



# [<sup>18</sup>F]NaF Manufacture via the ABT BG75 system. Imaging and Biodistribution studies.

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## SUMMARY:

A comparative imaging study was performed for sodium fluoride F-18 injection dose ([<sup>18</sup>F]NaF) manufactured with the ABT BG75 system and a commercial source. [<sup>18</sup>F]NaF manufactured from the ABT BG75 system installed at UNC in less than 30 minutes (ready-to-use, isotonic and sterile syringe) passed the established release criteria for visual inspection, radiochemical identity/purity, radionuclidic identity/purity, pH, sterility and bacterial endotoxins. Imaging and biodistribution results based on standard uptake value (maximum and mean) showed that the accumulation *in vivo* is equivalent between ABT dose and commercial dose for all conditions tested.

## I. BACKGROUND:

Sodium Fluoride F-18 or [<sup>18</sup>F]NaF was initially introduced in 1962,<sup>1</sup> and its indication as a bone imaging agent to define areas of altered osteogenic activity was approved by FDA in 1972. The pharmacokinetics of this tracer depends on the rates of bone uptake and elimination from the circulation via renal excretion. In particular, for [<sup>18</sup>F]NaF there is a high and rapid bone uptake accompanied by very rapid blood clearance<sup>2,3</sup>. The resulting imaging characteristic is a high bone-to-background over a short time, which is very favorable for a radiotracer as imaging agent. Unfortunately at the time of its introduction in the 60 to 70's, the available technology was not ready to get the most out of this tracer due to the limitation of Anger type  $\gamma$ -cameras, scintigraphy.

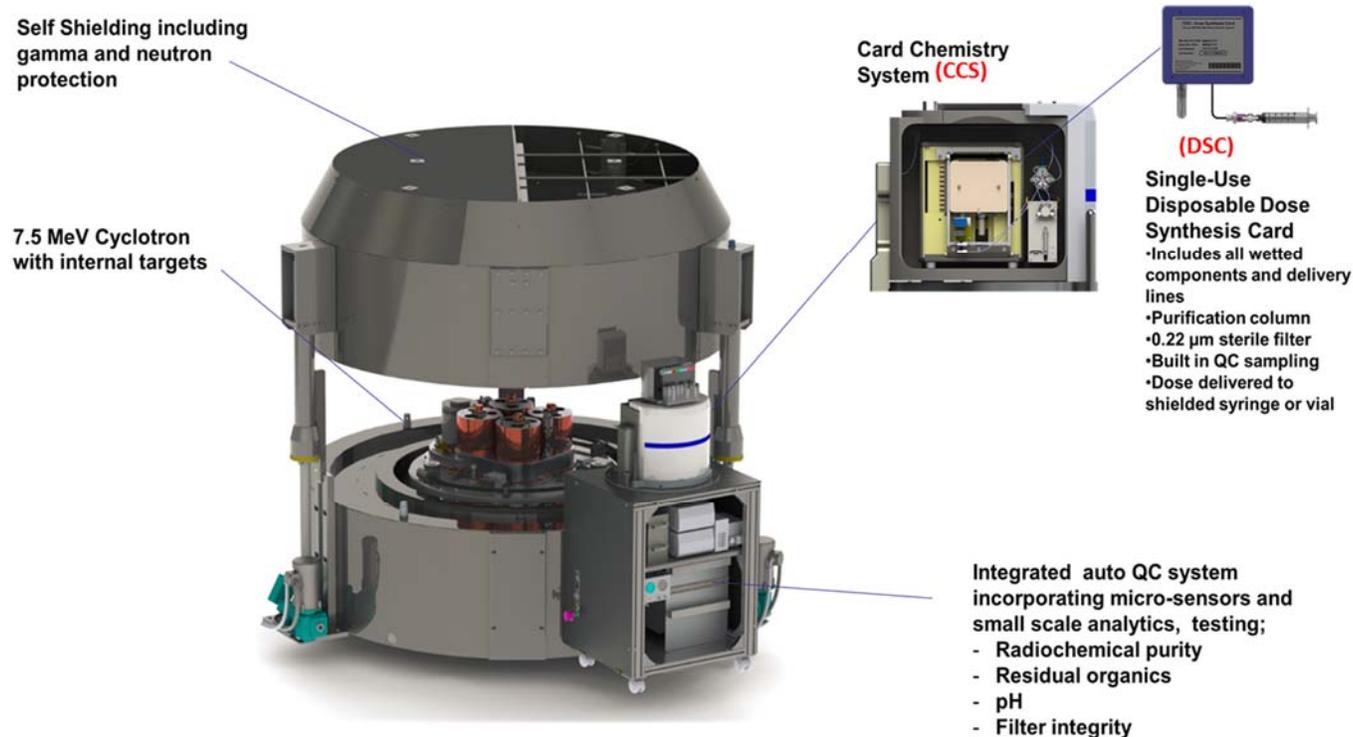
Since the 1990's there has been widespread availability of PET and PET/CT cameras in the US which has favored a renewed interest in [<sup>18</sup>F]NaF for imaging malignant, metabolic, degenerative, traumatic and inflammatory bone diseases using either PET or PET/CT. The improved sensitivity and specificity of a bone scanning is superior to the standard planar bone scintigraphy performed with technetium diphosphonates, as reported for bone metastases in several occasions<sup>4-8</sup>. To note the combined use of [<sup>18</sup>F]NaF/[<sup>18</sup>F]FDG for assessment of metastatic bone disease is a current topic of research (and interesting debate)<sup>9</sup>. In addition, applications of [<sup>18</sup>F]NaF in imaging atherosclerotic plaque<sup>10</sup> and as a marker for valve calcification activity in aortic stenosis<sup>11</sup> underscore the value of this radiotracer in a wider array of applications beyond the current approved indication.

