACBC demonstrated 6:1 tumor to tissue uptake, little renal excretion, and high pancreatic uptake.

Subsequently a carbon-11 ACBC radiotracer for human imaging was developed for the Oak Ridge National Laboratory high energy 86 inch cyclotron by Washburn (v). ^{11}C-ACBC was first tested in 3 rats implanted with hepatomas, 2 hamsters implanted with pancreatic adenocarcinomas and 5 rats with *S. aureus* abscesses. All the tumors demonstrated high radiotracer uptake with little renal excretion. None of the abscesses had significant radiotracer uptake. More recently, a new synthesis was adapted for the small Siemens RDS 112 medical cyclotron by Goodman et al (vi,vii) for human investigation. ^{11}C-ACBC has since been studied with an emphasis on pancreatic and brain tumor imaging. Results on a total of 82 human subjects have been reported (viii,ix,x,xi). Intense ^{11}C-ACBC uptake was documented in a wide variety of tumors including brain, bronchogenic cancer, lymphoma and metastases to bone from breast and lung cancer. Due to relatively high uptake in liver, liver metastases were visualized as cold defects, and there was a variable appearance of pancreatic tumors due to normal pancreas uptake. There was relatively little renal excretion. No toxicity was reported.

Pilot data published by Hubner in 1995 demonstrated uptake in brain tumors but not infarcted brain from stroke (xii). This suggests active transport into tumor and not mere uptake due to a disruption in the blood-brain-barrier. Also, uptake in brain tumor of ^{11}C-ACBC lends itself to two-compartment Gjedde-Patlak modeling. Unfortunately, carbon-11 has only a 20 minute half-life, making preparation and imaging impractical in most settings.

<u>Development of ^{18}F-FACBC</u>

In 1999, Dr. Mark Goodman's laboratory at Emory University reported on synthesis of an ACBC analog, anti-[^{18}F]FACBC, where a fluorine atom replaces a hydrogen atom. A triflate precursor is then radiolabeled with fluorine-18, yielding ^{1} anti-[^{18}F]FACBC (xiii-xvi). This radiotracer seems to demonstrate similar activity to ^{11}C-ACBC, yet with the advantage of the 110 minute half-life of fluorine-18. This longer half-life allows sufficient time for incorporation of flourine-18 into the radiopharmaceutical during manufacture, quality control, multi-dosing from a single batch production, more practical imaging protocols, and shipment to facilities with PET or coincidence cameras without on-site cyclotrons.

<u>Preliminary Work with ^{1} anti-[^{18}F]FACBC</u>

Under the auspices of the Emory University Radioactive Drug Research Committee (RDRC) and Institutional Review Board (IRB), a total of 24 human subjects with brain tumors, 6 human subjects with suspected renal tumors, 2 human subjects with prostate cancer and 8 rats with implanted gliomas have been studied without

reported toxicity. Approximately 6:1 tumor to-brain uptake ratios were noted. In the animal studies, there was early renal uptake, which cleared by 60 minutes.

Twenty-four human subjects to date with brain tumors also underwent total body imaging with anti-[^{18}F]FACBC after the brain scan was completed. As with ^{11}C-ACBC, high liver and pancreas uptake was present. Most importantly, the anti-[^{18}F]FACBC whole body scan demonstrated minimal renal and bladder activity compared to FDG. Arterial blood sampling and metabolite analysis in five subjects demonstrated that no metabolites of the radiotracer were found in blood.

In addition, unpublished data from Dr. Mark Goodman's laboratory at Emory University in which a monkey was injected with [1] anti-[^{18}F]FACBC establishes that no metabolites of the radiotracer were found in blood or urine. Lack of metabolites is a desirable characteristic for any imaging radiotracer. Also, unpublished data from Dr. Goodman's laboratory presented below in Table 1 demonstrates inhibition of anti-[^{18}F]FACBC uptake in various in-vitro cancer cell lines by an "L" but not "A" transport inhibitor, suggesting that there is an active amino-acid transport mechanism involved in tumor uptake.

Table 1

% Dose Uptake (expressed in CPM/1E+06 cells) of Radiolabeled Amino Acids in Human Tumor Cell lines incubated for 30 min at 37 °C

	Anti-FACBC H125 (Lung)	Anti-FACBC DU145 (Prostate)	Anti-FACBC SKOV3 (Ovarian)	Anti-FACBC A549 (Lung)	Anti-FACBC MDA MB468 (Breast)	Anti-FACBC U87 (Glioma)	FDOPA[4] H125	F DOPA[4] DU145
Control	7.1	16.5	6.9	15.9	20.2	4.4	3.3	8.8
ACS[1]	0.29	0.29	0.06	0.17	0.24	0.06	0.70	2.0
BCH[2]	**4.4**	**4.2**	**1.17**	**3.5**	**5.8**	**2.0**	**0.22**	**1.8**
MeAIB[3]	5.6	13.9	6.6	18.4	19.3	4.5	2.2	6.5

[1] 10 mM
[2] 10 mM "L" amino acid transport inhibitor
[3] 10 mM "A" amino acid transport inhibitor
[4] "L" amino acid transport standard

More recently, in a biodistribution study on a rat prostate cancer model, a comparison of the uptake of ^{18}F-FACBC and ^{18}F-FDG into normal tissues and a orthotopic implanted prostate tumor was performed. The results of the study are shown in Tables 2-5. The study demonstrated that ^{18}F-FACBC uptake into the orthotopic implanted prostate tumor was 3 times higher than that into normal prostate region. Furthermore, the uptake ratio of tumor to bladder was about 30 times higher than that of ^{18}F-FDG. Thus, we expect that ^{18}F-FACBC is suitable for imaging prostate cancer in humans.

Table 2.

% Injected Dose/gram tissue of ^{18}F-FACBC in normal tissues and a orthotopic implanted prostate tumor of nude rats

	ORTHOTOPIC		NORMAL NUDE RAT
	15 min	60 min	60 min
BLOOD	0.38 ± 0.021	0.30 ± 0.013	0.32
BRAIN	0.14 ± 0.017	0.27 ± 0.007	0.31
THYROID	0.76 ± 0.122	0.71 ± 0.054	0.80
HEART	0.58 ± 0.117	0.46 ± 0.022	0.54
LUNG	0.78 ± 0.109	0.58 ± 0.029	0.67
LIVER	1.84 ± 0.323	1.12 ± 0.086	1.55
SPLEEN	0.87 ± 0.075	0.63 ± 0.037	0.70
KIDNEYS	0.87 ± 0.085	0.62 ± 0.019	0.86
STOMACH	0.62 ± 0.247	0.73 ± 0.087	0.66
S.INTESTINE	0.82 ± 0.156	0.58 ± 0.066	0.63
L.INTESTINE	0.33 ± 0.051	0.28 ± 0.014	0.27
ADRENAL GLAND	1.03 ± 0.210	0.58 ± 0.053	0.76
TESTIS	0.19 ± 0.018	0.26 ± 0.019	0.28
BONE	0.50 ± 0.026	0.41 ± 0.046	0.50
SKIN	0.49 ± 0.025	0.53 ± 0.058	0.72
MUSCLE	0.24 ± 0.019	0.34 ± 0.017	0.41
PANCREAS	2.63 ± 0.154	2.42 ± 0.158	2.68
BLADDER/URINE	**0.42 ± 0.138**	**0.77 ± 0.119**	**1.15**
CARCUS	63.17 ± 2.555	71.06 ± 1.008	64.75
LYMPH NODE	0.41 ± 0.120	0.27 ± 0.032	0.38
PROSTATE (NORMAL)	**0.46 ± 0.044**	**0.37 ± 0.058**	**0.41**
PROSTATE (TUMOR)	**1.23 ± 0.215**	**1.12 ± 0.194**	
PROSTATE (WHOLE)	0.56 ± 0.072	0.48 ± 0.023	0.41
VESICULAR GLAND	0.42 ± 0.051	0.33 ± 0.036	0.35
	n=3	n=3	n=2

Table 3.

