Imaging Cancer Biomarkers

Xavier Tizon at Oncodesign looks at integrating pharmacology and imaging in preclinical oncology drug development

The last 10 years has seen major discoveries in cancer research, particularly in the field of technological investigation tools. The identification of original targets on which a large number of compounds are being tested *in vitro* is leading to the emergence of new active drugs. However, these new drugs have shown many of the limitations of conventional drug development processes, which have been inherited from the development of cytotoxic compounds. Firstly, developing an innovative drug is very expensive and time-consuming (costing around $1 billion and taking on average two to five years). Secondly, treatment with targeted therapies does not necessarily induce tumour shrinkage. To be able to make decisions about a candidate drug, assays are needed that measure their efficacy in terms of biological consequences on cancer cells and tumour microenvironment. Another point is that targeted therapies have shown disappointing results in patients; many Phase III clinical trials have failed to show the benefits of these new drugs, with high attrition rates mainly caused by unexpected toxicity and lack of efficacy.

The drug selection process is partly performed using animal models that are as close as possible to the targeted therapy. Extending the use of biomarkers throughout this process has been suggested to make this process more effective. A biomarker is defined as 'an objectively measured indicator of a biological or pathological process, or of pharmacological response to treatment' (1).

Biomarker applications can be divided into four categories:

- Diagnosis – ill or healthy? Diagnosis biomarkers are mostly used for extension assessment and disease staging. One exception is the search for metastases, which can be performed on small animal models
- Patient selection/stratification – should the patient receive treatment or not and if so, with which drug? These biomarkers are intended to identify particular genetic and/or phenotypic characteristics of the tumour that are relevant to guide the choice of drug to be used
- Efficacy – is the treatment working? Efficacy biomarkers identify biological changes in the tumour associated with the effect of the drug on tumour cells or other tumour tissue
- Pharmacodynamics (PD) – which dose/schedule? By measuring the effect of the drug on the tumour and the body, PD biomarkers help in deciding if the conditions in which the drug has been administered (dose, schedule and order of combination) have led to success

THE USES OF PHARMACO-IMAGING

Technological advances over the past 20 years have provided cancer researchers with dozens of methods to analyse tumours (including genomics, proteomics, metabolomics and microscopy). Among these methods, imaging biomarkers have one major advantage: they give readouts from live, intact organisms with sufficient resolution for studying biological processes *in vivo*. Additionally, they are mostly non-invasive, allowing for repeated sampling and thus measuring dynamic changes over periods of time. Their translation towards the clinic is quite easy, as imaging scanners used on small animals are very similar to these used in clinical studies. Imaging also has great potential to assess the heterogeneity of biochemical processes within the tumour because it provides a spatial and temporal mapping of these processes.

Non-invasive imaging technologies are being used increasingly in drug discovery and development. Across therapeutic areas, imaging endpoints are showing promise as quantifiable measures of compound efficacy and disease response to treatment. Access to these technologies is increasing through the establishment of in-house imaging centres and CROs. In this brief review we will try to explore, through some examples, how pharmaco-imaging – the use of imaging for pharmacological enquiry – can help drug development produce faster, more objective answers (2-5).

Does the Drug Hit its Target?

Advances in genomics, proteomics and chemistry have accelerated the development of compounds aimed at specific molecular targets associated with disease. Numerous inhibitors of kinases, receptors and proteinases are currently under active development. Imaging of these targets is crucial for development in order to measure their expression and their interaction with the administered drug. It

is also crucial in order to be able to select patients that will most probably respond favourably to treatment: thus lowering costs and minimising failures of late phase clinical trials. The main constraint is the low concentration of these targets, requiring very high sensitivities from measurement techniques.

Optical imaging is one modality of choice for target imaging. In principle, photons emitted by a probe are measured after excitation with an external light source (as in fluorescence imaging) or as a result of a biochemical reaction between an enzyme and its substrate (bioluminescence imaging). Both techniques are low-cost, small footprint and high-throughput, therefore they are well suited for pharmacology research. The results are fairly quantitative, meaning that relative light levels can be compared within an experiment, but the technology is difficult to translate to humans because of the low depth of penetration of photons that are imaged. The main applications are cell tracking, target imaging (receptors, enzymes and pathway-based molecules) and efficacy assessment for orthotopic or transgenic models. For example, the use of reporter gene imaging *in vivo* is rapidly emerging as a powerful tool to monitor gene expression.

