DURAMETRIX™

An *In Vivo*, whole-body approach with utilities in the oncology space including cytotoxicity survey, drug safety, simultaneous determination of antitumor efficacy and off-target cytotoxicity, comparison among drug candidates for prioritization.

Background: Cytotoxic anticancer drugs and treatments target tumor cells but also adversely affect susceptible off-target tissues. The efficacy of many treatments is often limited by the tolerance. It is also a challenge to effectively and timely compare drug candidates for decisions of prioritization and elimination. Further, existing standard toxicology measures can and do miss serious cytotoxic effects, which can potentially cause serious risks for the health and safety of patients. There is a great need for methodologies that complement existing measurements by enabling the characterization of cytotoxic effects of oncological treatments *in vivo*, in a whole-body and near real time fashion. The ability to simultaneously assess antitumor efficacy and collateral tissue damage in a systemic and timely fashion provides an opportunity to obtain critical information for the characterization of new drug candidates and therapies.

Our technology: In the current document, we present an *in vivo* imaging tool for characterizing chemotherapeutic agents/treatments in preclinical settings with spatiotemporal measurements on tissue damage induced by cytotoxic effects. The data allow semi-quantitative comparison among drug candidates or different treatments (between monotherapies as well as mono- vs. combination therapies). The outcome of these studies enables expediated decision making for determining efficacious and safe candidates for prioritization while eliminating those with poor performance and excessive toxicity.

This *in vivo* imaging technology is dedicated to and optimized for assessing tissue damage systemically, including tumor and nontumor tissues. The technology detects molecular signatures of cell death, including apoptosis, as an important manifestation of terminal cellular response to cytotoxic stimuli. Compared to other molecular and biochemical assays which detect changes in signaling, gene expression, metabolism, etc., cell death is an unambiguous marker for cytotoxicity. This near-universal marker allows for the detection of multiple forms of cell death regardless of causes or pathways. This is particularly important because there is extensive signaling cross talk among mechanisms of cell death in which different cell/tissue types respond differently to cytotoxic stimuli, and wherein the propensity toward one mode of cell death over another may differ. Such an imaging technique is therefore of practical advantage where tumor kill and off-target tissue damage can be assessed simultaneously and systemically as opposed to a particular pathway or mode of cell death. This approach is applicable without prior knowledge of drug effects, thus serving as a useful survey for tissue susceptibility in response to drug toxicity.

Our imaging technique targets phosphatidylethanolamine (PE) for its capacity in detecting cell death as a surrogate marker for tissue damage. PE is a type of aminophospholipid which is sequestered inside viable cells but is externalized and becomes accessible when a cell is dead or dying. The proprietary imaging agent, ^{99m}Tc-duramycin, binds PE with relatively high affinity and specificity, and with favorable pharmacokinetics. The relatively high target uptake and fast background clearance are favorable properties for whole-body imaging applications.

Areas of applications: Our technology detects a near universal molecular marker for cell death; it is thus potentially applicable for a broad range of therapeutic and pathological changes as a result of

tissue damage from cytotoxic drugs and treatments. Valuable data can be derived from specific tissues/organs or in a whole-body fashion. Applications include but are not limited to the following:

- Assessment of organ-specific tissue damage, such as drug-induced liver injury (DILI), renal toxicity, myelosuppression, cardiotoxicity, etc.
- Whole-body survey for tissue/organ susceptibility to cytotoxicity without prior knowledge.
- Determination of antitumor efficacy and prioritization of drug candidates.
- Simultaneous measurements of antitumor efficacy and cytotoxicity-induced off-target damage.
- Optimizing drug dosing/regimens for maximized efficacy and minimal off-target damage.
- Characterizing the therapeutic efficacy and adverse side effects of oncologic treatments, including chemotherapies, radiation therapies, immunotherapies (CAR T, antibodies, immunomodulators), viral therapies.
- Preemptive assessment on drug safety for minimizing black box warning risk.
- Providing comparative data among drugs/treatments (prioritization among drug candidates, comparing mono- versus combination therapies, etc.) Other benefits of the technology include:
- The technology is aligned with the principles of the 3Rs (Replacement, Reduction and Refinement).
- By being minimally invasive, the technology enables continuous studies in the same animals over time with repeated measurements. As such, the technology is compatible with both acute and longitudinal studies. After the completion of the imaging study, the animals can be transported back to the customer for further evaluations as needed.
- With data obtained using this technology, the information can help reduce the attrition rate by determining potential safety issues early.
- The technology can help identify efficacious and safe drug candidates for prioritization while eliminating ineffective and/or overly toxic candidates in a more streamlined R&D process.

