Critical National Need Idea: Tracking Drugs to Diseased Tissues By Accelerator Mass Spectrometry for Personalized Medicine

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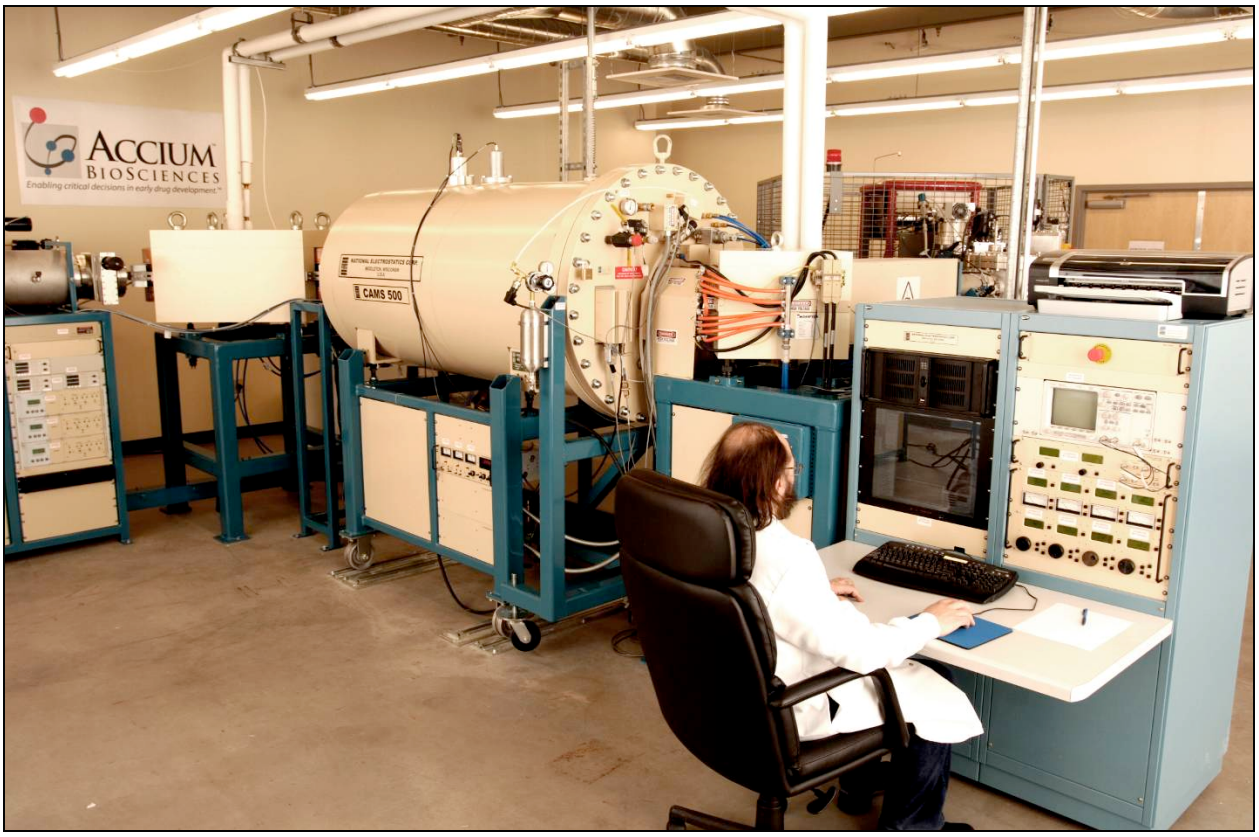
Abstract. The accelerator mass spectrometer (AMS) is used to measure trace amounts of carbon-14-labelled compounds in human tissues and fluids in a process known as "microdosing." The AMS is, therefore, capable of tracking one or more drugs safely in humans to determine which chemical entities reach the diseased tissues in individuals who may have different responses to different compounds. Personalized medicine has a new tool in the AMS for empirically measuring the likely effectiveness of each medication, whether given in a cocktail (combination therapy) or as a single drug (monotherapy). This technology does not rely on biomarkers, genomics, proteomics, or other types of predictive methods. Instead, the AMS is able to quantify the actual (*in vivo*) delivery of drugs to the diseases on an individual basis.