Positron emission tomography (PET) is a nuclear imaging technique that also has great potential to measure specific biological endpoints directly relevant to a particular target. To label a given compound and study its biodistribution, PET isotopes have to be chosen depending on the half-life and size of the compounds they will be linked to. In order to match the pharmacokinetics (PK) of the labelled compound with the half-life of the isotope itself, small molecules with shorter half-life can be labelled with short-lived isotopes such as ^{11}C ($T_{1/2}$ = 20 minutes) and ^{18}F ($T_{1/2}$ = 110 minutes), whereas antibodies have to be labeled with longer-lived isotopes. For example, ^{124}I ($T_{1/2}$ = 4.2 days) or ^{64}Cu ($T_{1/2}$ = 12.7 hours) have been used to label anti-erbb2 antibodies to select patients for therapy with Herceptin in the treatment of breast cancer (6). One of the main advantages of labelling a drug with PET is that the sensitivity of the technique allows for tracer amounts of the drug to be administered, thus causing no toxicity and making it feasible early in the development process, when only small amounts of the compound can be produced. Being a quantitative imaging modality, PET is also used to acquire dynamic data for PK studies, or site-specific occupancy study, in which an isotope-labelled ligand such as an existing PET tracer is used, which competes for the target with the specific drug under investigation.

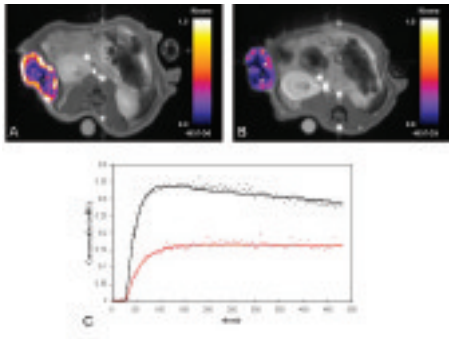
Is the Drug Having any Biological Effect?

Once evidence has been gathered that the drug hits its target, it is especially important to establish a well-defined relationship between PK and PD properties to select the best drug candidate for clinical development. The Pharmacodynamic/Pharmacokinetic Technologies Advisory Committee of Cancer Research UK recommends the development of noninvasive 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 specific molecular targets (7). At this stage, information is gathered that can help identify the mechanism of action, the nature and intensity of the biological effect, and also prepare for early clinical trials by giving an indication of response to treatment.

Angiogenesis, the process whereby new blood and lymphatic vessels are formed from pre-existing vasculature, plays a pivotal role in tumour development and metastasis. Inhibiting angiogenesis has represented the first strategy for development of anticancer targeted therapies (8). Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is based on the temporal and spatial changes in signal intensity following the rapid injection of gadolinium chelates to provide information on tumour perfusion, vessel density and permeability. It allows for the quantification of pharmacodynamic effects of anti-angiogenic agents and their relationship to the administered dose. In DCE-MRI studies, images are acquired rapidly to dynamically follow the extravasation of an injected contrast agent into the tumour

Figure 1: Results from a DCE-MRI experiment performed on Nude rats bearing MDA-MB-231 human breast tumour xenografts and treated with Sorafenib

Image acquisition was performed just before the first treatment and three days after treatment onset. K^{trans} parameter maps superimposed on morphological images before (A) and after (B) treatment. Mean Gd-DTPA uptake curves in tumour rim (circles) 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.

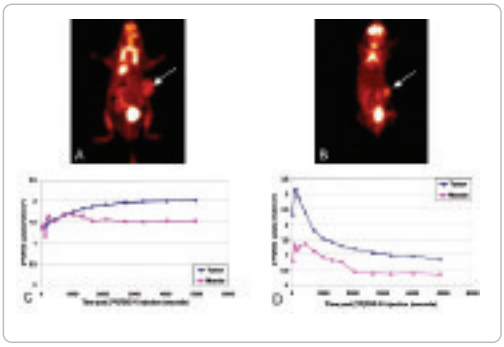


tissue (see Figure 1). The scientific debate is still active on methods to analyse and interpret the measured dynamic signal, which contrast agent is the most useful, and on the reliability of multi-site studies. However, it is now the most widely used technique in the preclinical and early clinical evaluation of antiangiogenic and antivascular agents. Avastin (Bevacizumab, Roche, Switzerland), Nexavar (Sorafenib, Bayer, Germany), and Sutent (Sunitinib, Pfizer, US) are the first three FDA-approved compounds where DCE-MRI documented efficacy in both preclinical and early clinical phases.