% Injected Dose/gram tissue of ^{18}F-FDG in normal tissues and a orthotopic implanted prostate tumor of nude rats

	ORTHOTOPIC		NORMAL NUDE RAT
	15 min	60 min	60 min
BLOOD	0.710 ± 0.086	0.230 ± 0.085	0.190
BRAIN	1.640 ± 0.197	2.260 ± 0.130	2.240
THYROID	0.890 ± 0.203	0.890 ± 0.387	0.820
HEART	1.800 ± 0.513	2.040 ± 0.783	1.750
LUNG	0.720 ± 0.250	0.640 ± 0.092	0.470
LIVER	0.700 ± 0.093	0.260 ± 0.038	0.230
SPLEEN	0.730 ± 0.045	0.860 ± 0.068	0.710
KIDNEYS	1.350 ± 0.164	0.560 ± 0.045	0.530
STOMACH	0.380 ± 0.105	0.450 ± 0.066	0.390
S.INTESTINE	1.100 ± 0.064	1.220 ± 0.102	0.950
L.INTESTINE	0.220 ± 0.021	0.260 ± 0.019	0.210
ADRENAL GLAND	1.690 ± 0.410	0.920 ± 0.433	1.050
TESTIS	0.480 ± 0.016	0.840 ± 0.065	0.790
BONE	0.490 ± 0.037	0.570 ± 0.023	0.500
SKIN	0.570 ± 0.006	0.410 ± 0.039	0.330
MUSCLE	0.180 ± 0.014	0.160 ± 0.024	0.140
PANCREAS	0.400 ± 0.072	0.340 ± 0.053	0.220
BLADDER/URINE	**13.390 ± 0.480**	**29.860 ± 1.259**	**21.040**
CARCUS	59.190 ± 1.148	45.990 ± 1.581	44.280
LYMPH NODE	0.720 ± 0.117	0.690 ± 0.052	0.870
PROSTATE (NORMAL)	**0.900 ± 0.417**	**0.550 ± 0.179**	**0.500**
PROSTATE (TUMOR)	**0.940 ± 0.184**	**1.080 ± 0.382**	**0.000**
PROSTATE (WHOLE)	0.880 ± 0.311	0.690 ± 0.119	0.500
VESICULAR GLAND	0.390 ± 0.037	0.310 ± 0.084	0.190
	n=3	n=3	n=2

Table 4

% Injected Dose/gram tissue of FACBC and FDG in tumor and normal prostate

		15 min		60 min		Normal 60 min
		Avg	SD	Avg	SD	Avg
Whole prostate	FDG	0.88	0.31	0.69	0.12	0.50
	FACBC	0.56	0.07	0.48	0.02	0.41
Normal region	FDG	0.90	0.42	0.55	0.18	0.50
	FACBC	0.46	0.04	0.37	0.06	0.41
Tumor region	FDG	0.94	0.18	1.08	0.38	NA
	FACBC	1.23	0.22	1.12	0.19	NA

Table 5

Ratio of tumor to normal region and normal nude rat prostate

		TRACER	PROSTATE	
			VS. NORMAL REGION	VS. NORMAL NUDE RAT PROSTATE
ORTHOTOPIC	15 MINS	FDG	1.04	-
		FACBC	2.67	-
	60 MINS	FDG	1.96	2.16
		FACBC	3.03	2.73

Table 6.

Ratio of Tumor to Urinary bladder including urine

		TRACER	% D/organ	% D/gm
TUMOR ORTHOTOPIC	15 MINS	FDG	0.09	0.0010
		FACBC	0.10	0.0011
	60 MINS	FDG	0.11	0.0013
		FACBC	0.10	0.0015
BLADDER ORTHOTOPIC	15 MINS	FDG	13.39	0.0646
		FACBC	0.42	0.0018
	60 MINS	FDG	29.86	0.0682
		FACBC	0.77	0.0028
VS BLADDER ORTHOTOPIC	15 MINS	FDG	0.0067	0.0157
		FACBC	0.2381	0.6287
	60 MINS	FDG	0.0037	0.0186
		FACBC	0.1299	0.5225

Table 7.

Ratio of tumor to lymph nodes, bone, muscle and brain

		TRACER	vs LN	vs BONE	vs MUSCLE	vs BRAIN
ORTHOTOPIC	15 MINS	FDG	1.31	1.92	5.22	0.57
		FACBC	3.00	2.46	5.13	8.79
	60 MINS	FDG	1.57	1.89	6.75	0.48
		FACBC	4.15	2.73	3.29	4.15

Finally, the NCI DCIDE program approved a request by Dr. Mark Goodman to perform toxicity studies on anti-FACBC and the only detectable unlabeled side product, syn-1-amino-3-hydroxycyclobutane-1-carboxylic acid, identified in the production batch vial. The results of the toxicity studies are presented in Section 12.8

We plan to use PET-CT anti-[^{18}F]FACBC metabolic imaging for a single research purpose. We plan to use PET-CT imaging to obtain SUV measurements of anti-[^{18}F]FACBC uptake in tumor and surrounding tissue. We hypothesize that anti-[^{18}F]FACBC tumor uptake will be proportional in part to the greater of L-type amino acid transporters on membranes of tumors in comparison to surrounding normal tissue. By scanning subjects with a variety of tumor types SUV measurements can be made and correlated to presence of tumor and grade.

The primary purpose of obtaining SUV measurements is to assess the, pharmacokinetics, pharmacodynamics, specificity and sensitivity of anti-[^{18}F]FACBC tumor uptake in brain and systemic tumors. The long term goal is to identify which tumor types are best suited for anti-[^{18}F]FACBC PET-CT imaging. Such inforamtion could then be used for non-invasive clinical screening for cancer, detection of tumor recurrence and assessment of therapy. The primary purpose of the current IND will be to: a. establish that particular PET-CT protocols yield reliable results in small populations of subjects. B. to assess if the PET-CT protocols are reliable in single subject analysis.

Our previous human experience with anti-[^{18}F]FACBC has shown it to be a remarkably safe radiopharmaceutical (see section 12.9 for previous human experience). Prior experience by the investigators of this IND confirm that anti-[^{18}F]FACBC produces no side-effects. The key risk related to this compound is the exposure to radiation. This issue is addressed in the later sections of this IND.

Our IND for anti-[^{18}F]FACBC is to conduct research studies in humans to determine the pharmacokinetics, pharmacodynamics, specificity and sensitivity of anti-[^{18}F]FACBC tumor uptake in brain and systemic tumors. The IND is not intended to obtain marketing approval. These research studies will always be performed with human investigation committee review and approval. We are certain that the potential benefits of these investigations outweigh the risk in human subjects. The plan duration of this IND is 5 years.

SECTION 12.4

GENERAL INVESTIGATIONAL PLAN

Rationale:

Anti-[^{18}F]FACBC is a radiopharmaceutical used for imaging tumors (xiv-xviii). The magnitude of tumor is dependent on the expression of L-type amino acid transporters on the tumor cell membrane and the relative metabolic activity of the tumor cells (ii). We plan on using SUV measurements of tumor uptake to detect primary tumors and metastasis. This will be done by having patients undergo dynamic PET-CT scans following administration of anti-[^{18}F]FACBC .

The use of anti-[^{18}F]FACBC as a tumor imaging agent has been established in preliminary studies at Emory University (xv-xviii). PET-CT with imaging with anti-[^{18}F]FACB may have advantages that are particularly suitable for detecting tumors in particular brain tumors. After surgical resection and/or radiotherapy of brain tumors, conventional imaging methods such as CT and MRI do not reliably distinguish residual or recurring tumor from tissue injury due to the intervention and are not optimal for monitoring the effectiveness of treatment or detecting tumor recurrence (xx,xxi). The leading PET agent for diagnosis and imaging of neoplasms, 2-[^{18}F]fluorodeoxyglucose (FDG), also has limitations in the imaging of brain tumors. Normal brain cortical tissue shows high [^{18}F]FDG uptake as does inflammatory tissue which can occur after radiation or surgical therapy; these factors can complicate the interpretation of images acquired with [^{18}F]FDG(xxii-xxiv). A number of reports indicate that PET and SPECT imaging with radiolabeled amino acids better defines tumor boundaries within normal brain than CT, MRI or [^{18}F]FDG, allowing better planning of treatment (xxv,xxvi). Additionally, some studies suggest that the degree of amino acid uptake correlates with tumor grade, which could provide important prognostic information (ii,xxvii,xxviii).

Indications:
We plan to use the measurements for RESEARCH studies of human cancer. Subject selection will depend on the design and aims of each tumor paradigm.

Drug Evaluation:
We are interested in assessing the utility of this radiopharmaceutical for detecting brain and systemic tumors. The evaluation will be organized per tumor organ type. Each paradigm will assess a minimum of 20 subjects, and it is expected that 5 different protocols will be evaluated per year. All of these protocols will rely on the same imaging protocol and data analysis methods.

Evaluation of anti-[^{18}F]FACBC for radiochemical purity, sterility and pyrogenicity is described in details in Section 12.7 of this application.

Clinical Trials – Year 1.

- We plan to study up to 100 cancer patents.

Risks to Human Subjects:

Anti-[^{18}F]FACBC toxicology has been performed by NCI and has no known toxicological effects or pharmacological effects in the tracer doses employed. This is discussed in Section 12.8. This tracer has been used in preliminary studies in 32 patients. The major risk of this and other positron emitting tracers is the exposure to low level ionizing radiation. This is discussed in Section 12.6.

SECTION 12.5

INVESTIGATORS BROCHURE

This investigation will be performed only at the Emory University PET Imaging Center. A formal brochure will not be prepared for distribution. Instead, all potential coinvestigators at the Emory PET Imaging Center will receive a copy of our general and supplemental applications to the HIC. These define our standard protocols. Specific answers to the questions of this section are fully addressed in the protocols of Section 12.6.

