Integrating Pharmacology and Imaging in Preclinical Oncology Drug Development

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Introduction

The last ten years have seen major discoveries in cancer research particularly in the field of investigation techniques. The identification of original targets on which a large number of compounds are being tested in vitro leads to the emergence of new active drugs. The drug selection process is partly performed using animal models that are as close as possible to the targeted malignancy. In this context, imaging techniques using small-animal dedicated imaging devices play an essential role.

Imaging techniques designed for in vivo use include: X-ray computerized tomography (CT), ultrasound (US), magnetic resonance imaging (MRI) and spectroscopy (MRS), positron emission tomography (PET), single photon emission computed tomography (SPECT), and new optical technologies such as near-infrared fluorescence imaging (NIRF). These non-invasive modalities are increasingly used in preclinical studies using animal models to assess drug distribution or biomarker levels for tumor staging or treatment follow-up. All of these imaging modalities can accelerate the preclinical development of new drugs and some are also directly transferable from the animal model to the clinic. Among these, MRI / MRS and PET are complementary technologies allowing quick and repeated access to morphological and functional information in vivo. The selection of the imaging modality varies with the question to be answered and the performance of the imaging device (sensitivity, spatial and temporal resolutions). The main objective is to deliver new active drugs to the clinicians earlier and with more accuracy.

Clearly, there is a need to produce new drugs with novel mechanisms of action. Today, it takes approximately 12 years and $1-2 billions to bring a drug from laboratory to FDA approved product. The drug development process needs to move more efficiently and quickly while minimizing costs, to rapidly identify the most promising candidates and to identify and cease those projects that are failing before too much money has been invested. In the development of new targeted therapies, a number of key issues need to be addressed:

- **Does the drug reach active concentrations in blood and tumor to induce the intended biological effect?**
- **Does the drug hit the selected molecular target? (1, 2, 3) (Figure 1).**

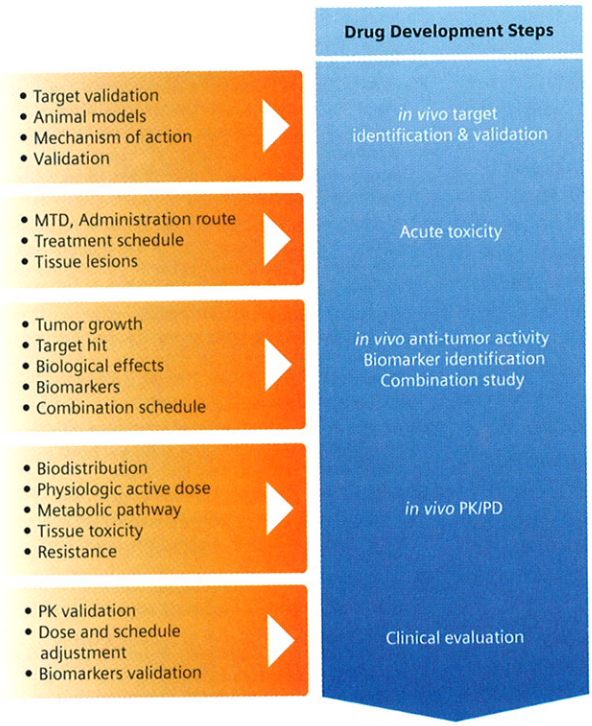


Figure 1. Drug development process. MTD is the maximum tolerated dose.

The use of imaging biomarkers in cancer drug development is rapidly being adopted by pharmaceutical and biotech companies to obtain improved pharmacological endpoints. It is especially important to establish a well-defined relationship between pharmacokinetic (PK) and pharmacodynamic (PD) properties to select the best drug candidate for clinical development. Many pharmacological endpoints in clinical routine are invasive, requiring repetitive sampling. To reduce this invasiveness and to choose the best timing for sampling, we argue the importance and potential value of functional and molecular non-invasive imaging techniques. The purpose of this review is to discuss, on the basis of examples focusing on MRI and PET, the ability of functional imaging to meet researchers' requirements and to evaluate all possibilities offered by translational research to validate and transfer these techniques from the preclinical field to the clinic.