Currently the use of [<sup>18</sup>F]NaF PET is being evaluated by the National Oncologic PET Registry (NOPR) to assess its impact in patients with suspected or known osseous metastases in any cancer type. A recent report provides further evidence that [<sup>18</sup>F]NaF PET can "change management of patients, either as a secondary test after <sup>99m</sup>Tc-diphosphonate bone scanning or as a first imaging study"<sup>12,13</sup>.

The University of North Carolina at Chapel Hill owns a BG75 system that is currently pursuing an aNDA application for [<sup>18</sup>F]FDG manufacture. The BG75 system is self-shielded and comprises a small, 7.5 MeV positive ion cyclotron, and automated synthesis (Card Chemistry System, CCS) and quality control modules (Fig. 1). The central idea on the BG75 system is the use of a single-use and sterile card (DSC) to make a dose of radiotracer, at any time is required (dose-on-demand). Reagents are loaded in a metering sub-assembly located at the top of the CCS and then measured/transferred into the inside of a sterile DSC where manufacture occurs in a close system, the dose is then purified using a solid phase column and transferred to a sterile syringe via a non-vented 0.2  $\mu$ m filter. An equivalent BG75 system has been producing

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[<sup>18</sup>F]FDG and [<sup>18</sup>F]F<sup>-</sup> for pre-clinical and R&D purposes for more than two years in the University of Oklahoma Health Sciences Center<sup>14,15</sup>.



**Figure 1:** BG75 system showing its main components.

On this study we aimed to use the capabilities of the system to manufacture on-demand [<sup>18</sup>F]NaF using the DSC configuration. The DSC was adapted for purification of the incoming [<sup>18</sup>F]F<sup>-</sup> radioisotope from the cyclotron and purification via solid phase extraction. The sterile product obtained in syringe was compared with commercial [<sup>18</sup>F]NaF by injecting a mice cohort alternatively in two days and further analyzing statistical differences between the groups.