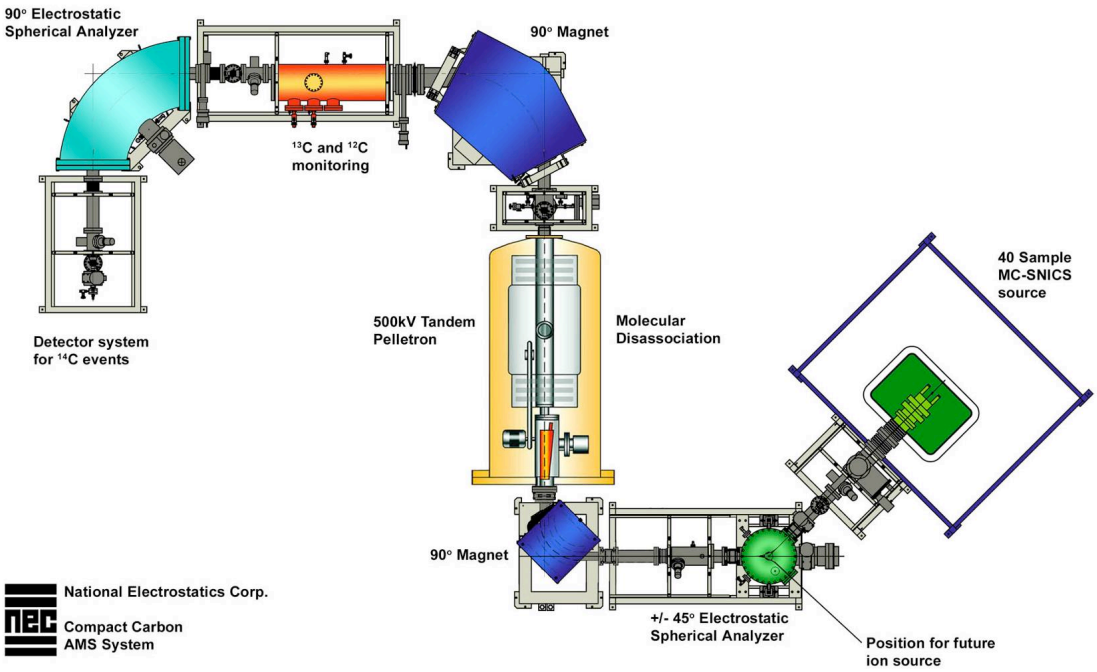
Dr. Ugo Zoppi operates Accium's 500,000-volt Accelerator Mass Spectrometer

Introduction. Personalized medicine is the use of molecular analysis to best manage an individual's disease or predisposition to specific diseases. Typically, personalized medicine relies on the expression levels of each patient's genes to predict the optimal therapy through the selection of specific medications and doses. It is widely held in the research community and healthcare industry that personalized medicine will both reduce medical costs and greatly increase positive outcomes of treatments. The societal advantages of successful personalized medicine are savings of billions of dollars and countless lives. Personalized medicine also promises to reduce the pain and suffering in individuals by eliminating unnecessary drugs and procedures that could lead to toxic effects and even death.

Existing Tools for Personalized Medicine. Genes (DNA and associated transcribed RNA), proteins, and metabolites are the usual suspects for personalized medicine, spawning areas of studies known as genomics, proteomics, metabolomics, and pharmacogenetics. By looking at individual variations in gene expression, scientists and physicians hope to better predict the responses to different medical treatments or to determine which individuals are at greatest risks for specific diseases. While specific antibody tests exist for relatively few diseases and genetic variations, such as HER2 expression in breast cancer, the main tool for personalized medicine is the microarray, which allows the screening of thousands of RNA transcripts, proteins, or metabolites. For DNA or reverse transcribed RNA, high throughput DNA sequencers may be used to generate the same types of information as microarrays. Tandem mass spectrometers (which are different than AMS) are also used to identify and quantify biological molecules that are studied for personalized medicine. These tools provide a correlation between an individual's genetic background or biomarkers and specific diseases using *in vitro* methods. However, the actual responses *in vivo* could vary because of non-genetic factors, such as diet or other drugs.

