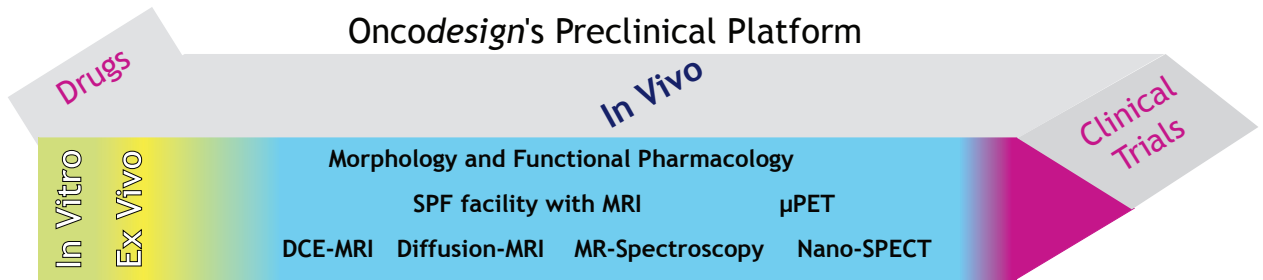


The expertise to obtain translational data from images useful for clinical development

Oncodesign's Preclinical Platform



Biomarkers are now recognized for greatly helping drug development in both preclinical and clinical settings: decision-making is based on strong science. Imaging biomarkers can be used to objectively measure and evaluate normal biological processes, pathogenic processes, or biological responses to a therapeutic treatment. The use of appropriate imaging modalities yields rapid and non-invasive access to morphological, functional and molecular information.

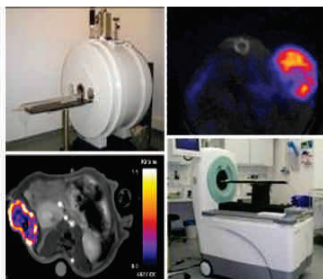
Key Benefits

- Non-invasive and serial tumor monitoring
- Characterization of drug mechanism of action
- Early and predictive evaluation of drug potency
- Translational biomarkers to be included in phase I/II clinical trials

Pharmaco-Imaging Biomarkers in Preclinical Drug Development

Candidate selection

- *In-situ* biodistribution of the radiolabeled drug
- Efficacy
- Comparison of selected candidates on validated imaging assays



Clinical biomarker selection

- Dose
- Mechanism of Action
- Feasibility of the clinical set-up implementation
- Time for clinical response