## II. METHOD:

### II.a. [<sup>18</sup>F]NaF manufacture:

Non-carrier [<sup>18</sup>F]F<sup>-</sup> was produced through the <sup>18</sup>O(p,n)<sup>18</sup>F nuclear reaction by irradiation ( 15 – 20 min) of a 95% enriched [<sup>18</sup>O]H<sub>2</sub>O target (280 μL) with a 7.5 MeV proton beam (3.5 – 5.0 μA). The [<sup>18</sup>F]F<sup>-</sup> was transferred to the DSC and passed through a strong cation exchange column to trap any potential radionuclidic impurity. [<sup>18</sup>F]NaF solution (volume 3.0 mL, in 0.9% NaCl) is delivered in a syringe after final sterilization using a non-vented 0.22 μm filter. Synthesis of the radiotracer was performed automatically after transfer from the cyclotron using the BG75 software. Sterile water for injection from the BG75 kit (ABX advanced biochemical compounds) was used to dilute the concentrated [<sup>18</sup>F]NaF. Sodium chloride, injection USP grade solution 23.4% was used as received as an in-card reagent (Abraxis Pharmaceutical Products).

Quality Control of the doses produced was performed manually following USP monograph for “Sodium Fluoride F18 Injection”.<sup>16</sup> Radiochemical Purity and Identity were determined using a RHM-monosaccharide (hydrogen form, Phenomenex) using sodium fluoride (#S299-100, Fisher Scientific) as a standard and a Bioscan FC3300 radioactive detector. Radionuclidic Purity was determined using a multi-channel analyzer with NaI(Tl) detector for fresh and decayed samples. Sterility samples were sent to WuXi Apptec to be inoculated within 30 hours according to USP general chapter <71> sterility tests.

## **II.b. Imaging Study Protocol**

*Animals:* Twelve normal male athymic nude mice (6-8 week age) were purchased from UNC mouse breeding core facility. Mice were randomly divided in two groups with N=6 for each group. Mice in Group-1 were injected with commercial [<sup>18</sup>F]NaF on Day-1, and injected with ABT [<sup>18</sup>F]NaF on second day. Mice in Group-2 were injected with ABT dose on Day-1, and commercial dose on second day. Mice were allowed to acclimatize for one week before the imaging study. All the animal procedures were approved by UNC IACUC committee.

*Imaging procedure:* Mice were anesthetized by isoflurane (1.5% mixed with oxygen) inhalation and tail vein catheter was placed. About 9.25MBq (~0.1 ml in volume) of [<sup>18</sup>F]NaF was injected via tail vein catheter. Remaining activity in the catheter and needle was measured to calculate net injected activity for each animal. Mice were allowed to recover from anesthesia and returned back to the cage after dose injection. At 40min post injection, mice were re-anesthetized using isoflurane. Bladder was manually expressed to minimize bladder signal before scans. A small animal PET/CT system (eXplore Vista, GE Healthcare, Inc.) was used to perform PET/CT scans. Mice were placed on the cradle with the four limbs fastened to fixed molds to ensure consistent positions between scans. CT scan was performed before PET to provide anatomical reference and attenuation correction. A 15min PET scan was then started at 1 hour after the dose injection. Mice injected with the ABT dose on the first day were injected with the commercial dose on the second day, and total of 12 mice were imaged in two consecutive days. After imaging, mice were euthanized with tissues collected and counted in a gamma counter. Images were reconstructed using 2D OSEM algorithms with random, scatter, and attenuation corrections. Standardized uptake value (SUV) was calculated voxel-wise based on injection dose and body weight.

*Image analysis:* PET images were first aligned with CT images for each mouse. Bone volume in mouse torso (between the end of the sternum and start of the tail bone) was segmented from CT images using threshold method. Mean and maximum SUV on the bone volume was calculated from the PET images. Volumes of interest (VOIs) on the following organs, including liver, kidney, heart and muscle, were also manually drawn and mean uptake was measured from the PET images.

## **III. RESULTS:**

Ten (10) doses of non-carrier added [<sup>18</sup>F]NaF were successfully produced at UNC in the BG75 system from sterilized DSC's in a range of activities 10 – 19 mCi, all the doses in syringes passed the release criteria established by USP as seen in Table 1.