SECTION 12.6a

PROTOCOLS

All PET-CT imaging protocols use the following scanning method.

PET Scanning Procedure

1) The patient will be placed in the tomographic gantry for a CT scan to be utilized for anatomic imaging and correction of emission data. This takes approximately 1 minute.
2) The patient will then receive a bolus of ^{18}F-FACBC injected intravenously over 2 min.
3) The dosage will be 10.0 mCi (3.70×10^8 Bq).
4) A dynamic sequence of the organ of interest PET imaging lasting 120 minutes is then obtained with

- 6 consecutive 30 second scans followed immediately by
- 4 consecutive 3 minute scans followed immediately by
- 10 consecutive 10 minute scans followed immediately by
- 1 static 5 minute scan giving a total dynamic organ scan imaging time of 120 minutes

Image Analysis

1) The images will be reconstructed with a Hann filter (1.0 X Nyquist frequency) giving an approximate in plane resolution of 6.0 mm.
2) The influx rate constant of ^{18}F-FACBC from plasma to tumor and will be calculated using the Patlak technique.
3) The images will be analyzed by regions of interest related to pertinent organ and lymphatic structures and the tumor to determine tissue time activity curves. Standardized uptake values (SUV) will be calculated with the standard Emory software protocol.

Functional Brain Mapping-General Protocol:

A. Objectives and Purpose

The objectives of this study are to (a) determine the functional organization of normal patterns of brain activity during performance of specified neurobehavioral tasks, b. to assess changes of these patterns in pathologic conditions, and c. to identify tasks that are sufficiently robust to determine patterns of brain activity in single subjects. These observations will be obtained in normal subjects to establish a reference data set and in patients with neurologic disorders to determine how their brains adapt and reorganize after injury or during degeneration. Characterization of the functional activity of the human brain at this microscopic scale is critical in order to understand the mechanisms underlying nervous system plasticity and its functional recovery.

Specific tasks will be performed by each subject during PET scanning. Typically, ten subjects are examined. The data is analyzed for evidence of task related differences for the population as a whole. Once these areas are identified on a population basis, post-hoc analysis will be performed on each individual subject to determine if there are significant changes on individual subject PET images. If the latter is present, then the test will be deemed sufficiently sensitive for the assessment of this particular behavior of individual subjects. This has obvious relevance as a potential clinical tool.

B. Patient Selection (David)

C. Study Design (David)

D. Risks

PET scanning has four direct risks: catheterization, low dose radiation exposure, phlebotomy, and adverse reactions to the injectate. The actual scanning procedure itself during which the subject's body is in the large ring of detectors poses no physical or psychological risks to the subject.

Anti-[^{18}F]FACBC is administered in tracer doses and there is no significant mass effect from the water. Total dosage is determined by the radiation burden of the compound. Up to twelve 10 mCi doses will given. This corresponds to a peak organ exposure of **XX** rads per year to the pancreas and a whole body exposure of **XX** rads per year. A complete summary of radiation dosimetry is included in Section 12.10.

E. Observations and Measurements

Each subject will be referred by a primary care and physician and medical history evaluated by the IND P.I. to ensure the subject is appropriate for the inclusion criteria of the specific imaging protocol. Clinical data will be entered into a computer database. PET-CT results will be linked to clinical findings in that database. All results will be recoded and blinded to name to respect subject confidentiality.

F. Clinical Monitoring

Each subject will have the heart rate, temperature, respiratory rate and clinical symptoms monitored prior to injection and after injection. Clinical data will be reported on a clinical reporting form and also stored in our clinical database.

SECTION 12.6b

FDA Form 1572

SECTION 12.6C

FACILITIES DATA

All aspects of this investigation are to be performed at Emory University School of Medicine. See Section 12.6A and FDA Form 1572, Section 4, for the name and address of the research facility to be used. The PET scanner is located in the Emory PET Imaging Center. There are two PET scanners that can be used. The scanners are a GE PET-CT Model LS whole body and brain scanner. Data can be acquired in either 2D or 3D mode. The second scanner is a CTI HRRT brain scanner. It acquires contiguous tomographic planes of 2 mm separation. Each scanner is operated by its own work station and dedicated advance computational software.

The Emory PET Imaging Center is located on the first floor of the Emory University Hospital. This building is located on the Emory Health Sciences Campus. The cyclotron is a Siemens/CTI 11 MEV negative ion self-shielded device capable of delivering dual isotopes. It produces C-11, O-15, N-13 and F-18 for synthesis of tracer compounds. The cyclotron is located in the basement of the Emory University Hospital. The cyclotron area is connected to the PET Imaging suites by a pneumatic tube for rapid delivery of radioisotopes to the PET facility. Data analysis is performed in the laboratories of the investigators of this proposal. They are located in (a) the Nuclear Medicine Clinic of the Emory University Hospital, and (b) the Woodruff Memorial Research Building adjacent to the hospital. These buildings are all connected by fiber optical infra rapid data transfers.

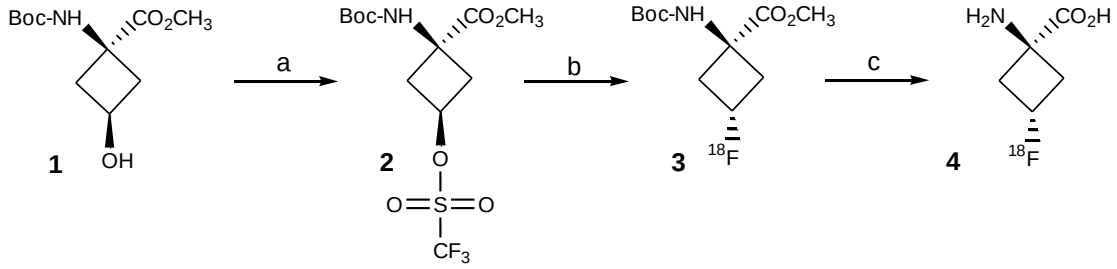
SECTION 12.6d
Institutional Review Board Data

All protocols in the IND will be reviewed by the Emory University Human Investigations Committee. A copy of the approval notification for a specific protocol follows.

SECTION 12.7
Chemistry, Manufacturing and Control Data

Automated radiosynthesis

The preparation of *anti*-[^{18}F]FACBC is shown below in Scheme 1.



a) $(CF_3SO_2)_2O$, pyridine,CH_2Cl_2; b) $K^{18}F$, K_{222},K_2CO_3, 90°C; c) 4N HCl, 120°C.

Scheme 1.

The precursor *syn-1*-(*N*-(*tert*-butoxycarbonyl)amino)-3-[[(trifluoromethyl)sulfonyl]oxy]-cyclobutane-1-carboxylic acid methyl ester **(2)** was prepared in the Emory University PET radiopharmacy. The alcohol **7** (10 mg, 0.04 mmoles) was use to prepare the labeling precursor **2** (14 mg, 91 %) as described previously (xiv). ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s) 3.02-3.13 (4H, m) 3.81 (3H, s) 5.31 (1H, broad s) 5.37-5.41 (1H, m).

The automated radiosynthesis of *anti*-[^{18}F]FACBC (**4**) was carried out in a CTI chemical process control unit (CPCU) with a computer interface. The setup and flow diagram of the CPCU are shown in **Figure 1**. Prior to the start of synthesis, solutions containing the appropriate reagents were loaded into glass v-vials which were sealed with silicone rubber septa held in place with aluminum crimp tops. Each vial was equipped with an inlet line for nitrogen gas to pressurize the vial and Teflon line outlets for delivery of the vial's contents to vessel 1, vessel 2 or the trap/release cartridge within the CPCU.

Vessels 1 and 2 were 12 mL conical vials with side arms that allowed pressurization of the vessels with nitrogen gas to transfer the contents of the vessels. These vessels were placed in the CPCU with stopper assemblies that contained the Teflon lines from appropriate v-vials. Teflon lines connecting vessels 1 to vessel 2 were connected with a Silica SepPak (preconditioned with 10 mL of diethyl ether) under the control of the pinch valve V1. Vessels 1 and 2 were independently heated by lowering the vessels into preheated oil baths.

The ion retardation (IR) column assembly, consisting of a 7 x 120 mm bed of AG 11 A8 ion retard resin, a neutral alumina SepPak Plus (preconditioned with 10 mL water) and an HLB Oasis cartridge (preconditioned with 10 mL ethanol then blown dry with 20 mL air), was rinsed with 60 mL of sterile water and then attached to the outlet line from vessel 2 and the dose vial. The trap/release cartridge was activated with 1 mL of water followed by 2 mL of air and then placed in the CPCU under the control of valve R1. After initial setup of the CPCU, subsequent steps of the procedure were controlled remotely via a computer terminal.