Drug development

In vivo Target Identification & Validation

PET
BIOLUM/FLUO

Acute Toxicity

MRI/MRS

In vivo Antitumor Activity

Biomarker Identification
Combination Study
Preclinical Phase II

MRI/MRS
PET/SPECT
BIOLUM/FLUO

In vivo PK/PD

PET/SPECT

Phase 0

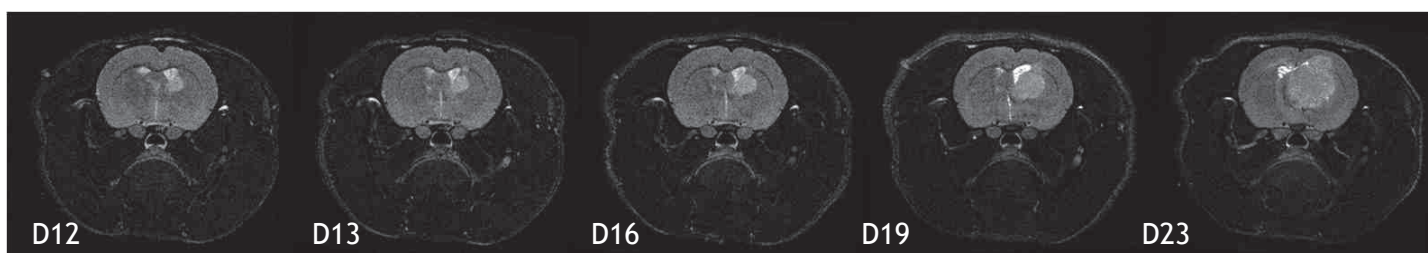
MRI/MRS
PET/SPECT

Translational Imaging Biomarkers at Oncodesign

Application	Imaging Modalities	Endpoints	Information	Models of choice
<ul style="list-style-type: none"> Evaluation of anti-angiogenic / antivascular effect of therapies 	<ul style="list-style-type: none"> DCE-MRI with Gadolinium-based or novel contrast agents Susceptibility-weighted MRI* 	<ul style="list-style-type: none"> K^{trans}, iAUC Blood volume (BV) & Vessel Size Index (VSI) 	<ul style="list-style-type: none"> Evaluation of the vascular status 	<ul style="list-style-type: none"> Calu-6 U-87 MG HCT 116 MDA-MB-231
<ul style="list-style-type: none"> Assessment of tumor & metastatic burden Phenotyping 	<ul style="list-style-type: none"> Morphological MRI ^{18}F-FDG-PET Diffusion-Weighted MRI* SPECT (HDP) 	<ul style="list-style-type: none"> Tumor volume Number, location of metastases Apparent Diffusion Coefficient (ADC) 	<ul style="list-style-type: none"> Number and size of individual metastasis Tumor volume Qualitative & quantitative information on tumor heterogeneity 	<ul style="list-style-type: none"> CMEL-5 U-87 MG
<ul style="list-style-type: none"> Assessment of tumor proliferation 	<ul style="list-style-type: none"> ^{18}F-FLT-PET* 	<ul style="list-style-type: none"> Standard Uptake Value (SUV), relative uptake 	<ul style="list-style-type: none"> DNA synthesis 	<ul style="list-style-type: none"> U-87 MG HCT 116
<ul style="list-style-type: none"> Assessment of tumor metabolism 	<ul style="list-style-type: none"> ^{18}F-FDG-PET ^{11}C-Choline-PET or ^{18}F-Fluoroethylcholine-PET* 1H MR spectroscopy 	<ul style="list-style-type: none"> Standard Uptake Value (SUV), relative uptake Relative concentration of molecules of interest 	<ul style="list-style-type: none"> Glucose metabolism Membrane synthesis 	<ul style="list-style-type: none"> U-87 MG CWR-22

* Translational biomarkers developed at Oncodesign to monitor response to anticancer therapy

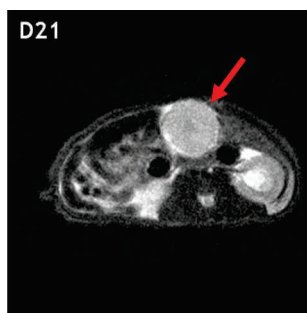
Morphological Magnetic Resonance Imaging (MRI)



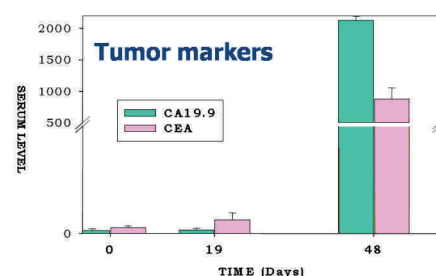
Serial monitoring of the growth of an orthotopically xenografted human glioblastoma (U-87 MG) by T2-weighted MRI in *Nude* Rats. Imaging timepoints in days post tumor implantation.

Morphological imaging applications:

- Anatomical imaging of soft tissues
- Non-invasive follow-up of tumor growth (example: OT models)
- Early detection of antitumor efficacy
- Use of contrast agents to enhance tumor visibility
- Identification of tumor location and evaluation of disease extension (metastases)



Tumor volume at D21: 1.06 cm³



Earlier evaluation of tumor growth by MRI in comparison to blood markers in *Nude* rats bearing orthotopic BxPC-3 human pancreatic tumors.

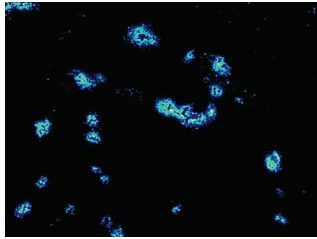
Dynamic Contrast-Enhanced Magnetic Resonance Imaging (DCE-MRI)

Applications:

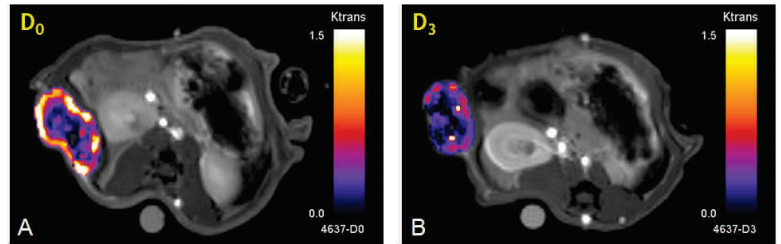
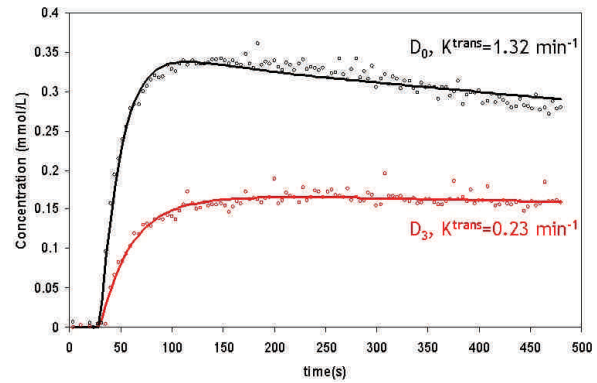
- Characterize tumor perfusion and microvascular permeability
- Support proof of concept / mechanism of action
- Optimize biologically active dose and scheduling in combination therapies
- Identify biomarkers of efficacy for anti-angiogenic or anti-vascular drugs

DCE-MRI protocol:

- Baseline image acquisition
- Bolus injection of Gadolinium chelate
- Dynamic follow-up of contrast agent uptake in the tumor
- Quantitative and standardized image analysis using a software developed at Oncodesign
- Analyzed parameters:
 - Initial Area Under the Curve (iAUC)
 - K^{trans} tumor mapping



Vessel permeability observed after Hoechst injection



Results from a DCE-MRI experiment performed using *Nude* rats bearing MDA-MB-231 human breast tumor xenografts and treated from D0 with Sorafenib. K^{trans} parameter maps superimposed on morphological images before (A) and after (B) treatment with corresponding uptake curves (top panel), showing the effect of Sorafenib on vascular status.

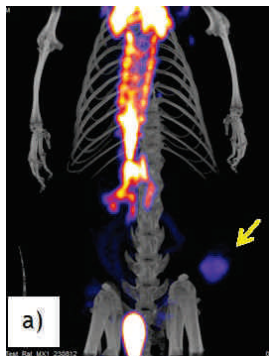
Control/validation with gold standard immuno-histochemistry assays:

- Semi-quantitative analysis to evaluate neo-angiogenesis and vessel maturity (CD31/ α -SMA co-staining)
- Functional assay to measure the number and size of vessels and to characterize vascular permeability (Hoechst/Dextran-FITC)

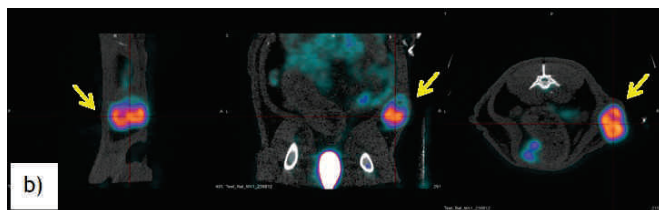
Positron Emission Tomography (PET)

PET Applications:

- Assessment of tumor glucose metabolism as a biomarker of efficacy (^{18}F -FDG)
- PD biomarker using dedicated, MoA-optimized tracers (proliferation: FLT, hypoxia: FMISO...)
- PET tracer development studies using our Nanocyclix[®] platform
- *In vivo* high-content screening of drug candidates
- Phase 0 studies (micro-dosing studies) in collaboration with the Clinical Investigational Center in Dijon.



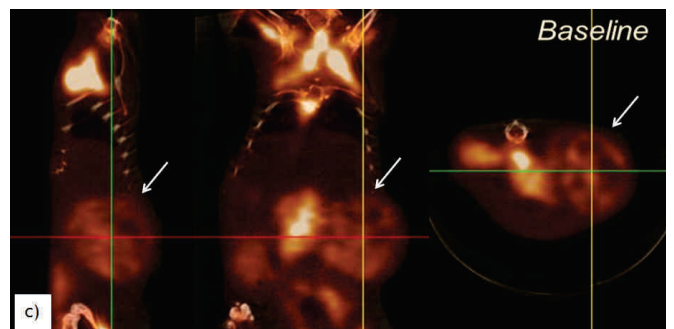
(a, b) ^{18}F -FDG / PET (overnight fasting, 39 MBq, 40 min uptake) in MX-1 tumor-bearing RH *Nude* rat (triple negative tumors).