The testing of diseased cells taken from patients is sometimes used to predict the responses of the tumors or diseased organs to medications. This so-called *ex vivo* method tries to determine drug sensitivity and resistance of cancer or other diseased cells that are removed from the body and are grown in incubators. ChemoFX by Precision Therapeutics is an example of a testing service that conducts *ex vivo* measurements of killing by various drugs of tumor cells taken from patients and grown in the lab. *Ex vivo* data may not be clinically relevant because the cultured cells are given artificial growth conditions and drugs whose levels may not be at the same concentrations as at the tumor site within the body (*in vivo*). Factors of the patient, such as age, diet, hormones or biorhythm, and the microenvironment of the tumor cells cannot be mimicked accurately in the lab incubator. Many older patients are on other drugs for different conditions, which cannot be simulated in the laboratory. A better method is needed.

AMS and Drug Testing. The accelerator mass spectrometer (AMS) is a highly-sensitive and accurate instrument for counting atoms. The AMS at Accium has a 500,000-volt accelerator that is specifically optimized for separating isotopes of carbon (masses 12, 13, and 14). This instrument is capable of measuring 14C at a level of less than one in a trillion carbon atoms, which is the ambient amount of this isotope in our environment. The half-life of 14C is 5,700 years, so it is virtually stable for drug testing purposes. The AMS can accurately measure 10,000 14C atoms above the ambient level. This instrument is 1000 times more sensitive than tandem mass spectrometers and is 1,000,000 more sensitive than scintillation counting of the 14C radioactive decay.



The instrument (the Accium AMS is diagrammed above) has been mainly employed for radiocarbon dating of historical and archeological artifacts for half a century. However, in the last decade, the AMS has been used for drug testing in humans, because AMS is the most sensitive tool for measuring compounds without the use of signal amplification methods. Dr. Ali Arjomand, president and founder of Accium, conducted the first human study in 1998 with 14C folic acid at the University of California, Davis. Subsequently, in the pharmaceutical industry, many 14C-labelled compounds have been administered into humans at trace amounts (100 nanocuries typically) and have been tracked with the use of the AMS to determine pharmacokinetics, metabolite profiles, absorption, and other parameters of clinical testing. This added level of 14C is considered non-radioactive in the body and poses virtually no health risk, since it is less than the amount of 14C naturally found in humans.

Metabolite profiling with the AMS is particularly relevant for personalized medicine, because both processes use the same separation technology. Individuals may modify drugs at different rates or to different compounds because of variations in liver enzymes or other biochemical factors. By using high performance liquid chromatography (HPLC), blood, urine and other patient samples can be fractionated prior to AMS analyses to measure the amount of each modification of the drugs. Many drugs are chemically protected (as "pro-drugs") and require activation within individuals before these compounds are functional. HPLC and AMS can measure this activation. Furthermore, the same separation strategy has been used (see below) to quantify the localization of two different drugs administered at the same time to determine the distribution in tumors of each compound from a cocktail or combination therapy.

The techniques for using the AMS to study drug candidates in humans are well established and are known as microdosing, Phase 0, or exploratory IND methods. The FDA and government bodies in many parts of the world endorse the use of microdosing to obtain human data that can be used to predict qualities of new chemical entities entering Phase I clinical trials.

Accium is one of four companies worldwide that offer AMS services to the pharmaceutical industry. However, Accium may be unique in applying the AMS to personalized medicine and has filed a patent application on these approaches in 2007.

Tracking Drugs in Humans. In 2006 Accium conducted an AMS study to detail where a drug candidate would go in a mouse model. The animal was given a 14C-labelled compound. Later, various tissues (including brain, liver, muscle) and fluids were measured by AMS for the amounts of the molecule. Importantly in this study, the chemical entity did not cross the blood brain barrier. While this experiment involved sacrificing the mouse, it proved that very little tissue is needed for quantifying the 14C drug candidate and, therefore, that minimally evasive methods could be used in human patients to follow the fate of drugs to the specific disease sites.