The synthesis began with the addition of 5 mg of Kryptofix in 1 mL of acetonitrile from vial 1 to vessel 1. This step was immediately followed by the delivery of 30 to 44 GBq of H[^{18}F]F in 800 μL of [^{18}O]H_2O to the trap/release cartridge T/R. The activity was released from T/R with 0.6 mL of water containing 0.9 mg of potassium carbonate and delivered to vessel 1 as K[^{18}F]F. This operation was controlled by the pinch valve R1 which provided either an open path from the cyclotron through the trap/release cartridge to waste or an open path from vial 5 (potassium carbonate solution) through the trap/release cartridge to vessel 1. The mixture of

[^{18}F]fluoride and Kryptofix in vessel 1 was heated to 110 °C for 7 minutes with nitrogen gas flow to evaporate the solvent. The 2 mL portion of acetonitrile from vial 2 was then added, and the contents of vessel 1 were heated at 110 °C for an additional 8 minutes to completely dry the K[^{18}F]F. After a 2.5 minute cooling period at ambient temperature, the triflate precursor, *syn-1*-(*N*-(*tert*-butoxycarbonyl)amino)-3-[[(trifluoromethyl)sulfonyl]oxy]-cyclobutane-1-carboxylic acid methyl ester, (**2**) from vial 3 was added to vessel 1 and then heated for 10 minutes at 90 °C. After incorporation of [^{18}F]fluorine, a 3 mL portion of diethyl ether from vial 4 was transferred to vessel 1. The pinch valve V1 on the line from vessel 1 to vessel 2 was opened, and the contents of vessel 1 were transferred through the silica SepPak to vessel 2. The ether solution in vessel 2 was evaporated with nitrogen gas flow at 110 °C for 2 minutes. This transfer of ether from vial 4 to vessel 1 and then through the silica SepPak into vessel 2 followed by evaporation was repeated 2 more times to maximize recovery of the radiolabeled intermediate (**3**) from the silica SepPak. The final portion of ether was evaporated to dryness for 3 minutes under the same conditions to avoid the presence of diethyl ether in the final product.

After completing the evaporation of the ether solution in vessel 2, the oil bath for vessel 2 was heated to 120 °C for the deprotection of the ^{18}F-labeled intermediate, *anti-1*-(*N*-(*tert*-butoxycarbonyl)amino)-3-fluorocyclobutane-1-carboxylic acid methyl ester. A 1.5 mL portion of 4 N HCl was delivered from vial 6 to vessel 2 and heated at 120 °C for 20 minutes. The aqueous hydrosylate was allowed to cool for 1 minute and then diluted with approximately 4 mL water from vial 7. The aqueous solution in vessel 2 was then transferred to the IR column assembly. The product *anti*-[^{18}F]FACBC (**4**) was eluted in series through the ion retard resin, the alumina SepPak Plus and the HLB Oasis cartridge. The elution was performed with three successive portions of ~ 4 mL water transferred from vial 7 to vessel 2 to the IR column assembly. The radiolabeled product eluting from the column assembly passed through a 0.22 μm sterile filter into a dose vial containing 0.7 mL of 23.4 % saline previously equipped with 2 syringes to draw samples for quality control.

Quality control of anti-[^{18}F]FACBC

The collected production batch of *anti*-[^{18}F]FACBC was subject to much the same quality control as [^{18}F]FDG. A 0.5 mL sample was drawn for sterility testing inoculations, and a second 0.5 mL sample was drawn for the remaining QC tests: pH, radiochemical purity, pyrogenicity, and Kryptofix content. The batch vial was visually inspected for clarity and tested for radionuclidic identity. Radiochemical purity was determined by radiometric TLC utilizing silica gel plates or chiral plates with a mobile phase of 20:5:5 acetonitrile:methanol:water.

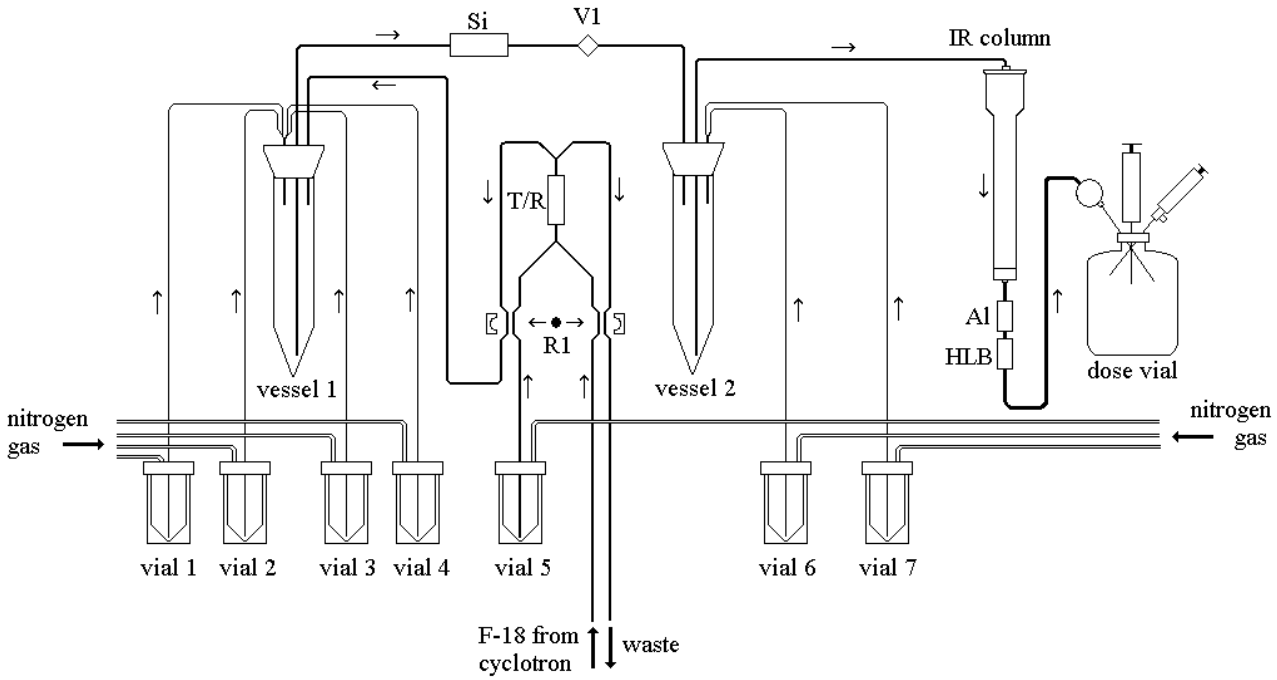


Figure 1. Diagram of the CPCU setup for production of *anti*-[^{18}F]FACBC. Chemistry occurs in vessels 1 and 2 while reagents are delivered from v-vials 1 through 7 at the appropriate times under positive nitrogen pressure. Arrows indicate direction of flow. Oil heating baths for vessel 1 and vessel 2 are not shown.

Vial 1: 5 mg Kryptofix in 1 mL of acetonitrile; 2 mL v-vial.
Vial 2: 2 mL of acetonitrile; 5 mL v-vial.
Vial 3: 12-15 mg of triflate precursor **8** in 1 mL of acetonitrile; 2 mL v-vial.
Vial 4: 10 mL of diethyl ether; 10 mL vial.
Vial 5: 0.9 mg potassium carbonate in 0.6 mL water; 2 mL v-vial.
Vial 6 : 1.5 mL of 4N hydrochloric acid; 5 mL vial.
Vial 7: 15 mL of sterile water; 15 mL vial.

T/R: Trap/release cartridge.
R1: Trap/release pinch valves. Operates in one of two positions: 1) closes line from vial 5 to T/R and line from T/R to vessel 1 during delivery of hydrogen[^{18}F]fluoride from cyclotron to T/R or 2) closes line from cyclotron to T/R and line from T/R to waste during deliver of potassium carbonate from vial 5 through T/R to vessel 1.

Si: Silica SepPak preconditioned with 10 mL of diethyl ether.
V1: Pinch valve. Opened during delivery of diethyl ether from vessel 1 to vessel 2 through **Si**, closed during delivery of the contents of vessel 2 to the IR column.

IR column: 7 x 120 mm bed of AG 11 A8 ion-retardation resin rinsed with 60 mL sterile water.
Al: Alumina N SepPak preconditioned with 10 mL of sterile water.
HLB: HLB Oasis cartridge preconditioned with 10 mL ethanol followed by 20 mL air.

Dose vial: 30 mL sterile vial containing 0.7 mL of 23.4 % saline with 0.22 μm sterile filter, 1 mL syringe with stopcock vent for sterility sample, and 0.5 mL syringe for other quality control assays.

Emory P.E.T. Radiopharmacy Emory University Hospital 1364 Clifton Road, NE EG-45 Atlanta, GA 30322 404-712-7930	Batch Production Record for: **[F-18]FACBC** Origin: 9/02 R. J. Crowe, RPh; Revised: 2/04 Approved: _____ Version VI	**SOP: FACBC-P001.6**

Date of Production:_____ Batch Number:_____ Operator:_____

^{18}F Production:

1. Go to *Target Zone #4* from **Produce Labeled Product** (from Main Screen) and select Test Bombardment-Auto Load.

2. Enter and record desired beam current and bombardment time.

   Beam Current:_____uA (20-33); Bombardment Time:_____minutes.