The summary of the imaging results obtained is seen in Table 2, in general there was no difference in terms of radiotracer accumulation in normal bone between the dose coming from either the ABT BG75 system or a commercial source. No statistical difference was observed between the injection sources according to unpaired t-test between two groups ( $p=0.95$ ), or paired t-test between the two injections on the same animal ( $p=0.94$ ) Biodistribution data in major organs and bones also showed no significant differences between the two injection sources (Figure 2). Visually

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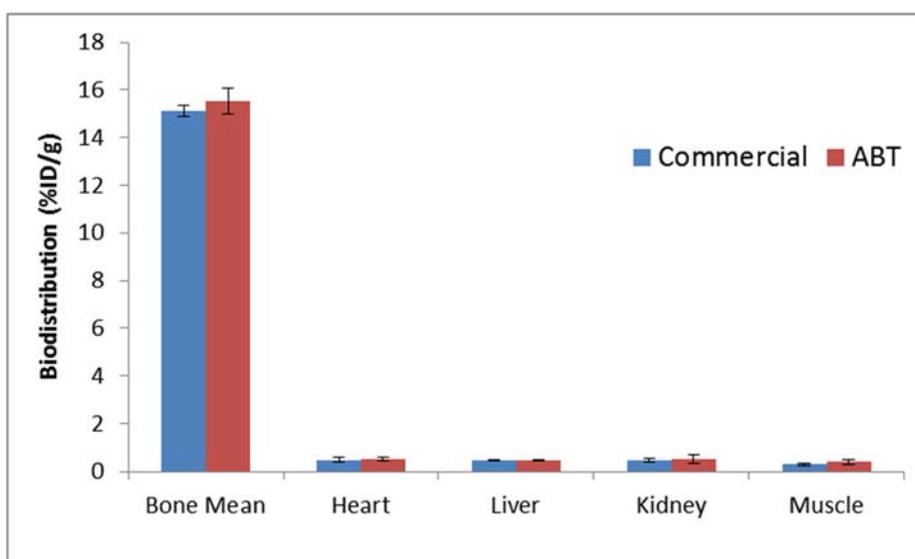
speaking there was no difference between the volume rendered PET/CT images from mice injected with [<sup>18</sup>F]NaF from a commercial source and from the BG75 system (Figure 3).

**Table 1:** Summary of Quality Control data for [<sup>18</sup>F]NaF produced with the ABT BG75 system.

QC Test	Release Criteria	Results
Appearance	Clear, Colorless, free of particulates	Pass
pH	4.5 – 8.0	5.0 – 6.0
Radiochemical Purity	> 95%	> 99%
Radiochemical Identity	Retention time matches standard	Pass
Radionuclidic Purity	> 99.9%	> 99.9%
Radionuclidic Identity	T <sub>1/2</sub> = 105 – 115 min	106 – 111 min
BET	< 17.5 EU/mL	< 10.0 EU/mL
Sterility	Sterile	Sterile

