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Human microdosing for the prediction of patient response

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Microdosing allows study of the behavior of drugs via the administration of doses so low they are unlikely to produce whole-body effects, but of sufficient concentration to allow the determination of absorption, distribution, metabolism and excretion (ADME). Microdosing was recently reviewed elsewhere [1,2]; although most effort to date has focused on pharmacokinetic (PK) predictions to accelerate drug development, microdosing was born out of molecular toxicology research [3-10] and is now extending to medical diagnostics applications. Here, we provide a brief overview of microdosing for drug development, followed by a description of recent efforts to implement prognostic and predictive diagnostics aimed at improving detection and treatment of human disease.

For drug development, the promise of microdosing is to reduce the resources spent on nonviable drug candidates and the amount of testing done on animals prior to first-in-human studies. Such 'Phase 0' studies are generally conducted before Phase I in order to screen candidate compounds. Selection criteria for advancing a compound from Phase 0 to Phase I typically include PK analysis, with an assumption that the data will be predictive of the kinetics at higher pharmacological doses. It is expected that metabolism of microdoses will be increasingly emphasized in the future, particularly in light of recent US FDA and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidance on metabolites in safety testing.

Although microdosing has been performed using PET for imaging and LC coupled to MS for some compounds, most studies utilize ultra-sensitive accelerator mass spectrometry (AMS) for detecting ^{14}C-labeled compounds in biological samples. Briefly, AMS works by breaking down the molecules in a sample and isolating an element of interest, such as carbon in the form of graphite, which is then quantified in a small particle accelerator. If the drug is labeled with a rare isotope such as ^{14}C, the ratio of radiocarbon to total carbon in the particle beam is used to calculate the concentration of the drug in blood, tissue, cells and subcellular components such as protein and DNA. Although carbon is the most commonly assayed element, owing to the low natural background of ^{14}C, other elements exist as mixtures with rare isotopes that can be useful for biomedical AMS applications, including ^{3}H and ^{41}Ca [11-13]. Human volunteers are administered the drug of interest at levels typically approximately 100-times lower than the anticipated therapeutic dosage and not exceeding 100 μg per dose. The amount of radioactivity administered is so low that the

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tissue samples are considered below the regulatory threshold for definition as radioactive. For example, the human body contains approximately 100 nanoCurie (nCi) of radiocarbon, so a transient exposure to 200 nCi of a ^{14}C-labeled compound is considered very safe – essentially comprising a negligible level of radiation and chemical exposure.

A critical issue for Phase 0 studies is whether the PK and other parameters are linear between the microdose and the therapeutic dose, which is required for the technique to be predictive of higher doses. There is emerging evidence that most compounds have linearly proportional PK over a 100-fold or greater concentration range [14,101]. Several validation studies demonstrated good predictability and, despite skepticism from the pharmaceutical industry, there appears to be a strong scientific rationale for microdosing [15]. However, much work remains to be carried out in order to determine a set of rules to accurately predict which compounds are most appropriate for microdosing and to exclude *a priori* from consideration compounds that will not have linear PK over the desired dose range. The pharmaceutical industry is now cautiously testing microdosing, particularly for speciality applications such as compounds used at concentrations that are difficult to detect in tissues by other means [16].

Microdosing has recently emerged as a strategy for medical diagnostics [13,17-19]. Advantages of such an approach include:

- *In vivo* analysis of drug disposition to gain phenotypic information at safe chemical and radiation doses;
- Ease of comparison to genotype assays that will result in higher predictive power compared with either assay alone;
- Elimination of uncertainty regarding the linearity of PK and metabolism parameters, since only those compounds with a defined microdose to therapeutic dose relationship will be used for the diagnostics.

Potential challenges of microdosing-based diagnostics include:

- The expense per assay;
- Insufficient throughput;
- Undefined regulatory and reimbursement pathways.

Accelerator mass spectrometry currently requires conversion to graphite by hand and some instruments cost up to US$7–8 million. Development of automated sample processing and the use of small instruments costing less than US$1 million will go far in alleviating the cost issue. For example, Vitalea Science and the Swiss Federal Institute of Technology developed a compact AMS instrument that was specifically designed for the pharmaceutical sciences [102]. There is no theoretical reason why AMS sample handling cannot be automated. Skipper and coworkers at the Massachusetts Institute of Technology developed a laser-induced combustion system for $^{14}CO_2$ production that is now commercially available [20-22]. The resulting $^{14}CO_2$ can be detected via a gas ionization source as part of an AMS system. The throughput of the system is sufficiently rapid to enable analysis of HPLC and GC fractions, although with some loss of sensitivity and reproducibility compared with graphite-based analysis, owing to decreased ion currents and other technical issues. Automation of graphitization has yet to be realized, owing to practical difficulties with handling submilligram masses of material during serial high temperature and gas-handling steps, but such issues are really just engineering problems.

The costs of the AMS assays will ideally be reimbursed by insurance, which is a new paradigm for covering the expense of producing and analyzing such samples. This may be

carried out by stacking current procedural terminology (CPT) codes, numbers assigned to every task and service a medical practitioner may provide to a patient for reimbursement, or developing new assay-specific CPT codes. Regarding throughput, a single laboratory technician can produce up to 300 samples per day. Some AMS instruments can run in excess of 20,000–40,000 samples per year, which is reasonable throughput for clinical diagnostics applications. Our group is interested in using AMS to predict which cancer patients will respond to platinum-based chemotherapy, which was used to treat approximately 300,000 patients in the USA in 2008. Performing microdosing assays on many thousands of patients represents a substantial yet realistic level of throughput increase compared with current methods. From a business standpoint, a compelling motivation is the potential for NDA approval of the isotope-labeled drug, which could provide the AMS vendor exclusivity for marketing the assay, even if the unlabeled analog was previously FDA approved.