Using the AMS for personalize medicine departs from microdosing studies in many important ways. First, the compounds have already been tested for safety and efficacy and have gotten FDA approval. Second, Accium can determine which of many drugs are reaching the target tissues in human patients with a disease (not limited to healthy volunteers, which are used for Phase 0 or microdosing studies). Third, a 14C tracer may be combined with a larger "cold" amount of the drug(s) being tested, in case the tracer does not behave in the same manner in the body as a nearly therapeutic dose, because the toxicology profiles of the approved drugs are known.

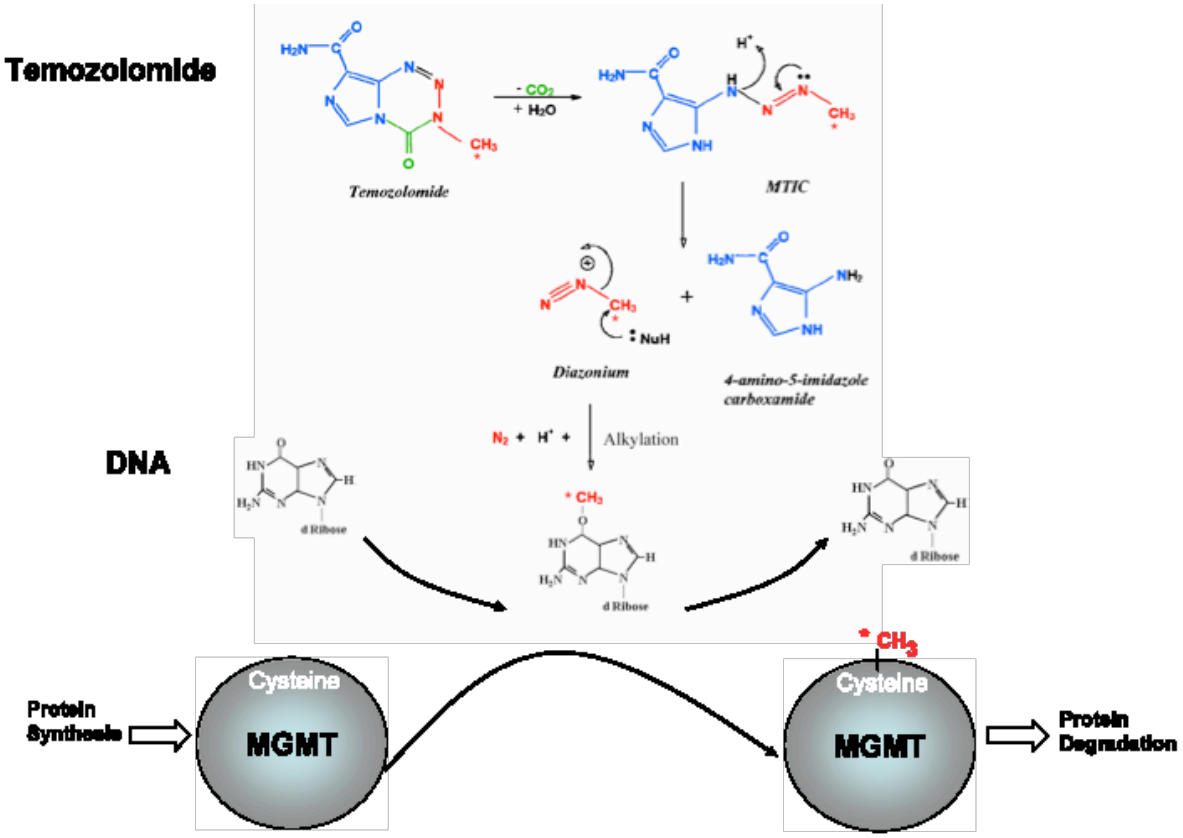
Accium proposes to conduct *in vivo* testing of drugs on tumors and other diseased cells, using the AMS and the expertise of medical collaborators. The advantage over genomics and proteomics is that the AMS results are empirical and show the actual uptake or elimination of the drugs by diseased cells. The Accium data are not correlations of biomarkers with specific medical conditions. The empirical results from AMS take into account genetics, phenotype, and unknown factors that may be relevant to the disease on an individual basis. Unlike the *ex vivo* testing methods of cultured human cells, the Accium technology uses the person to provide the growth environment for the cancer cells (and not artificial incubators and culture media).