3. Verify successful load of target with [^{18}O]Water:_____ml (0.900ml).

4. Record Start and End of Bombardment times: SOB:_____hours; EOB:_____hours.

5. During bombardment, print (‘*Control P’*) the following two pages: **Production of [F-18] Fluoride** and the **GET_VALUES** from the CD_TEST directory. Attach to Batch Record.

Operator:_____

Pre-Chemistry Preparation:

1. Initialize CPCU #2 via the **READYCPCU2** command from the CD_FACBC directory. Follow the prompts to place the vessels and oil baths in the correct position.
2. Visually inspect CPCU, delivery lines, and pinch valves for potential malfunctions.
3. Clean CPCU and sliding tray with 70% isopropyl alcohol.
4. After sufficient time for CPCU to ‘warm up’ (~20 min.), record the oil bath temperatures from the Omega controller display:

   Left Bath:_____°C (~90) Right Bath:_____°C (~110)

   Operator:_____

CPCU Setup:

1. Obtain and install two foil wrapped depyrogenated glassware vessels (SOP FACBC-P002).
2. Obtain and install two cleaned (SOP FACBC-P003) FACBC-type stopper assemblies, **Type A** goes in ***Left*** holder and **Type C** goes in ***Right*** holder.

ReagentVial Setup:

1. Obtain foil wrapped depyrogenated reagent vial glassware package (SOP FACBC-P002).

2. Mark the 1 ml v-vials #1, #3, and #5.
3. Mark the 5 ml v-vial #2.
4. Mark the 10 ml serum vial #4.
5. Mark the 5 ml disposable vial #6.
6. Mark the 20 ml vial #7.

7. Place prepared two-hole septa on the reagent vials and apply aluminum crimp seals. Check that seal seat is good and the crimp rings are not mangled.

8. Add the appropriate amount of the appropriate reagent to the appropriate vial as outlined below and record the amounts used.

After adding the appropriate reagents as prescribed, place the numbered vials in their corresponding numbered positions. Insert the reagent delivery lines of the vessel stopper assembly thru a septa hole in each vial so that the open end of the line rests on the bottom corner of the reagent vials. Attach the correct pressurizing line to the corresponding correct vial by inserting line just into the septa. Insert the "KCO3" line (emanating from the 4-way pinch valve) into reagent vial #5 as described above.

Vial #1: 0.5 ± 0.1 ml Krytofix $K_{2.2.2.2.}$ solution (10mg/ml, SOP M002S) and 0.5 ± 0.1 ml Acetonitrile

$K_{2.2.2.2}$:_____ml Acetonitrile:_____ml Operator:_____

Vial #2: 2.0 ± 0.1 ml Acetonitrile

Acetonitrile:_____ml Operator:_____

Vial #3: 16 ± 1.5 mg Triflate in 1.0 ± 0.1 ml Acetonitrile

Triflate:_____mg Acetonitrile:_____ml Operator:_____

Vial #4: 8.0-10.0 ± 0.5 ml Ether

Ether:_____ml Operator:_____

Vial #5: 0.22 ml freshly prepared Potassium Carbonate solution, 4.0 mg/ml (*see following note*) and 0.38 ml SWFI

Potass. Carb. Soln.:_____ml SWFI:_____ml Operator:_____

***Note:* 4.0 mg/ml Potass. Carb. Soln. Prep:** *Prepare daily in excess, weigh ~20mg KCO_3 and add SWFI to bring concentration of solution to 4.0 mg/ml.*

KCO_3:_____mg SWFI:_____ml Operator:_____

Vial #6: 1.5 ± 0.1 ml aqueous 4N Hydrochloric Acid *(Prepare from 6N HCl, see FACBC-P004)*

4N HCl:_____ml Operator:_____

Vial #7: 12.0 ± 1.2 ml 0.9% Sodium Chloride, USP

0.9% NaCl:_____ml Operator:_____

Ion Retard Purification Column Preparation:

1. Flush FACBC (SOP FACBC-C001) column with 30 ml of Sterile Water for Injection, USP.

   SWFI:_____ml Operator:_____

2. Condition Alumina SepPak with 10 ml of SWFI.

   SWFI:_____ml Operator:_____

3. Condition the HLB cartridge with 10 ml of Ethanol, USP followed by 30 ml of Air.

   EtOH:_____ml Air:_____ml Operator:_____

4. Connect the items above in sequence (column, attach Alumina SepPak to bottom of column, HLB to bottom of Alumina SepPak) and flush with 30-40 ml of SWFI.

   SWFI:_____ml Operator:_____

Silica SepPak Preparation:

1. Condition Silica SepPak with 10 ml Ether, USP and leave 'wet'.

   Ether:_____ml Operator:_____

2. Install the Silica SepPak into the transfer line connecting Vessel 1 and Vessel 2 ensuring that the gummy tubing line on the exit end of the SepPak resides in the ether pinch valve (R151).

Preparation of Fluoride-18 extraction column:

1. Slightly trim each end of extraction column to 45° to facilitate ease of insertion into ends of gummy tubing.
2. Flush extraction column dropwise with 1.0 ml of SWFI followed by 2.0 ml of air.
3. Attach gummy 'In' line from pinch valve to bottom of extraction column, place column in holder and attach 'Out' line to top of extraction column.
4. Connect $^{18}F^-$ gummy delivery line to the peak tubing on the stopper assembly of Vessel 1.

SWFI:_____ml Air:_____ml Operator:_____

^{18}F Measurement and Synthesis:

1. At EOB, choose **MAKE_FACBC** from the CD_TEST menu. Follow the computer screen prompts to unload activity into v-vial.

2. Measure and record activity in v-vial and delivery time and activity remaining after transfer to CPCU:

Delivery Time:_____ ^{18}F Activity:_____mCi ^{18}F Remaining:_____mCi

3. Follow screen prompts to deliver activity to CPCU. Record start of synthesis (SOS) and end of synthesis (EOS) below (see italics in 5. below):

SOS Time:_____ EOS Time:_____ Operator:_____

[SOS= Time of elution from T/R cartridge; EOS=Beginning of product transfer/collection]

4. Approximately 45 minutes into synthesis the computer screen will display a message for the Operator to manually raise the Right Oil Bath temperature from 110 to 120°C. [On the Omega controller for the right oil bath for CPCU 2, press and hold the '∗' button and press the '↑' button until the display reads 120°C].

Adjusted Right Oil bath Temp:_____°C Operator:_____

5. Synthesis time is ~70 minutes, at which point, the computer will prompt the operator to open the stopcock on the Batch collection vial. Follow the computer screen prompts to begin transfer into collection vial. *Due to the variable time required for complete transfer, record the time (end of hydrolysis, beginning of transfer/collection) as EOS above.* The Program automatically turns off the CPCU and returns the computer to the Main screen. Once activity has been collected, follow prompts to end synthesis program.

Return right oil bath temp returned to 110°C:___(check) Operator:_____

6. Indicate and explain any deviations from normal, semi-automated synthesis, if none, write 'None'.

Deviations:

Operator:_____

This marks the end of the semi-automated synthesis production of FACBC. The FACBC collection vial is assayed and the amount produced is recorded on the Quality Control Tests information sheets (QC summary record, production/dispensing record, pyrogen test record). Attach all QC records, QC result printouts, and Rx Dispensing records together. Attach Production Materials Log, computer production printouts, and XLS Production Yield Record to the Production Batch Record.

Operators sign below to verify correctness and completeness of required documentation and certify production batch vial has been labeled in accordance with SOP FACBC-L001.