Several promising clinical applications of AMS for diagnostics recently evolved from research efforts by UC Davis and Lawrence Livermore National Laboratory (LLNL) [103]. Vitalea Biosciences [102], a UC Davis spin out that licenses LLNL AMS technology, is developing an assay for vitamin B12 absorption in humans [17]. The bioavailability of vitamin B12 is important for normal health. Absorption of the vitamin is abrogated in pernicious anemia, an autoimmune disease that destroys parietal cells in the stomach that secrete intrinsic factor. Intrinsic factor is crucial for normal absorption of B12, so a lack of intrinsic factor, as seen in pernicious anemia, causes a vitamin B12 deficiency. The assay features oral microdosing with ^{14}C-labeled vitamin B12, produced as a biologic in which the radiocarbon label is incorporated biochemically from the growth media. The assay may make possible routine human characterization of B12 absorption with an essentially negligible radioactivity exposure and a single drop of blood generated from a finger prick. The assay is expected to prove particularly useful in gerontology, where B12 malabsorption is widespread and a potential cause of dementia.

A research effort led by Darren Hillegonds (LLNL) and Primo Lara (UC Davis) involves a 30-patient clinical trial aimed at using ^{41}Ca as a marker of bone-remodeling processes during cancer treatment. The current study resulted from earlier work led by Robert Fitzgerald (The University of California, San Diego, CA, USA), which concluded that ^{41}Ca is a feasible serum-based measure of bone perturbations in end-stage renal disease patients [13]. Calcium is rapidly taken up by bone and can be monitored by AMS-based urinalysis over many months following a single ^{41}Ca microdose. The goal of the study is to monitor bone turnover rates in hormone refractory prostate cancer patients with confirmed bone metastasis. The hypothesis is that a urinary ^{41}Ca assay utilizing AMS will be useful for risk assessment, disease staging, measurement of therapeutic efficacy and sensitive detection of progressive bony disease. Patients in the trial are administered 100 nCi of ^{41}Ca orally and followed over 18 months by periodic measurements of ^{41}Ca in urine and blood sampling for molecular prostate-specific antigen analysis. The expectation is that high bone turnover is prognostic or predictive of poor outcome compared with normal turnover.

Our research, in collaboration with Kenneth Turteltaub and Mike Malfatti at LLNL, is aimed at predicting which lung and bladder cancer patients will respond to platinum-based chemotherapy. Platinum-based drugs, such as cisplatin, carboplatin and oxaliplatin, are frequently used to treat solid cancerous tumors. However, response rates are as low as 25–30% for nonsmall-cell lung cancer and 50% for bladder cancer. There exists an unmet medical need for an assay to predict which patients will respond to chemotherapy. The major mechanism of cytotoxicity is the formation of covalent drug–DNA complexes, known as adducts. We are conducting clinical microdosing studies aimed at determining the feasibility of using platinum–DNA adducts as markers of chemoresistance, which is based upon our preclinical studies with carboplatin and oxaliplatin [18,19] and extensive clinical

data from patients administered therapeutic doses of chemotherapy. DNA damage data from blood and tumor biopsy samples from up to 100 patients will be compared with clinical outcomes such as progression-free survival, overall survival and side effects after initiation of standard platinum-based chemotherapy. In addition, a handful of rationally chosen gene-expression markers will be measured by quantitative real-time PCR in order to compare genotype to phenotype for each patient. We expect that the formation and repair of DNA damage induced by the platinum-based microdose will be predictive of the damage caused by the therapeutic dose, which is already established as an important determinant of outcome [23].

While it remains to be seen whether microdosing is a truly viable strategy for diagnostics, the approach has the potential to enable personalized medicine for the treatment of a variety of diseases and justifies further research and development.

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Biographies

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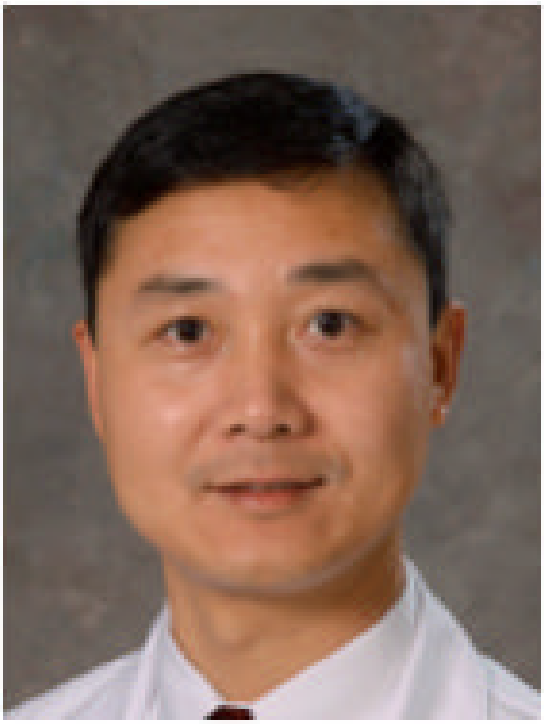

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