Brain Cancer. The use of AMS to track drugs to diseased tissues is most important for cancer. While many forms of cancer exist, Accium plans to focus first on brain cancer, breast cancer, melanoma, and leukemia. The Company has a research collaboration agreement with the Swedish Neuroscience Institute (a division of Swedish Medical Center in Seattle, WA) to apply the AMS technology to glioblastoma multiforme (GMB). This very lethal form of brain cancer is usually treated with temozolomide (TMZ). However, nearly half of the patients do not respond favorably to this drug. The reason for non-response to TMZ is believed to be due to the tumor cells' ability to counteract the chemical effect of TMZ, which is an alkylating reagent that modifies DNA.

GMB is an aggressive cancer. By the time a person exhibits symptoms and is diagnosed with the disease, a large tumor exists in the brain. Surgery will be performed to remove the cancer mass. Typically, after surgery, the patient is given TMZ for several weeks as the first-line treatment. However, as stated above, TMZ will not help half of the patients, so valuable time is lost in waiting to determine if the medication is the right one. Accium and Swedish Neuroscience are planning to administer 14C TMZ to patients with GMB prior to surgery. Although completely invasive, this procedure poses no additional risk to the patient, because the tumor has to be removed. The tracer amount of 14C TMZ will be allowed to distribute through

the body and to cross the blood brain barrier to reach the tumor. After localization of the 14C TMZ, Accium will measure how much drug reached the tumor cells and, very importantly, how much 14C is chemically integrated into the cancer DNA.

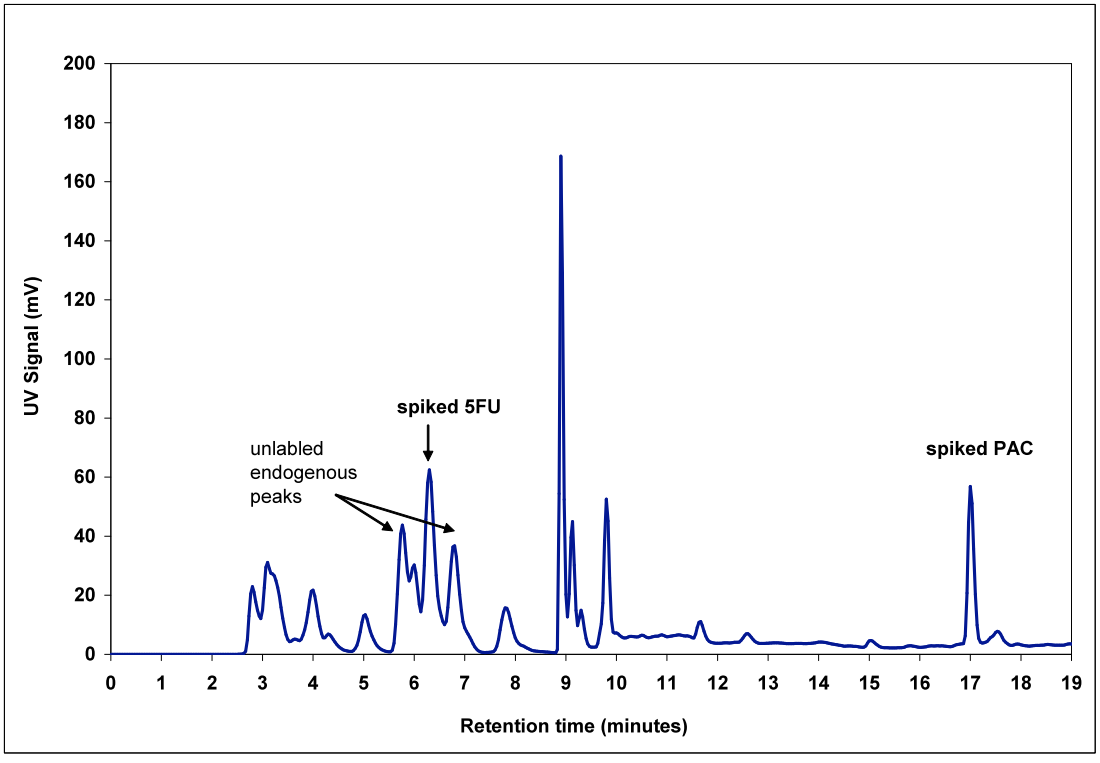
TMZ is an alkylating agent that transfers a methyl group to DNA. Cancer cells that are resistant to TMZ may either fail to take up the compound or may remove the methyl group (by specific demethylation enzymes, such as MGMT). Accium and Swedish had 14C TMZ synthesized with the carbon-14 on the methyl group that is transferred. In experiments with human brain cancer cell lines, the collaborators found that 14C TMZ caused tumor cell DNA *in vitro* to acquire 14C in a time-dependent manner. Thus, the methyl group was transferred to the genetic material. The National Institutes of Health gave a Phase I SBIR grant to Accium to conduct TMZ experiments in mice with human malignant brain tumors, so-called xenografts. These pilot experiments are designed to provide data for subsequent human studies.