Non-invasive imaging technologies to support the drug development process

For decades, anatomical imaging with CT or MRI has facilitated drug development in oncology by providing quantifiable and objective evidence of response to therapy. In recent years, metabolic imaging with [^{18}F] fluorodeoxyglucose–PET (FDG-PET) became an important tool for oncologists to detect treatment response earlier. MRI can assess tumor size and structure and provide func-

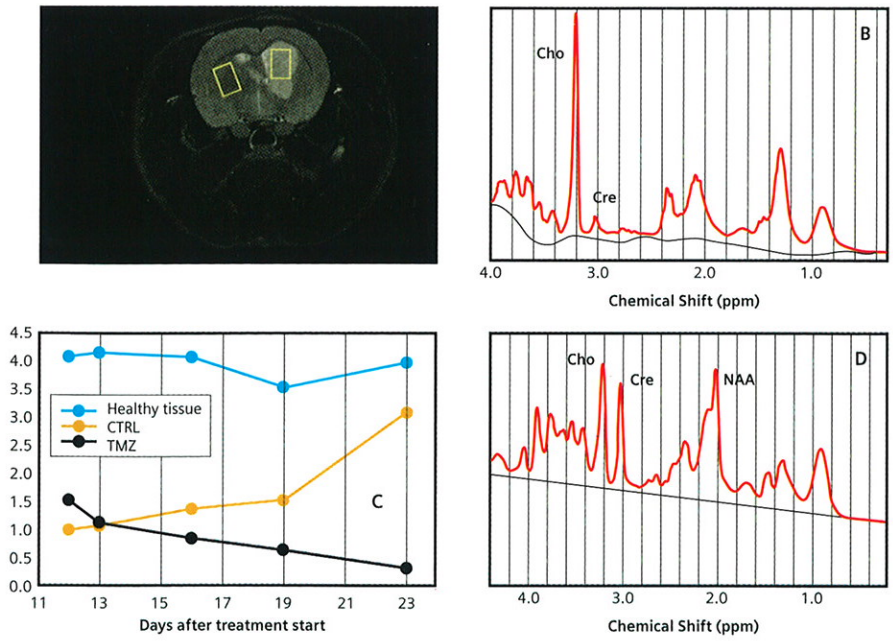


Figure 2. T2-weighted anatomical images showing localization of U-87 MG orthopically xenografted glioma in nude rats along with tumor and contralateral voxels for spectroscopic data acquisition (A). Evolution over time of ^{1}H metabolites, NAA over choline ratios (C). The NAA/choline ratio increased in the temozolomide-treated rats, while it decreased in the non-treated rats. ^{1}H MR spectrum from glioma without treatment (B) and after treatment with temozolomide (D).

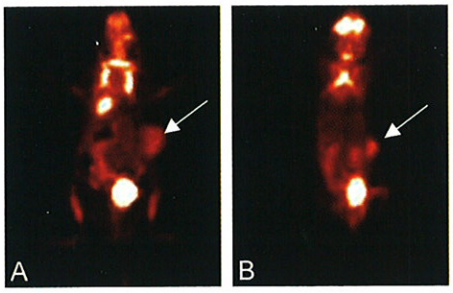
tional information such as tumor perfusion and permeability of the microcirculation. Dynamic Contrast-Enhanced MRI (DCE-MRI) is based on the temporal and spatial changes in signal intensity following the rapid injection of low molecular weight Gadolinium chelates to provide information on tumor perfusion, vessel density and permeability, and blood volume. Larger molecular weight Gd-based contrast agents or iron oxide nanoparticles may also be used to evaluate blood volume, vessel size and permeability, but are not yet available for clinical trials of anticancer drugs. DCE-MRI is now systematically used for biomarker identification of the efficacy of anti-angiogenic and anti-vascular compounds (12). Diffusion-weighted MRI (DW-MRI) measures changes in the diffusion properties of water molecules in living tissue and could be used to study tumor microenvironment at a physiological level. It has been used as an early indicator of response to classical cytotoxic, chemo- or radio-therapies (4, 5, 6). At cellular and molecular levels, the current clinical imaging techniques are MRS and PET. Both techniques can be used to directly monitor drugs pharmacokinetics and biodistribution when containing appropriate nuclei with magnetic properties (MRS) e.g. 5-FU detected by ^{19}F-MRS (7) or a radionuclide (PET) e.g. ^{11}C-temozolomide (8). Endogenous metabolites measured by ^{1}H-MRS (N-acetylaspartate, citrate, choline, lactate) or to a lesser extent by ^{31}P-MRS (adenosine triphosphate, inorganic phosphate) have been used particularly in brain and prostate malignancies to quantify tumor metabolism and bioenergetic status changes during treatment (9). FDG-PET, reflecting tumor glucose metabolism, or with ^{18}F-fluorothymidine (FLT), reflecting DNA synthesis, provides relevant information regarding treatment response. Changes in tumor PET tracers uptake may precede changes in tumor size. Both FDG and FLT-PET enable early prediction of success in the treatment course and enable the determination of the viability of residual masses (10). PET can also be used to measure specific biological endpoints that are directly relevant to a particular target, for example using ^{124}I or ^{64}Cu-labeled anti-erb b2 antibody to select patients for therapy with Herceptin in the treatment of breast cancer (11). To further illustrate the role of imaging technologies in drug development, examples of our own and collaborative works will be described in more detail.