_____	_____	_____
Signature	Initials	Date

_____	_____	_____
Signature	Initials	Date

Attach exact copy of Batch Production Vial Label below:

A. Tests that must be Complete at Time of Product Release FACBC Lot No.: _____

Radiochemical Identity and Purity: Radio-TLC

(Perform According to SOP Q002): **Radiochemical Pass □ Fail □**

Identity

R_f ^{18}F-FACBC: _____ (≅ 0.27) **Radiochemical Pass □ Fail □**

Purity

R_f external stnd: _____
(not required daily; see SOP) **(≅)**

% Radiochemical Purity: _____ % **≥ 90%** **Performed By: _____**

Radionuclidic Identity: Ten-Minute (Tx) Decay (Perform according to SOP Q005):

t_0: _____ A_{t0}: _____mCi t_x: _____ A_{tx}: _____mCi **Δt = 10 min** **Lab Timer: □**

Decay

Factor: 0.939
$A_{tc} = A_{t0}$ x D.F. A_{tc} = _____mCi

% Deviation = [(A_{tx} - A_{tc}) ÷ A_{tc}] x 100

% Deviation = _____ % **must be within ± 3%** **Pass □ Fail □**

Performed By: _____

Chemical Purity: Gas Chromatography (Perform According to SOP Q003)
Calculate according to the formula: µg/ml x Batch Volume(ml) ÷ 1000 = mg/batch

Ether: Pass □ Fail □ _____µg/ml _____mg/batch ≤ 6.69

Ethanol: Pass □ Fail □ _____µg/ml _____mg/batch ≤ 4.93

Acetonitrile: Pass □ Fail □ _____μg/ml _____mg/batch ≤ 1.70

Performed By: _____ Batch Volume = _____ml

Final pH (Perform According to SOP Q014):

"Tri-Colored strip (pH 5-10) pH 6 stnd: □ pH 7 stnd: □

"Green" strip (pH 4-7) pH 6 stnd: □ pH 7 stnd: □

Final Product: acceptable range 5.0 – 8.0: **Pass □ Fail □**

Performed By: _____

Filter Integrity Test (Perform According to SOP Q016): **Pass** □ **Fail** □

□ **Millex: (≥ 50 psi) _____** □ **EPS: (≥ 40 psi) _____** **Performed By: _____**

Bacterial Endotoxins Initiated (Perform According to SOP Q007):

Performed By: _____ ***Yes*** □ ***No*** □

20 Minute Pyrogen Results: **Pass** □ **Fail** □

In The Event Product Fails Retest According to SOP Q007

Visual Inspection Behind Leaded Glass

(Clear, Colorless, solution) **Pass** □ **Fail** □

Performed By: _____

B. Tests Completed After Product Release

Chemical Purity: Kryptofix TLC (Perform According to SOP Q004):

R_f = _____

Performed By: _____ **Pass** □ **Fail** □

Bacterial Endotoxin Test 60 minutes: LAL (Perform According to SOP Q007):
Attach test record to batch record
Performed By: _____ **Pass** □ **Fail** □

In The Event Product Fails Retest According to SOP Q007

Sterility Test: Direct inoculation (Perform According to SOP Q040):

Innoculated By: _____ ***Date:*** _____

Product Released for Distribution

A batch may be released for distribution only according to SOP A009, **Quality Control Release Authority**

Product Released Yes ☐ No ☐

Date: _____

Signature for Release: _____

Quality Control Supervisor

SECTION 12.8
Pharmacology and Toxicology

F-ACBC (NSC-726817) / OH-ACBC (NSC-726816)

PRECLINICAL TOXICOLOGY SUMMARY

Prepared by:

Toxicology & Pharmacology Branch
Developmental Therapeutics Program
Division of Cancer Treatment & Diagnosis
National Cancer Institute

April, 2005

Preclinical Toxicology Studies for F-ACBC (NSC-726817) / OH-ACBC (NSC-726817)

I. INTRODUCTION

Many amino acids have been synthesized with positron-emitting labels and used to image tumors[1-6]. Those labeled with ^{11}C have the disadvantage of a relatively short half-life (20-minutes), and those that are naturally occurring are highly susceptible to metabolism, and their metabolites can greatly confound interpretation of the images. Aminocyclobutane carboxylic acid, ACBC, being an Aunnatural@ amino acid, is much less susceptible to metabolism, and radiolabeled metabolites, if they did occur, would be less likely to confound interpretation of the images. Also, labeling ACBC with ^{18}F has the added practical advantage of a 110-minute half-life. We have conducted preclinical toxicology studies with ^{19}F-labeled-ACBC to demonstrate that it has an appropriate margin of safety and appears suitable for clinical use.

Target doses for safety pharmacology studies and IND-directed toxicology studies were determined based on the following considerations: (1) the standard PET tracer dose will be 10 mCi/patient/scan (it is anticipated that patients will undergo one or several scans, depending on the clinical protocol); (2) the drug product will contain 1 μg of F-ACBC per mCi and 25 μg/mCi of OH-ACBC (unlabeled reaction product) per mCi; (3) thus, the mass dose per patient per scan will be 10 μg of F-ACBC and 250 μg of OH-ACBC; (4) per patient mass doses were normalized on a $\mu g/m^2$ basis as shown in Table 1; (5) maximum doses ($\mu g/m^2$) for animal studies were then set at 100 - 200 times greater than the anticipated patient dose for each component of the drug product (Table 1).

However, the results of the dose formulation analysis for the rat and rabbit studies indicated that the measured concentrations, particularly for the OH-ACBC component, differed significantly from the nominal values (Table 2). Since the basis for the discrepancy is still unknown, the no-observed-adverse-effect levels (NOAELs) are reported herein based on doses calculated from the measured formulation concentrations, not the target doses. Dose formulation analysis was not conducted for the safety pharmacology study in dogs.

II. MUTAGENICITY ASSAYS

In Vitro Salmonella Reverse Mutation Plate Incorporation Assay (IITRI Project No. 2073-003-001)

The potential mutagenic activity of F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) was investigated in the Salmonella Reverse Mutation Plate Incorporation Assay, which is an *in vitro* test designed to detect point mutations in bacterial tester strains induced by chemical agents. The mutagenic events are reverse mutations that cause histidine-requiring mutants to revert to their prototrophic (non-histidine requiring) state. The study was conducted in two parts such that an initial mutagenicity/cytotoxicity assay was conducted first, which was followed by a confirmatory assay. For both assays, F-ACBC/OH-ACBC was tested in *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 in the presence and absence of S9 metabolic activation. Concentrations of 0, 50, 100, 500, 2000 and 5000 μg/plate were used for the initial mutagenicity/cytotoxicity assay, whereas 0, 10, 20, 50, 500 and 2000 μg/plate were used in the confirmatory assay. In order for F-ACBC/OH-ACBC to be considered mutagenic in this assay, all of the four following criteria had to be met: (1) the number of revertant colonies per plate for the test article-treated samples had to exceed the upper limit of the historical range for the vehicle control for the particular tester strain; (2) the increase in the number of revertant colonies had to be 2-fold or greater than the control; (3) the increase in the number of revertant colonies had to be reproducible between the initial mutagenicity/cytotoxicity assay and the confirmatory assay; and (4) the increase in the number of revertant colonies had to dose-related over the range tested. The study was conducted in compliance with GLP regulations (21 CFR, Part 58).

In the range-finding assays, F-ACBC/OH-ACBC did not exhibit a dose-related mutagenic response in any of the five tester strains in the presence or absence of S9 metabolic activation, and there was no evidence of cytotoxicity for any strain in the presence or absence of S9. While a two-fold or greater increase of revertants per plate was observed at the 50 μg/plate dose level for tester strain TA98 without S9, and at 500 μg/plate for tester strain TA1537 without S9, these responses were not considered drug-related since there was no dose-related mutagenic response. A two-fold or greater increase of revertants per plate was also observed at all dose levels for TA1537 with

S9, however this was not considered drug-related because the number of revertants per plate were within the historical range for the negative control for this tester strain.

In the confirmatory mutagenicity assay, cytotoxicity was not observed with doses up to 2000 μg/plate for any strain in the presence or absence of S9 activation. In addition, no dose-related mutagenic response pattern was observed in any of the five tester strains either in the presence or absence of S9.
The results of this study indicate that F-ACBC/OH-ACBC is non-mutagenic in the *Salmonella* Reverse Mutation Plate Incorporation Assay.

In Vivo **Micronucleus Assay in Rats**

The potential for F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) to induce chromosomal aberrations and spindle malformations *in vivo* was evaluated using the Micronucleus Assay in conjunction with the 14-Day Toxicity Study of F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) in Rats (IITRI Project No. 6336-141). Fischer 344 rats (15/sex/group) were treated with intravenous target doses of 1.25/0.05 and 5/0.2 mg/kg/d (7.5/0.3 and 30/1.2 mg/m^2/d) OH-ACBC/F-ACBC once a day for 14 consecutive days. Bone marrow was collected on study days 15 (10 rats/sex/group) and 29 (5 rats/sex/group) for preparation of bone marrow smears. Cytogenetic damage is indicated by the presence of micronuclei in polychromatic erythrocytes. The study was conducted in compliance with GLP regulations (21 CFR, Part 58).

No statistically significant (p<0.05) or dose-dependent increase in micronucleated polychromatic erythrocytes was noted for rats treated with OH-ACBC/F-ACBC relative to the vehicle control groups on day 15. However, a statistically significant decrease in micronucleated polychromatic erythrocytes was observed for females on day 29. Since this response was observed only after the 2-week recovery period and consisted of a decrease, rather than the anticipated increase, it was considered toxicologically irrelevant and unrelated to treatment. Rats treated with cyclophosphamide (positive control; males only) exhibited a statistically significant increase in micronucleated polychromatic erythrocyte counts on day 15 and day 29, as compared to the vehicle control group. This demonstrated that the assay was sensitive to detecting

mutagenicity. Therefore, OH-ACBC/F-ACBC was concluded to be negative in the rat micronucleus test at the doses administered.