The work on TMZ for brain cancer is an example of the use of AMS for selecting the best monotherapy (i.e., single drug). If a patient's tumor is shown to be resistant to TMZ because of the failure of the drug to reach the cancer cells or the elimination of the 14C methyl group from DNA, another medication or a combination therapy is expected to be given early after surgery, rather than several weeks later. The correct choice of treatment could greatly improve the therapeutic outcome for the patient. Tissue processing, DNA extraction, and AMS analysis could be routinely performed within one week of surgery, if an instrument is at least partially dedicated to measuring patient samples.

Combination Therapy. For many cancers, cocktails of drugs are given with the hope that one or more compounds are active against the malignancy and that combinations may have synergistic effects. For example, Avastin from Genentech is known to work in combination with some cancer drugs (but not all chemotherapeutics) in leading to better control of the tumors. While combination therapies may be better than monotherapies, knowing which drugs help would be valuable information to tailor treatments to reduce toxic side effects and to lower the medical costs. Accium proposes to use the Company's separation expertise for metabolite profiling of human samples to determine which drugs in a cocktail are the most likely to work on an individual basis.

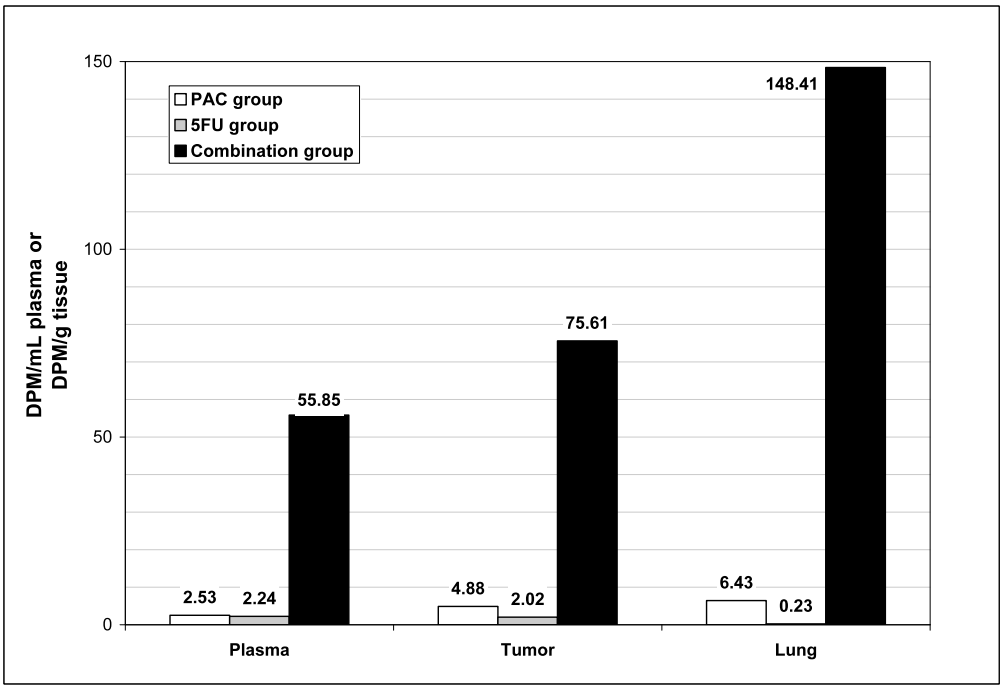
Using the microdosing approach for combination therapy, two or more 14C drugs may be co-administered into a patient at tracer amounts. The drugs can be well below the toxic levels, which would be known for FDA-approved treatments. After the compounds have had time to reach the cancer cells, patient samples are removed for AMS analyses to determine the amounts of each drug reaching the disease target. For breast cancer, a needle biopsy should provide enough cells for analysis. For melanoma, skin biopsies would be relatively non-evasive, whereas with leukemia, a simple blood draw is needed (with separation of the leukemic cells done with instruments that routinely sort for specific cell types). Before AMS analyses, the human samples have to be processed and separated with high performance liquid chromatography (HPLC) or other methods to measure independently each tracer drug. See figure below.