PET using fluoro-deoxyglucose (FDG) labelled with the ^{18}F isotope of fluorine, is now a well-established technique to visualise glucose metabolism in the tumour. FDG tumour uptake is correlated with the level of glucose transporter GLUT1 expression which will take up FDG into the tumour cells where it is phosphorylated by an hexokinase and not metabolised further, staying trapped inside the cell. 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 hours after the first dose, whereas tumour size reduction appeared several weeks later (9). From this day, many drugs have been evaluated by PET-FDG despite some limitations. For clinical applications, high uptake of FDG is measured in some normal tissues, such as the brain, and

Figure 2: (^{18}F) FDG uptake in human CWR-22 prostate tumours subcutaneously xenografted in Nude mice

Static images were recorded before (A) and eight weeks after surgical castration (B). (^{18}F)FDG uptake period was one hour. The tumour is indicated by a white arrow. (^{18}F)FDG dynamic scan recorded before (C) and eight weeks after castration (D). For both scans, mice received a single IV injection of 200 µCi (^{18}F)FDG after a six hour fasting period.



accumulation in inflammatory zones could influence the evaluation of tumour response to treatment. The main limitations are probably for preclinical applications where the fasting period for approximately six to 12 hours before FDG injection, in addition to anaesthesia maintenance between FDG injection and image acquisition, are very stringent conditions that could definitely modify the tolerance of small animals to the tested drug (see Figure 2). Additionally, some investigations have reported significant differences in ^{18}F-FDG uptake in various subcutaneous tumour xenografts. In tumours where radiotracer uptake is low, it may not be possible to assess the anti-tumour efficacy of a drug, with FDG as drug-related variations may be hardly detectable.

Link Between Effect and Disease

More nuclear imaging tracers, still in development or approaching approval, exist to measure apoptosis, hypoxia or other fundamental biological processes that are considered hallmarks of cancer. However, in some cases, the link is not always clear between the measured biomarker and the outcome, meaning that the biomarker is not predictive of outcome. If the hypothesised effect is measured and the drug does not seem to influence clinical endpoints (for example survival and quality of life), cancer cell exposure to the drug should be increased by increasing the dose, modifying the schedule of administration or adopting alternate delivery strategies. All of these interventions can be monitored with functional imaging. Alternatively, mechanisms of compensation or resistance can be investigated, using the same principles of molecular and biological imaging. It is currently an active research field that helps understand the pros and cons of antiangiogenic therapies (10).

The relative failure of more targeted compounds in clinical trials over the past 15 years has shown that it is most likely that no targeted compound will be used as a monotherapy. Additionally, the combination of biomarkers designed for two different compounds will not necessarily make for a good biomarker for the combination of the two compounds. It is therefore necessary to engage in research that will allow one to objectively assess the benefits of combinations, therefore reviving part of the cytotoxic paradigm.

CHALLENGES

Translational research aims at moving discoveries from preclinical research into clinical evaluation to better select the right drug for the right patient, and to help clinicians rapidly adapt their therapeutic strategy to tumour response. The two most famous examples of targeted cancer drugs, Gleevec and Herceptin, have exemplified the benefits of 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 major advances have to occur to evolve from a research endeavor for imaging to a high-throughput production system.

For decades, imaging has been used to illustrate disease but it is now mature enough to be used as a quantitative technique. What is needed is fit-for-purpose qualification of each imaging technique. That is, linking a disease-related biomarker with biology and clinical endpoints, through a graded evidentiary process dependent on the intended use. Qualification of imaging biomarkers requires a consensus around a set of recommendations on standardised imaging protocols, which would include suggestions on:

- How to design experiments in studies involving small animals, considering the number of animals, anaesthaesia, animal positioning and warming, and the use of support experiments like immunohistochemistry
- Imaging times (definition of the imaging time points which depends on tumour type, therapy type and imaging type)
- How to report imaging results, including the use of modern, non-parametric, multivariate analysis techniques
- How to ensure, by the use of QC procedures, the reliability of measurements; particularly for all small animal imaging modalities where there is a very high need for phantom development
- How to better understand limitations; by clearly defining the conditions in which the experiments are realised, it is easier to pinpoint their caveats

This would allow the comparing of results across sites and better translation between preclinical and clinical data. The consensus can be achieved through groups of experts taking the responsibility of setting standards, or by imaging consortia providing a framework for discussion among expert users (11-13). Solving all these very challenging problems requires broad and connected teams of multidisciplinary scientists involving clinicians, biologists, chemists, physicists and mathematicians. Again, this will only be possible through an active international collaboration among users from academia and the industry. Building a collaborative model out of which both public and private partners find benefits is also a difficult task.