Although the target dose levels of the OH-ACBC (NSC-726816) / F-ACBC (NSC-726817) mixture in this study were 1.25/0.05 and 5/0.2 mg/kg (7.5/0.3 and 30/1.2 mg/m^2), the dose formulation analysis results indicated that measured concentrations of the OH-ACBC component may have been 30% and 40% of nominal for the low and high dose, respectively. Measured concentrations of the F-ACBC component agreed with the nominal values. Since the basis for the discrepancy between measured and nominal values for OH-ACBC is still unknown, we report the NOAELs for the study as 0.2 mg/kg F-ACBC and 2 mg/kg OH-ACBC. F-ACBC and OH-ACBC doses of 0.2 mg/kg and 2 mg/kg, respectively, given once a day for 14 consecutive days did not induce chromosomal aberrations nor spindle malformations in rats. These doses are ~200-fold (F-ACBC) and ~90-fold (OH-ACBC) higher than the proposed human dose.

III. SAFETY PHARMACOLOGY STUDIES

Cardiovascular and Pulmonary Safety Testing of F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) in Beagle Dogs (Battelle Study G465536A). The objective of this study was to evaluate the cardiovascular and pulmonary safety of F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) when administered as a single intravenous bolus dose in Beagle dogs (2/sex/dose group). The test articles were combined to obtain a 1:25 ratio of F-ACBC to OH-ACBC and the mixture was dissolved in a sufficient volume of 0.9% sodium chloride for injection, USP to prepare the formulated solutions. Drug-treated dogs were administered a target dose of 0.053/1.32 mg/kg (1.06/26.4 mg/m^2) F-ACBC/OH-ACBC, and control animals received an equal volume (2 mL/kg) of vehicle. Cardiovascular data (systemic arterial blood pressures, heart rate, and ECG data) were collected for up to 60 hours post dosing via implantable radiotelemetry units. ECG interval measurements were made on the ECG waveforms. Pulmonary data (respiratory rate, tidal volume and minute volume) were collected continuously for approximately 4-5 hours post dosing. Clinical observations, body temperatures and clinical pathology were also evaluated. The study was conducted in compliance with GLP regulations (21 CFR, Part 58).

Mortality/Clinical Observations: No deaths occurred in this study. There were no test-article related abnormal clinical observations.

Body Weights/Body Temperatures: There were no test article-related changes in body weights or body temperatures.

Clinical Pathology: There were no test article-related changes in hematology or clinical chemistry, including C-reactive protein, serum amyloid A and troponin T.

Cardiovascular Effects: There were no apparent or statistically significant differences in blood pressure or heart rate for test article-treated animals (males and females combined) compared to vehicle-treated animals during this study. However, minor blood pressure alterations were observed for test article-treated females compared with vehicle-treated females. In these animals, systemic pressures appeared to track higher than vehicle during the daytime periods, and lower than vehicle during the evening hours. The relevance of these minor non-statistically significant blood pressure alterations is questionable.

Evaluation of ECG interval data revealed no apparent or statistically significant differences in the test article group as compared to vehicle, and there were no alterations in ECG rhythm or morphology that could be attributed to the test article.

Respiratory Effects: There were no apparent or statistically significant alterations in respiratory rates, tidal volume or minute volume in this study.

Conclusions: No treatment-related effects on cardiovascular or respiratory function were observed in dogs dosed with a 0.053/1.32 mg/kg ratio of F-ACBC to OH-ACBC (1.06/26.4 mg/m^2). This dose is 200-fold higher than the proposed human dose.

Neurological Safety Assessment in Rats. Neurotoxicology assessments [functional observational battery (FOB) evaluations] for rats were done in conjunction with the 14-Day Toxicity Study of F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) in Rats (IITRI Project No. 6336-141). Fischer 344 Rats (15/sex/group) were treated intravenously with target doses of 1.25/0.05 and 5/0.2 mg/kg/d (7.5/0.3 and 30/1.2 mg/m^2/d) OH-ACBC/F-ACBC once a day for 14 consecutive days. Neurological toxicity evaluations were

conducted on rats prior to dosing (pre-study), and on days 14 (10/sex/group) and 28 (recovery rats; 5 /sex/group). The following FOB parameters were monitored: home cage observation (e.g. tremors, convulsions, biting, vocalizations, posture, fur appearance), arousal/anxiety when handheld, open field mobility/gait, reactivity to sensory stimulation (e.g. visual, auditory, tactile, pain), hindlimb extension, catalepsy, forelimb and hindlimb grip strength, righting reflex, footsplay, body temperature and body weights. The study was conducted in compliance with GLP regulations (21 CFR, Part 58).

A statistically significant decrease (<3%) in mean body temperature was observed for males in the high dose group on day 14, relative to the control group. This slight decrease was not considered toxicologically relevant. Body temperatures for all treated animals were comparable to controls on day 28. No other statistically significant or otherwise noteworthy changes were observed for any of the FOB parameters during the dosing or recovery periods.

Although the target dose levels of the F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) mixture in this study were 1.25/0.05 and 5/0.2 mg/kg (7.5/0.3 and 30/1.2 mg/m^2), the dose formulation analysis results indicated that measured concentrations of the OH-ACBC component may have been 30% and 40% of nominal for the low and high dose, respectively. Measured concentrations of the F-ACBC component agreed with the nominal values. Since the basis for the discrepancy between measured and nominal values for OH-ACBC is still unknown, we report the NOAELs for the study as 0.2 mg/kg F-ACBC and 2 mg/kg OH-ACBC. In conclusion, there was no evidence of neurological toxicity in rats dosed once a day for 14 consecutive days with F-ACBC and OH-ACBC doses of 0.2 mg/kg and 2 mg/kg, respectively. These doses are ~200-fold (F-ACBC) and ~90-fold (OH-ACBC) higher than the proposed human dose.

IV. IND-DIRECTED TOXICITY STUDIES OF F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) IN RATS AND RABBITS

14-Day Toxicity Study of F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) in Rats (IITRI Project No. 6336-141). The objective of this study was to evaluate the target organ toxicity of F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) and its reversibility

when administered intravenously to rats once a day for 14 days. The test articles were combined to obtain a 1:25 ratio of F-ACBC to OH-ACBC and the mixture was dissolved in a sufficient volume of 0.9% sodium chloride for injection, USP to prepare the formulated solutions. The OH-ACBC/F-ACBC mixture was administered at target dose levels of 1.25/0.05 and 5/0.2 mg/kg (7.5/0.3 and 30/1.2 mg/m^2) to male and female Fischer 344 rats (15/sex/group) once a day for 14 consecutive days. Control rats received an equal volume (2 mL/kg) of 0.9% sodium chloride for injection, USP once a day for 14 days. Cageside clinical observations were performed and recorded daily, and hand-held physical and clinical observations were performed during pretest, and weekly throughout the treatment and recovery periods. Body weights were measured twice weekly during the treatment and recovery periods. Hematology and clinical chemistry parameters were evaluated on days 8, 15 and 29. Functional observational battery (FOB) tests to determine neurological toxicity were performed during pretest, and on days 14 and 28. Organ weights were determined on days 15 and 29. A bone marrow micronucleus assay was conducted using bone marrow from rats euthanized on day 15 and day 29. Microscopic histopathology was performed on tissues from rats in the high dose (5/0.2 mg/kg OH-ACBC / F-ACBC) and control groups which were euthanized on day 15. The study was conducted in compliance with GLP regulations (21 CFR, Part 58). The results are summarized in Table 3.

Mortality/Clinical Observations: No deaths occurred, and no drug-related adverse clinical signs were observed after 14 days of dosing.

Functional Observational Battery: The results of the neurotoxicology assessments are summarized in the Neurological Safety Assessment in Rats.

Body Weights: There were no treatment-related changes in body weights or body weight gain during the treatment and recovery periods.

Hematology: Minimal changes in hematology parameters occurred that were considered toxicologically irrelevant and/or unrelated to treatment with the test articles.

Clinical Chemistry: Minimal changes in clinical chemistry parameters occurred that were not toxicologically relevant nor clearly drug related.

Organ Weights: No treatment-related effect on organ weights was observed in this study.

Micronucleus Assay: The results of the micronucleus assay are summarized in the *In Vivo* Micronucleus Assay in Rats.

Pathology: No treatment-related gross lesions were observed in tissues of rats on day 15 or 29, and no treatment-related microscopic lesions were observed on day 15 in the tissues of rats that were administered a target dose of 5/0.2 mg/kg/d OH-ACBC / F-ACBC. Tissues from recovery rats were not evaluated microscopically.

Conclusions: Intravenous administration of the OH-ACBC (NSC-726817) / F-ACBC (NSC-726816) mixture to rats at target dose levels of 1.25/0.05 and 5/0.2 mg/kg/d (7.5/0.3 and 30/1.2 mg/m^2/d) for 14 consecutive days did not result in any clearly dose-related, treatment-related and/or toxicologically significant effects on mortality, clinical observations, mean body weights, mean body weight gains, hematology, clinical chemistry, neurotoxicity (as measured by FOB), mutagenicity (bone marrow micronucleated erythrocytes) or organ weights. No treatment-related gross or microscopic lesions were observed in any of the tissues evaluated.