Human colon cancer cells were implanted in mice and grown to a small tumor. The (xenograft) mice were given 14C 5-fluorouracil (5FU) and 14C paclitaxel (PAC) in combination or as single treatments. After various times, the animals were sacrificed, and the tumor tissues were sent to Accium for AMS analyses. In the case of the combination treatment, HPLC was necessary to separate the 5FU from PAC, in a similar manner that metabolites are separated from

parent molecules. The appropriate HPLC fractions were further processed and analyzed by AMS. Accium was able to separate and quantify the two compounds from tumor, plasma, and lung material from the xenograft mice. The same techniques should be immediately applicable for the measurement of different drugs in cocktails in humans.

From this xenograft study with 5FU and PAC, Accium observed a strong synergistic effect of the two drugs in combination, compared to single treatments of each compound. See figure below, comparing single versus the two-drug cocktail. Such synergies may occur in humans but not necessarily in all patients. The AMS for personalized medicine may be able to sort out which combinations lead to synergies and are, therefore, potentially more beneficial (or more toxic) to individuals. Accidental overdoses due to combination treatments are common. A screening of patients for responses to cocktails is, therefore, very important, especially as more drugs and more combinations are used.



Accium conducted the same type of mouse study with a human breast cancer (xenograft), using 5FU and PAC. The tissues have been frozen and await AMS analysis. Breast cancer killed over 40,000 women in the U.S. in 2008. For personalized medicine, HER2 is used as a biomarker for tumors that are likely to respond favorably to Herceptin. However, even these tumors become resistant; and secondary treatments are needed. Accium is looking to work with one or more breast cancer researchers for conducting human studies of 14C tracer compounds given to women before needle biopsy. In practice, the biopsied material may first go to a pathologist for determining if a lump is malignant (20%) or benign (80%). AMS measurement could be performed for drugs reaching the malignant cells, whereas the benign tissue could be archived for later analysis, if necessary.

Melanoma and leukemia are two types of cancer that are prevalent and easily sampled for AMS work. As with the other types of malignancies, tracer amounts of 14C drugs in combination may be administered before the skin biopsy or blood draw. Many other forms of

cancer may be examined by this method to determine which drugs are most likely to work and which drugs should be eliminated or adjusted in dosage.

Infectious Diseases. Human pathogens and pathogen-infected cells can also be pre-treated with tracer amounts of 14C drugs to determine by AMS which compounds may reach the organisms or target cells at a therapeutic level. Malaria, trypanosomes, and other pathogens that chronically infect humans may be studied with approved and candidate drugs for localization at the site of the pathogen, using safe methods provided by the microdosing strategies.

Other Diseases. Patients with immune system diseases, diabetes, and other conditions may benefit from the application of tracer therapeutic agents. The use of the AMS for following the distribution of drugs in humans is very new and has been mostly limited to healthy volunteers in clinical studies. The instrument is a sensitive tool awaiting new applications in healthcare and other industries.