(c) ^{18}F -FDG / PET (overnight fasting, 15 MBq, 40 min uptake) in SC BT-474C tumor-bearing Balb/c *Nude* mice.



Bioscan BioPET/CT



Single-Photon Emission Computed Tomography

Applications:

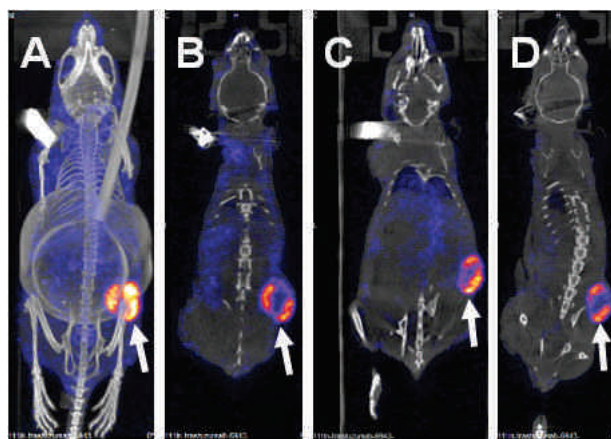
- Assessment of target expression
- Biodistribution
- Assessment of tumor proliferation/metabolism
- Monitoring of bone metastasis

SPECT Protocol:

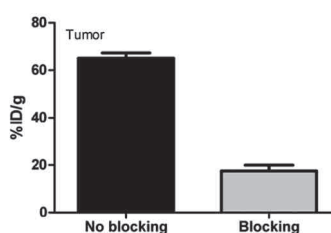
- Radiolabeling strategy
- *In vitro* assessment of the radiolabeled compound's biological properties.
- *In vivo* imaging:
 - Ability to accumulate in the tumor
 - PK parameters determination
 - Specificity of the labeled antibody

Radiolabeling offering

Oncodesign has the expertise to perform radiolabeling of antibodies and peptides, through specific radiochemistry units dedicated to radiometal chelation and access to a broad range of selected radioisotopes.



In vivo proof of concept for a novel chelate for antibody labeling. Uptake of DOTA-trastuzumab in BT-474 tumors (A:MIP, B:24h, C:48h, D:72h)



In vivo tumor targeting of a HER2-expressing tumor model: evaluation of the specificity of the labeled antibody.

Equipment & Software

MRI:

- Bruker PharmaScan® M-C 47/16AS (4.7 Tesla)
- Located in SPF facility
- Radio-frequency coils: mouse body and rat brain
- Cardiac and respiration gating
- Continuous isoflurane anesthesia and warming of animals

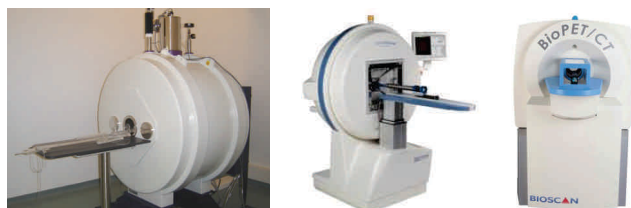


Image analysis:

- Image processing software for pixelwise or Region of Interest analysis using ImageJ
- Numerous ImageJ plugins have been developed in-house for parameter estimation and visualization

SPECT:

- Bioscan nanoSPECT/CT™
- High detection sensitivity and image resolution (SPECT: 1 mm / CT: 40 µm)
- Hot laboratory and access to a broad range of selected radioisotopes (¹¹¹In, ^{99m}Tc, ¹²³I, ¹²⁵I, ¹³¹I, ⁶⁷Ga)

PET:

- Bioscan BioPET®-CT
- High resolution and sensitivity for uniform resolution and superior image uniformity

The radiolabeling of your drug is achieved through state-of-the art facilities:

- GE 16 MeV cyclotron
- Radiochemistry units
- Specific radiometal chelation expertise for peptides and antibodies
- Good manufacturing practice (GMP)



References Available on our Website

- Review in European Biopharmaceutical Review, Mar 2011
- Review in Siemens Medical Solutions USA, Inc., Aug 2008
- 2012 EORTC Poster
- 2012 AACR Poster, #2447
- 2011 AACR Poster, #1764
- 2010 AACR Poster, #1367

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