Although the target dose levels of the F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) mixture in this study were 1.25/0.05 and 5/0.2 mg/kg (7.5/0.3 and 30/1.2 mg/m^2), the dose formulation analysis results indicated that measured concentrations of the OH-ACBC component may have been 30% and 40% of nominal for the low and high dose, respectively. Measured concentrations of the F-ACBC component agreed with the nominal values. Since the basis for the discrepancy between measured and nominal values for OH-ACBC is still unknown, we report the NOAELs for the study as 0.2 mg/kg F-ACBC and 2 mg/kg OH-ACBC. These doses are ~200-fold (F-ACBC) and ~90-fold (OH-ACBC) higher than the proposed human dose.

14-Day Toxicity Study of F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) in Rabbits (IITRI Project No. 6336-142). The objective of this study was to determine target organ toxicity of F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) and its reversibility when given intravenously to rabbits once daily for 14 consecutive days. The test articles were combined to obtain a 1:25 ratio of F-ACBC to OH-ACBC and the

mixture was dissolved in a sufficient volume of 0.9% sodium chloride for injection, USP to prepare the formulated solutions. The OH-ACBC/F-ACBC mixture was administered at target dose levels of 0.625/0.025 and 1.25/0.05 mg/kg (7.5/0.3 and 15/0.6 mg/m^2) to male and female New Zealand White rabbits (8/sex/group) once a day for 14 consecutive days. Control rabbits received an equal volume (0.5 mL/kg) of 0.9% sodium chloride for injection, USP once a day for 14 days. Clinical signs of toxicity were monitored daily, and body weights were measured twice weekly during the treatment period and once weekly during the recovery period. Hematology and clinical chemistry parameters were evaluated on days 8, 15 and 29. Microscopic histopathology was performed on tissues from all rabbits in the high dose (1.25/0.05 mg/kg/d) and control groups that were euthanized on day 15. The study was conducted in compliance with GLP regulations (21 CFR, Part 58). The results are summarized in Table 3.

Mortality/Clinical Observations: No deaths occurred in this study. All rabbits were described as clinically normal at all observation times.

Body Weights: There were no treatment-related alterations in body weights or body weight gain during the dosing or recovery periods.

Hematology: No treatment-related changes in hematology were observed.

Clinical Chemistry: No treatment-related changes in clinical chemistry were observed.

Organ Weights: No treatment-related effect on organ weights was observed in this study.

Pathology: No treatment-related gross lesions were observed in tissues of rabbits on day 15 or day 29, and no treatment-related microscopic lesions were observed on day 15 in the tissues of rabbits that were administered a target dose of 1.25/0.05 mg/kg/d. Tissues from recovery rats were not evaluated microscopically.

Conclusions: Intravenous administration of the OH-ACBC (NSC-726817) / F-ACBC (NSC-726816) mixture to rabbits at target dose levels of 0.625/0.025 and 1.25/0.05 mg/kg/d (7.5/0.3 and 15/0.6 mg/m^2/d) for 14 consecutive days did not result in any treatment-related effect on mortality, clinical observations, mean body weights, mean

body weight gains, hematology, clinical chemistry, or organ weights. No treatment-related gross or microscopic lesions were observed in any of the tissues evaluated.

Although the target dose levels of the F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) mixture in this study were 0.625/0.025 and 1.25/0.05 mg/kg (7.5/0.3 and 15/0.6 mg/m^2), the dose formulation analysis results indicated that measured concentrations of the OH-ACBC component were 40-80% and 50-120% of nominal for the low and high doses, respectively. Measured concentrations of the F-ACBC component were 86-110% of nominal for the low dose, and 110-170% of nominal for the high dose. Since the basis for the discrepancy between measured and nominal values is still unknown, we report the NOAEL for the study as 0.055 mg/kg F-ACBC and 0.625 mg/kg OH-ACBC. These doses are ~120-fold (F-ACBC) and ~55-fold (OH-ACBC) higher than the proposed human doses.

V. SUMMARY

A dose of 0.25/0.01 mg/person (0.132/0.005 mg/m^2, based on a 70 kg/person) OH-ACBC/F-ACBC is proposed for the clinical trials. In the cardiovascular and respiratory safety pharmacology study, dogs receiving a single target dose of OH-ACBC/F-ACBC at 1.32/0.053 mg/kg (26.4/1.06 mg/m^2) F-ACBC/OH-ACBC, a 200-fold higher dose than the proposed clinical dose, exhibited no treatment-related effects. In the 14-day toxicity studies, the NOAELs for rats were 2 mg/kg (12 mg/m^2) for OH-ACBC and 0.2 mg/kg (1.2 mg/m^2) for F-ACBC, doses which are 90-fold (OH-ACBC) and 200-fold (F-ACBC) higher than the proposed human doses, when compared on a daily basis. For rabbits, the NOAELs were 0.625 mg/kg (7.5 mg/m^2) for OH-ACBC and 0.055 mg/kg (0.6 mg/m^2) for F-ACBC. These doses were ~55-fold and ~120-fold higher, respectively, than the proposed human dose, when compared on a daily basis. When the total doses administered are compared, the NOAELs for OH-ACBC for rats and rabbits were 1270-fold and 800-fold higher, respectively, than the proposed clinical dose, and the NOAELs for F-ACBC for rats and rabbits were 3200-fold and 1700-fold higher than the proposed clinical dose. Hence, OH-ACBC/F-ACBC has an appropriate margin of safety for clinical use.

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TABLE 1. ESTABLISHMENT OF DOSES FOR *IN VIVO* STUDIES

Compound	Anticipated Human Dose*			200X Human Dose ($\mu g/m^2$)	Maximum Dose in Dogs (200X; $\mu g/kg$)	Maximum Dose in Rats (200X; $\mu g/kg$)	Maximum Dose in Rabbits (100X; $\mu g/kg$)
	μg/patient	$\mu g/kg$	$\mu g/m^2$				
F-ACBC	10	0.143	5.29	1,060	53	177	44.2
OH-ACBC	250	3.57	132	26,400	1320	4,400	1,100

*Based on administration of 10 mCi of drug product to a 70 kg adult patient

TABLE 2. TARGET DOSES OF OH-ACBC/F-ACBC VERSES CALCULATED DOSES OH-ACBC/F-ACBC

Lab/ Project No.	Species	Target OH-ACBC/F-ACBC Daily Dose (based on Nominal Values)		Calculated OH-ACBC/F-ACBC Daily Dose (based on Measured Values)	
		mg/kg	mg/m^2	mg/kg	mg/m^2
Battelle Study G465536A	Dogs	0	0	ND	ND
		1.32 / 0.053	26.4 / 1.06	ND	ND
IITRI Project No. 6336-141	Rats	0	0	0	0
		1.25 / 0.05	7.5 / 0.3	0.375 / 0.05	2.25 / 0.3
		5.0 / 0.2	30 / 1.2	2 / 0.2	12 / 1.2
IITRI Project No. 6336-142	Rabbits	0	0	0	0

		0.625 / 0.025	**7.5 / 0.3**	**0.25 / 0.022**	**3 / 0.26**
		1.25 / 0.05	**15 / 0.6**	**0.625 / 0.055**	**7.5 / 0.66**

ND = dose formulation analysis was not done

TABLE 3. SUMMARY OF 14-DAY TOXICITY STUDIES WITH F-ACBC (NSC-726817) / OH-ACBC (NSC-726816) IN RATS and RABBITS

Study Type	Lab/ Project No.	Vehicle	Species/ Strain	#/Sex/ Dose	Route/ Schedule	Target* OH-ACBC/F-ACBC Daily Dose		Target* OH-ACBC/F-ACBC Total Dose		Target Organs/Systems of Toxicity
						mg/kg/d	mg/m^2/d	mg/kg	mg/m^2	
Toxicity GLP	IITRI Project No. 6336-141	0.9% sodium chloride for injection USP	Fischer 344 rats	15	i.v./ d x 14	0	0	0	0	Toxicity: none
						1.25 / 0.05	7.5 / 0.3	17.5 / 0.70	105 / 4.2	Toxicity: none Lesions: h
						5.0 / 0.2	30 / 1.2	70 / 2.8	420 / 16.8	Toxicity: none Lesions: none
Toxicity GLP	IITRI Project No. 6336-142	0.9% sodium chloride for injection USP	New Zealand White rabbits	8	i.v./ d x 14	0	0	0	0	Toxicity: none Lesions: none
						0.625 / 0.025	7.5 / 0.3	8.75 / 0.35	105 / 4.2	Toxicity: none Lesions: h
						1.25 / 0.05	15 / 0.6	17.5 / 0.7	210 / 8.4	Toxicity: none Lesions: none

*Target doses of OH-ACBC/F-ACBC differed from the Calculated Doses - see Table 2

SECTION 12.9
Previous Human Experience

(Dave insert)

12.10 A Radiation Dosimetry

(Dave Insert)

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