Business Opportunity Costs. Accelerator mass spectrometers are getting smaller in size. Xceleron, the world's first provider of AMS services for microdosing, operates an instrument in York, UK, that would fill a basketball court. The Accium model occupies a 10-meter square room and was considered the compact AMS three years ago. The newest model is two by three meters but has an untested reliability record. Despite the size shrinkage, the cost of an AMS for carbon-14 analysis has been $1-1.5 million for several years. This high price is largely due to the small number of AMSs in productions, possibly as little as two or three per year for pharmaceutical use. Each instrument is assembled by hand and custom-made with a manufacturing time of 1.5 years. Each AMS is capable of measuring 25,000-100,000 samples annually, but higher throughput models that are less sensitive are being tested.

Like nuclear magnetic resonance, now MRI, the cost of an AMS is expected to drop as more instruments are manufactured for a growing market. The newer models have many fewer parts and may require less technical knowledge for operations. Accium is considering the purchase of another AMS. If the AMS becomes routinely used for personalized medicine, as described above, far more instruments will be needed even for research purposes than exist today. In addition to the AMS cost, appropriate housing for the instrument may be $100,000 to $200,000, depending upon the model purchased.

Accium or a contractor may manufacture the 14C cocktails for personalized medicine as kits for clinics. While each compound is expected to cost $100,000-250,000 per radioactive synthesis, 10,000 patient doses result from a one-millicurie chemical synthesis (at 100 nanocuries per patient). Therefore, the cost is $10-25 per test for each 14C compound.

Healthcare Costs. Patient costs for AMS analysis will depend largely on the number of samples, including fractions from the HPLC. Typically, AMS service providers charge $300-400 per AMS sample, plus additional fees for HPLC separations. A patient or doctor may want to examine 10 biopsy samples for large, complex tumors. If three drugs are compared simultaneously, a total of 30 AMS samples may be analyzed for a cost of $10,000. In contrast, for leukemia, a single population of cells may be analyzed for three different drugs at an AMS price of $1,000. The patients or insurers will also bear the costs of the 14C tracer cocktails, administration of the drugs, and collection of the diseased material. Tissue processing and HPLC are possibly additional expenses. If the AMS technology is automated, costs should drop

significantly. Improved instruments are being tested with higher throughputs, which could significantly drive down the price of AMS analyses.

Healthcare Benefits. With some drugs costing tens of thousands of dollars per year, personalized medicine in all forms should reduce unnecessary treatments and, therefore, serve as a large benefit to the healthcare system. The AMS should be viewed as an ultrasensitive tool for helping to determine the right treatment for each patient. AMS results need to be interpreted along with genetic and other data to increase the efficacy of medical treatment and to reduce unnecessary and possibly deleterious procedures and drugs. Identifying the right treatment as early as possible is the goal of Accium's AMS program for personalized medicine. For aggressive cancers and pathogens, time is extremely important. The days of trial-and-error to see what works for a disease should be ending soon. Accium expects several years of clinical testing to prove that drug delivery data obtained by the AMS tracer technology is valuable information that will result in improved therapeutic outcomes. If such knowledge becomes a routine part of treatment decisions, this technology could transform medicine as a novel way to individual care.

Conclusion. The accelerator mass spectrometer (AMS) has been successfully used to follow the distribution of tracer amounts of 14C-labelled compounds in humans for drug development. The same sensitivities and precision can be applied to approved drugs to determine which medications are most likely to work on human diseases on an individual basis. AMS is, therefore, a novel tool for personalized medicine that does not depend on genetic or protein markers. Instead, AMS analyses can determine if drugs get to the target cells empirically for individual diseases. Therefore, this technology inherently accounts for all genetic and environmental factors of the patient. In some cases, such as brain cancer and the drug temozolomide, the AMS is capable of measuring molecular changes *in vivo* caused by the drugs and to determine if the diseased cells are likely to be resistant to treatment. An AMS facility requires significant investment. However, like MRI instruments that are now routinely used in hospitals, AMS could become a standard tool for determining the optimal treatment of cancer and other diseases, as this technology becomes widely used and improved.