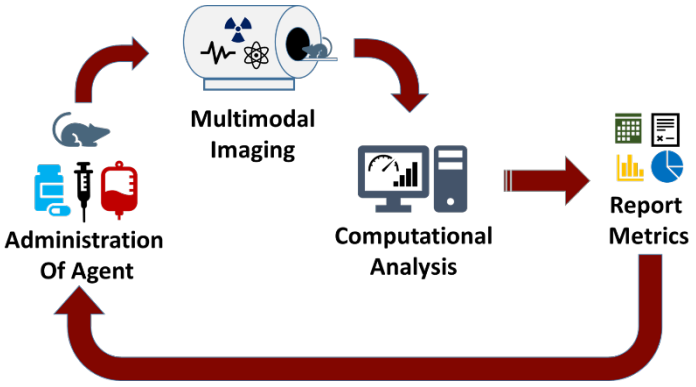

Figure 1. An overview of workflow. *In vivo* imaging data are acquired on preclinical animal models treated with drugs or treatments, where the data provide indicators for antitumor efficacy and toxicity-induced tissue damage in off-target tissues. The technology can be deployed for single drug assessments, comparison among multiple drug candidates, or mono- versus combination therapies. The data allow semiquantitative assessments of drugs and therapies. This technology can be applied iteratively along multiple stages along the drug R&D process for identifying safe and efficacious candidates while help eliminating ineffective and/or overly toxic compounds.

Demonstrations of utilities:

Showcase I: Assessing tissue damage induced by cytotoxic drugs.

Model: Sprague Dawley rats (male)
Drug: Methotrexate
Dosing: 100 mg/kg, single dose, i.v.