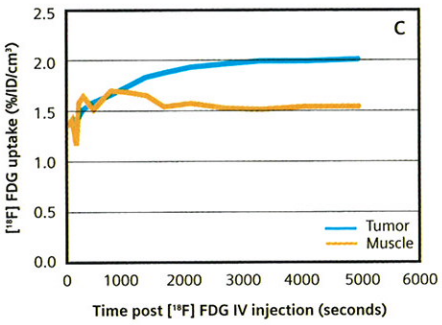
Tumor metabolism and cellular proliferation inhibition

Many anticancer treatments affect cell cycle and cellular metabolism. The most appropriate techniques to evaluate these biologic processes are proton MRS (^{1}H-MRS) and the FDG-PET for tumor metabolism and FLT-PET for tumor cellular proliferation. ^{1}H-MRS measurements of decreases in the levels of choline-containing compound following treatments have been shown to be predictive of response in brain, breast and prostate cancers (18,19). As an example, single voxel ^{1}H-MRS was used successfully to evaluate the anti-tumor activity of Temozolomide (TMZ) and radiotherapy (RT) in human orthotopic glioblastoma models in nude rats (Figure 2). A strong inhibition of tumor growth and prolonged survival were observed by TMZ treatment in both models while RT treatment had no or moderate effect on survival. The N-acetylaspartate to choline peak ratios increased significantly in TMZ treated rats, whereas it decreased in control and RT-treated rats. Monitoring tumor metabolism using ^{1}H-MRS was well suited to follow the growth of glioma and quantify the anti-tumor effect of TMZ with choline being the most pertinent biomarker (20).

FDG tumor uptake is correlated with the level of glucose transporter GLUT1 expression to take up into the tumor cells where FDG is phosphorylated by hexokinase. Glycolysis could be evaluated by FDG-PET reflecting the effect of drugs on cell metabolism. All have in mind the FDG-PET images of the first patients treated by Gleevec® where FDG uptake was significantly decreased as early as 24 hrs after the first dose, whereas tumor size reduction appeared several weeks later (21). From this day, many drugs have been evaluated by PET-FDG though this technique has some limitations. For clinical application, high uptake of FDG is measured in some normal tissues, i.e. the brain, and accumulation in inflammatory zones could influence the evaluation of tumor response to treatment. The main limitations are probably for preclinical applications where the fasting period for approximately 6-12 hours before FDG injection in addition to anesthesia maintenance between FDG injection and image acquisition are very stringent conditions that could definitely modify the tolerance of small animals to the tested drug.

Some investigations have reported significant differences in ^{18}F-FDG and ^{18}F-FLT uptakes in various subcutaneous tumor xenografts. In tumors where radiotracer uptake is low, it may not be possible to assess the anti-tumor efficacy of a drug, as radiotracer uptake variations may be hardly detectable (22). Tumor cell proliferation and response to treatment have been assessed by PET using FLT trapped in the cells after phosphorylation by thymidine kinase 1, which is up-regulated during the S phase of the cell cycle (23). The potential advantage of FLT over FDG could be the possible increased sensitivity to cytostatic properties of targeted therapies, which often block cell division with a low influence on glucose metabolism (24), but this hypothesis needs additional supporting data.





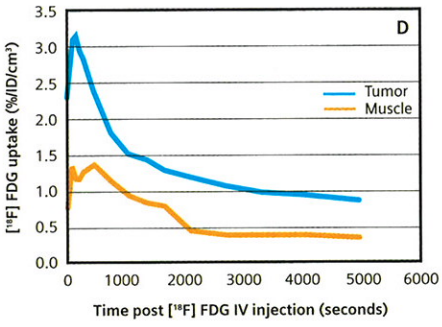


Figure 3. [18F]FDG uptake in human CWR-22 prostate tumors subcutaneously xenografted in Nude mice. Static images were recorded before (A) and eight weeks after surgical castration (B). [18F]FDG uptake period was 1 hour. The tumor is indicated by a white arrow. [18F] FDG dynamic scan recorded before (C) and eight weeks after castration (D). For both scans, mice received a single IV injection of 200 µCi [18F]FDG after a 6 hour fasting period.