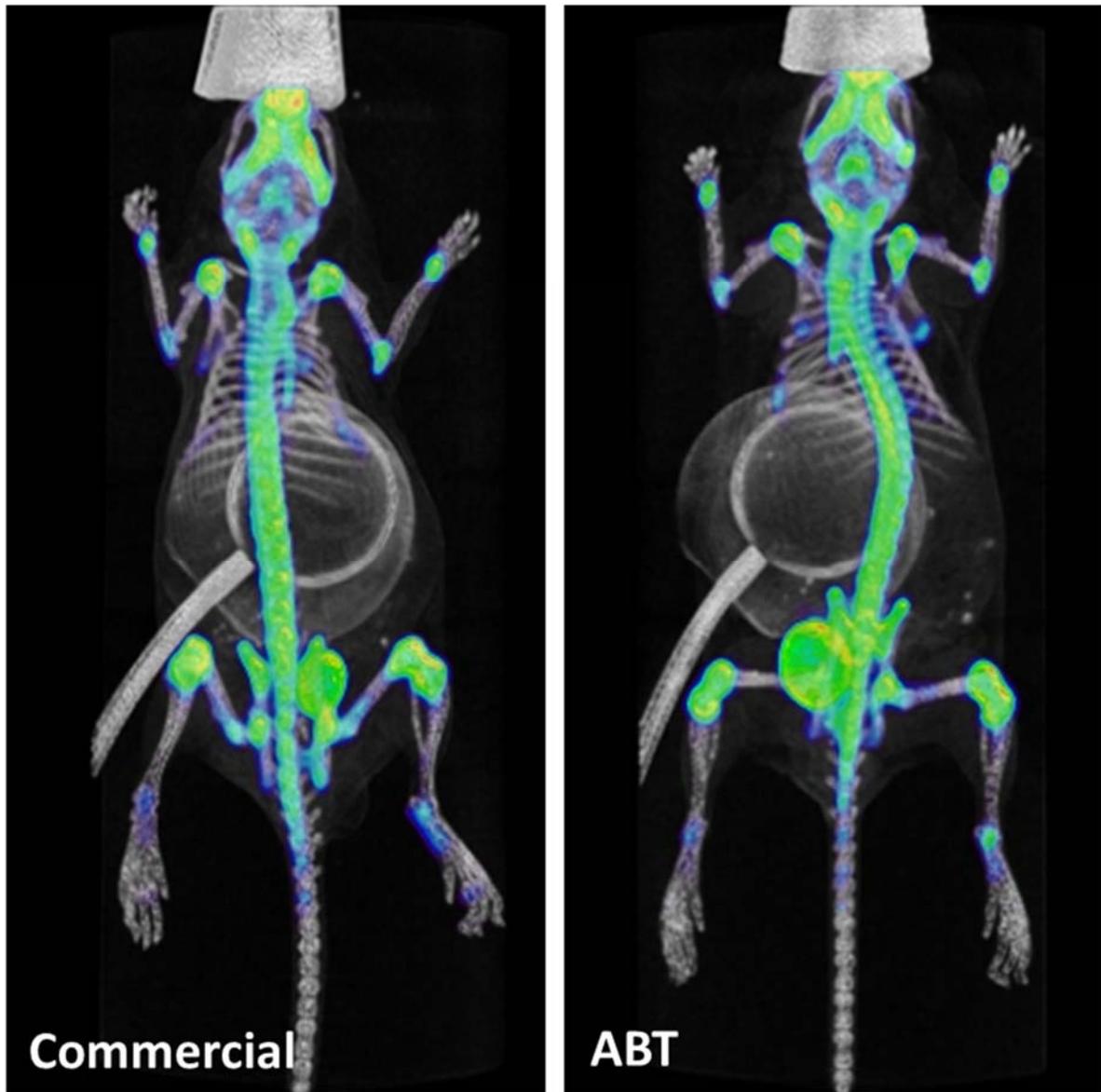
**Table 2:** Summary of imaging data for injected mice with [<sup>18</sup>F]NaF.

	ABT	Commercial	Different	P-value (t-test)
<b>SUV-Mean</b>				
average	3.83	3.82	NO	0.94
SD	0.36	0.31		
N	12	12		
<b>SUV-Max</b>				
average	15.5	16.1	NO	0.47
SD	1.9	2.1		
N	12	12		



**Figure 2.** Biodistribution of [<sup>18</sup>F]NaF in normal mice. There were no significant differences on the normal biodistribution between doses manufactured from ABT BG75 system and the commercial dose.

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**Figure 3.** Volume rendered PET/CT images from mice injected with [<sup>18</sup>F]NaF from a commercial source (left) and from the BG75 system (right).

#### IV. CONCLUSIONS:

Automatic manufacture of [<sup>18</sup>F]NaF passing all appropriate release tests and within the configuration of the DSC was achieved using a custom made card and script. Imaging-based biodistribution study showed equivalent distribution on normal mice between the dose manufactured from the ABT system and the commercial dose.

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