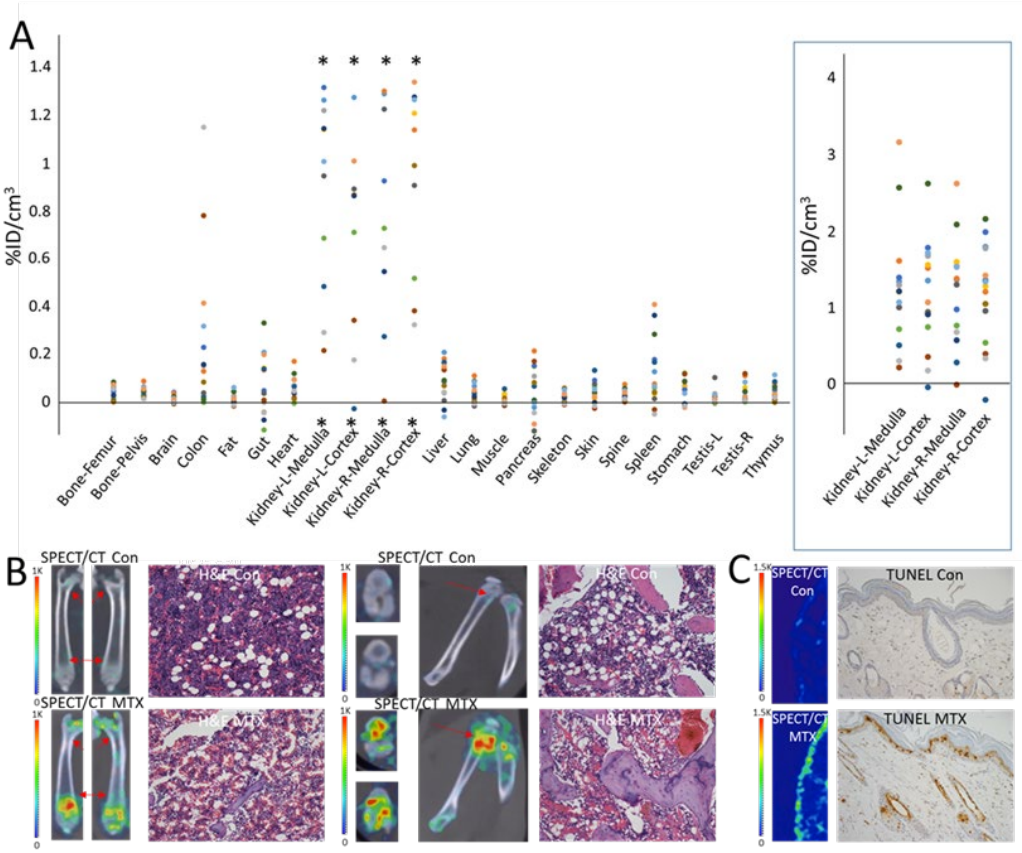

Figure 2. In vivo single time point study of methotrexate treated rats (n = 15) at 24 hours. A) Scatter plot of net change in radioactivity uptake in terms of percentage of injected dose per cm3 in methotrexate treated (n = 15) over the mean of nontreated control tissues (n = 15). *Signal changes in the kidney are plotted at a different scale in the box on right. B) SPECT/CT fusion images show gut signals in control versus methotrexate treated animals as indicated. Corresponding H&E and TUNEL images are shown. C) SPECT/MRI fusion images of lungs from control and methotrexate treated animals demonstrate an elevated signal intensity in the lungs. The corresponding H&E images are presented on the right panel. D) SPECT/CT fusion images of the femurs and knees in control and methotrexate treated animals are presented. Significant signal elevation was detected in the symphysis of the bones and knee joints. H&E stained section of femoral bone demonstrates a depletion of hematopoietic cells in bone marrow of methotrexate-treated animals. E) SPECT images of skin obtained from the control and methotrexate treated animals with corresponding TUNEL images as indicated.

Showcase II: Assessing tissue damage induced by cytotoxic drugs.
Model: Sprague Dawley rats (male)

Drug: Cisplatin
Dosing: 2.5 mg/kg, single dose, i.p.

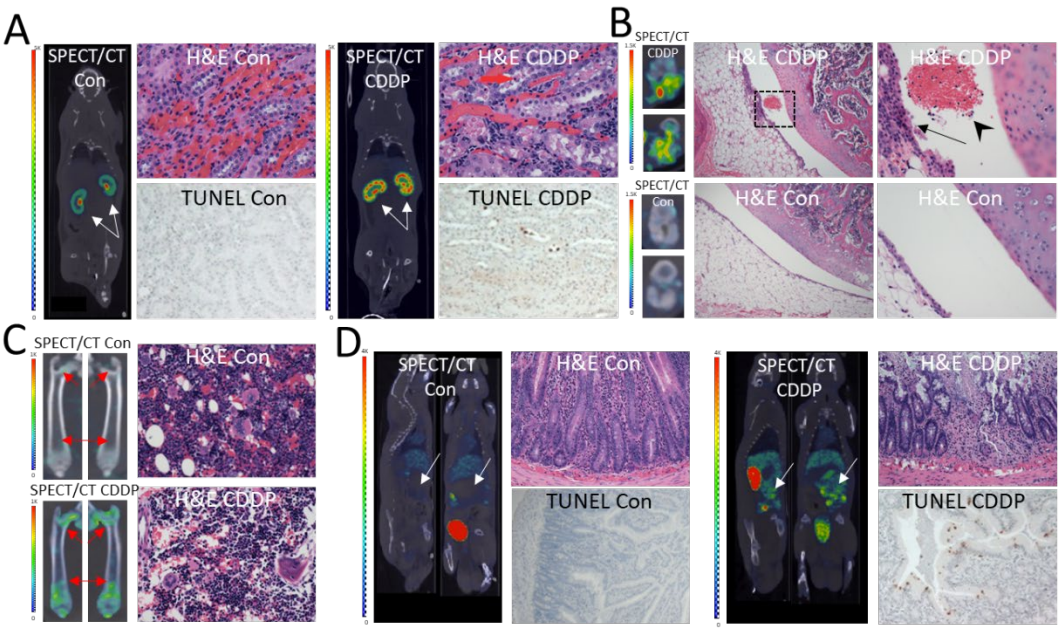

Figure 2. Assessment of cisplatin-induced tissue damage. A) Cisplatin (CDDP) treatment caused significant signal elevation in renal cortex as validated by corresponding histopathology (TUNEL staining for apoptotic nuclei). B) Knee joint damage was identified by in vivo imaging as focal signals, which was confirmed by histopathology with injury to the joint lining and the presence of thrombus. C) Images of the femurs in control and treated animals are presented. Significant signal elevation was detected in the symphysis of the bones. H&E stained section of femoral bone demonstrates a depletion of hematopoietic cells in bone marrow of treated animals. D) Damages to the guts were identified with in vivo imaging as seen in corresponding TUNEL micrographs (apoptotic nuclei were stained positive with the deposition of brown pigment).