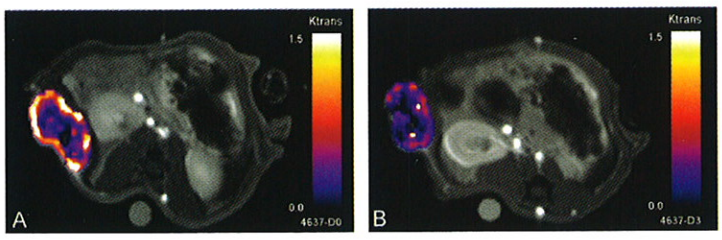
As FDG-PET has lower sensitivity for slow growing and metabolically less active tumors like hormone-dependent prostate tumors, new PET tracers are needed. One research program of the Laboratory for Preclinical Imaging and Imaging Technology of the Werner Siemens-Foundation (Tuebingen, Germany) is the selection of novel PET tracers for prostate cancers. They demonstrated that human hormone-independent tumor xenograft models, also compared to clinical findings in humans, showed very different pharmacokinetics and uptake characteristics for [18F] FLT, [18F]FDG, [11C]Choline and [18F]FECh. Subsequently, they investigated PET tracers uptakes in xenografted hormone-dependent human prostate tumor models. In baseline studies, they found faint uptake in tumors imaged with [18F]FECh, no tumor tracer uptake with [11C]choline and moderate [18F]FLT and [18F]FDG uptakes. Surgical castration induced a decrease of [18F]FDG tumor-to-muscle ratios (Figure 3) and variable [18F]FLT tumor-to-muscle ratios depending on the tumor model (25).

Angiogenesis and vascular function inhibition

Angiogenesis, the process whereby new blood and lymphatic vessels are formed from pre-existing vasculature, plays a pivotal role in tumor development and metastasis. Inhibiting angiogenesis represents the first strategy for development of anticancer targeted therapies (12). As mentioned in previous section, DCE-MRI allows for the quantification of pharmacodynamic effects of anti-angiogenic agents and their relationship to the administered dose (Figure 4). In DCE-MRI studies, images are acquired rapidly to dynamically follow the extravasation of an injected contrast agent into the tumor tissue. It is now the most widely used technique in the preclinical and early clinical evaluation of anti-angiogenic and anti-vascular agents (12), with 75 anti-angiogenic agents in clinical trials at present (13). Avastin® (Bevacizumab, Roche, Switzerland), Nexavar® (Sorafenib, Bayer, Germany), and Sutent® (Sunitinib, Pfizer, USA) are the first three FDA-approved compounds where DCE-MRI was documented in both preclinical and early clinical phases.

Vascular endothelial growth factor (VEGF) plays a key role in tumor angiogenesis by stimulating the proangiogenic signaling of endothelial cells via activation of VEGF receptor (VEGFR) tyrosine kinases, making VEGF and VEGFRs attractive therapeutic targets. KRN951, a novel multiple tyrosine kinase inhibitor (Kirin Pharma, Japan and Aveo Pharmaceuticals, USA), showed a significant anti-tumor activity against a wide variety of human tumor xenografts (14). DCE-MRI revealed a correlation between Ktrans reduction, reflecting a modification of tumor perfusion/vascular permeability, and the anti-tumor activity of KRN951. Furthermore, in a dose-escalation phase I clinical trial, KRN951was active against renal, colon and lung cancers. DCE-MRI also indicated a decrease in tumor perfusion in selected patients (15). These studies suggest that DCE-MRI is useful in detecting early responses to KRN951 in a clinical setting.

In collaboration with the Grenoble Institute for Neurosciences (France), we have recently investigated the use of multiple MRI biomarkers to explore the vascular changes associated with the anti-tumor activity of Carmustine and Sorafenib in a human orthotopic glioblastoma model in nude rats. Blood volume (BV), vessel size index (VSI), apparent diffusion coefficient (ADC) and blood brain barrier permeability to a contrast agent (BBB perm.) were mapped in the whole tumor, at different time-points after treatment onset. VSI/BV and BBB perm. parameters were computed from T2, T2* and T1-weighted images using an intravascular contrast agent (Ferumoxtran-10, Sinerem®) and P846 (Gd-based contrast agent, Guerbet/AMAG Pharmaceuticals). Despite poor effects of Sorafenib and