Showcase III: Dynamic systemic tissue susceptibility to cytotoxic drugs.
Model: Sprague Dawley rats (male)
Drug: Cyclophosphamide
Dosing: 80 mg/kg, single dose, i.p.
Dynamic *in vivo* imaging: baseline and days 1, 3, 5 and 7 post treatment.

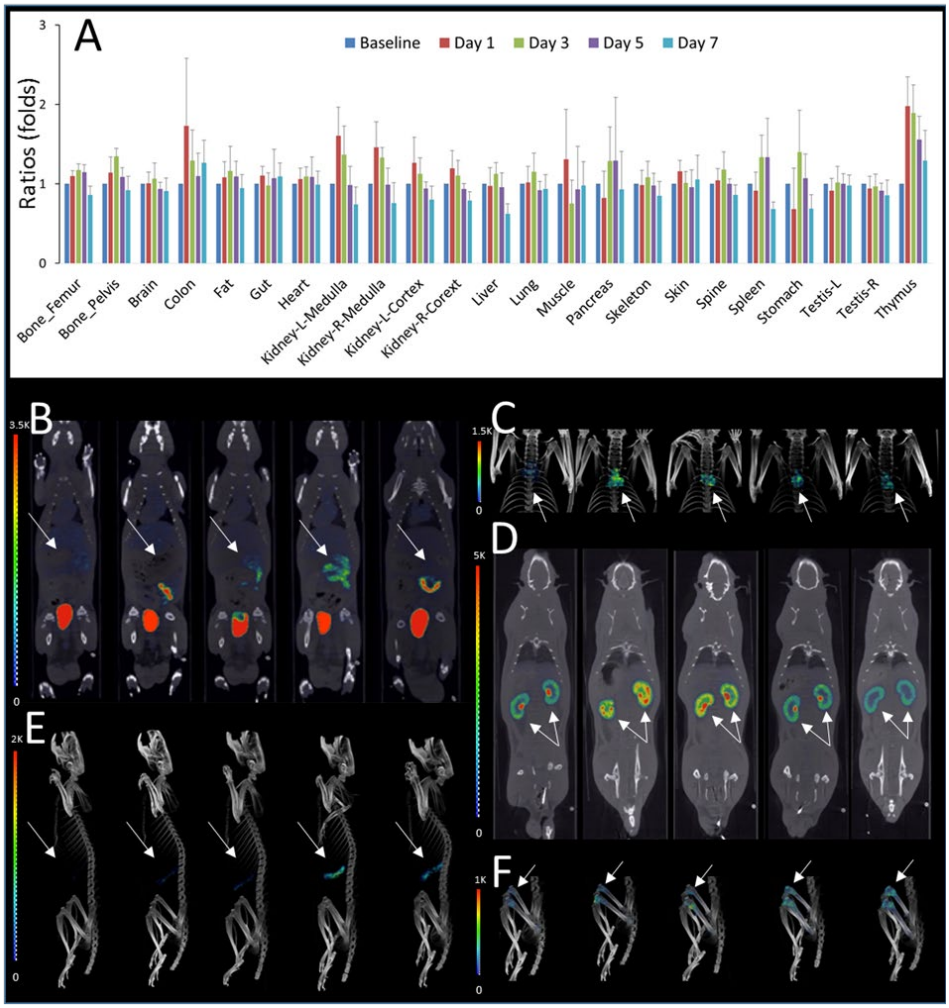

Figure 3. Dynamic imaging of cyclophosphamide treated rats. (A) Signal changes in each tissue at before drug administration, days 1, 3, 5 and 7 post cyclophosphamide treatment. Visual changes are shown in the gut (B), thymus (C), kidneys (E), spleen (E) and femurs/knees (F) over the course of 7 days after treatment. Note that the onset and progression of tissue injury differ in a spatiotemporal fashion.