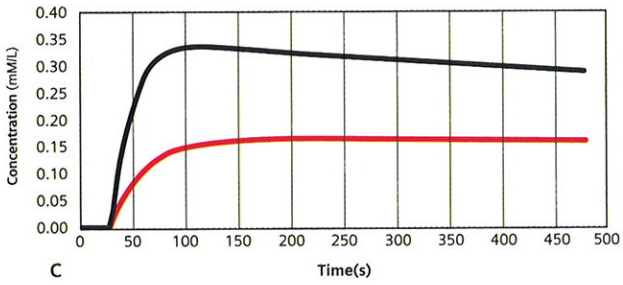


Figure 4. Results from a DCE-MRI experiment performed on nude rats bearing MDA-MB-231 human breast tumor xenografts and treated with Sorafenib. Image acquisition was performed just before the first treatment and 3 days after treatment onset. K^{trans} parameter maps superimposed on morphological images before (A) and after (B) treatment. Mean Gd-DTPA uptake curves in tumor rim with fitted PK model (solid line) before (black) and after (red) treatment (C). K^{trans} values are 1.23 s^{-1} before treatment and 0.32 s^{-1} after treatment.

Carmustine treatments on survival, MRI demonstrated a tumor growth inhibition induced by these drugs. ADC is affected by both treatments while VSI and BV were sensitive to the effect of Sorafenib only. Histological data confirmed the mean vessel density was highly decreased by Sorafenib treatment. Together, these results indicate that VSI, BV and ADC parameters would be of value to combine anti-angiogenic with cytotoxic therapies in glioblastomas (16, 17).

Perspectives of functional and molecular imaging for personalized medicine

Translational research aims at moving basic discoveries from preclinical research into clinical evaluation to better select the right drug for the right person and to help the clinician to rapidly adapt therapeutic strategy to tumor response. The two most famous examples of targeted cancer drugs, Gleevec® and Herceptin®, highlight the necessity of imaging biomarkers and surrogate pharmacological endpoints adapted to the mechanism of action of each drug. Even as pharmaco-imaging is now becoming an important tool in drug development, we believe that some major advances need to occur in order to evolve from a research endeavor to a high-throughput production system. This requires the integration of multiple imaging modalities (26), with huge volumes of data and the standardization of protocols through the construction of dedicated international consortia.

There are many possibilities to combine complementary data from multiple imaging modalities. Combining functional MRI and spectroscopy with PET paves the way for a new perspective in molecular imaging with great potential for clinical applications (27). Combined or hybrid technologies, such as PET/CT and SPECT/CT, incorporate both imaging modalities into one machine but conduct the two scans sequentially. The lack of uniformly structured data affects drug discovery and individualized medicine, all of which rely heavily on integrating and interpreting data sets produced by different experimental methods such as non-invasive imaging, high-throughput genotyping, DNA microarrays, protein arrays, and high-volume clinical data.

In this context, the most urgent challenge for the immediate future is to standardize imaging procedures for a better qualification of multiple biomarkers. There is now a real need to dedicate worldwide networks to develop consensus recommendations and progress in this key area. The Pharmacodynamic/Pharmacokinetic Technologies Advisory Committee of Cancer Research UK recommend the development of non-invasive methods that measure common biological processes - particularly proliferation, cell cycle status, apoptosis, invasion, and angiogenesis - affected by many different drug classes and considered as more cost-effective than those that measure a specific molecular target (28).

Translational research is a multidisciplinary field based on teams rather than individuals. The challenge is to build efficient consortia with individuals coming from different entities such as academia, big pharmas, biotechs and CROs and having different scientific backgrounds. In this context, Oncodesign, dedicated to the preclinical evaluation of cancer therapies, has developed in-house skills for small animal imaging and established partnerships with the Laboratory for Preclinical Imaging and Imaging Technology of the Werner Siemens-Foundation (Tuebingen, Germany), dedicated to bridge the gap between in vitro biomedical research and in vivo imaging; and with PHARMIMAGE, a pharmaco-imaging platform in Dijon (France). Many technological platforms have been built in the past five years to help drug manufacturers with the development of biomarkers in parallel to the development of therapeutic drugs. Today, a large panel of imaging technologies and imaging biomarkers are being developed and identified as surrogate endpoints of drug efficacy with different mechanisms of action in preclinical studies. The real validation will be achieved by integrating more data from clinical trials incorporating these noninvasive imaging biomarkers, which will need to be correlated with other classical biomarkers and patient survival.

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