PERSPECTIVES

Despite the great progress witnessed in the past 15 years, imaging biomarkers have only shown part of their potential. We give here three possible developments that we think are likely to occur in the mid- to long-term. First, salvation of abandoned drug programmes into biomarker programmes could be one consequence of the collaboration between the pharmaceutical industry and

biomarker/diagnostic-based companies on imaging programmes. Some Big Pharmas have already understood the potential of having gathered knowledge on a promising compound that has for example an unfavourable toxic profile: used as an imaging PET tracer, it could be injected at doses far below its toxic threshold and still be pushed to market as an imaging biomarker.

Secondly, we know that toxicity accounts for a significant part of failures of Phase III clinical trials, making it a major concern for the pharmaceutical industry. Imaging has the potential to offer methods to non-invasively measure the function of main organs (central nervous system, heart, liver, kidneys and lungs) in order to help identify the side effects of drugs earlier. The elevated cost of imaging techniques make it unsuited for early toxicity screening, but it can be highly effective at answering specific questions and may sometimes be the only practical means to obtain drug safety information in clinical trials.

Finally, it is recognised that imaging techniques are complementary rather than competitive. They provide information at different scales about various biological processes occurring within the tumour that are spatially or temporally linked. There is an increasing opportunity to use these techniques in conjunction. PET and CT are already combined in most PET scanners, some even offering trimodality PET/SPECT/CT imaging. A promising development currently underway is the PET/MRI scanner. One technology is using a PET sensor insert inside an MR scanner to acquire data by PET and MR simultaneously instead of sequentially as it is done with PET/CT, reducing imaging time and adding the exquisite tissue contrast provided by MRI to the extended functional and molecular information gathered by PET.

CONCLUSION

There is still, to date, no example of a marketed drug for which imaging has been used from the very beginning of its development and up to late clinical phases. Nevertheless, some success stories have been written, giving evidence of the great promises held by imaging biomarkers in drug development. The real expectation lies in the multivariate integration of data from clinical trials incorporating imaging, which will be analysed in context with other classical biomarkers and disease outcome.

About the author

A trained biomedical engineer, Xavier Tizon completed his PhD in medical image processing at Uppsala University, Sweden in 2004. His research focused on semi-automatic segmentation methods for the analysis of whole-body magnetic resonance angiography images. He then moved back to France, where he spent a year working as a postdoctoral researcher and teaching assistant in the medical imaging lab of the University of Burgundy in Dijon. Since June 2006, he has been the head of the imaging lab at Oncodesign, where he and his team routinely use magnetic resonance imaging and positron emission tomography to assess the efficacy of anticancer therapies in rodent studies using rats and mice. They work in close collaboration with several research institutions to develop and validate imaging biomarkers of pharmacological activity, dedicated to preclinical and clinical studies.
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References

1. Biomarkers Definition Working Group, Biomarkers and surrogate endpoints: preferred definitions and conceptual framework, *Clin Pharmacol Ther* 69(3): pp89-95, 2001
2. Gillies RJ, Bhujwalla ZM, Evelhoch J, Garwood M, Neeman M, Robinson SP *et al*, Applications of magnetic resonance in model systems: tumour biology and physiology, *Neoplasia* 2(1-2): pp139-151, 2000
3. Willmann JK, van Bruggen N, Dinkelborg LM, Gambhir SS, Molecular imaging in drug development, *Nat Rev Drug Discov* 7(7): pp591-607, 2008
4. Rudin M and Weissleder R, Molecular imaging in drug discovery and development, *Nat Rev Drug Discov* 2(2): pp123-131, 2003
5. Gambhir SS, Molecular imaging of cancer with positron emission tomography, *Nature Reviews Cancer* 2(9): pp683-693, 2002
6. Orlova A, Tolmachev V, Pehrson R, Lindborg M, Tran T, Sandström M *et al*, Synthetic affibody molecules: a novel class of affinity ligands for molecular imaging of HER2-expressing malignant tumors, *Cancer Res* 67(5): pp2,178-2,186, 2007
7. Workman P, Aboagye EO, Chung Y, Griffiths JR, Hart R, Leach MO *et al*, Minimally invasive pharmacokinetic and pharmacodynamic technologies in hypothesis-testing clinical trials of innovative therapies, *Journal of the National Cancer Institute* 98(9): pp580-598, 2006
8. O'Connor JPB, Jackson A, Parker GJM and Jayson GC, DCE-MRI biomarkers in the clinical evaluation of antiangiogenic and vascular disrupting agents, *Br J Cancer* 96(2): pp189-195, 2007
9. van den Abbeele AD, The lessons of GIST-PET and PET/CT: a new paradigm for imaging, *Oncologist* 13(2): pp8-13, 2008
10. Jain RK, Duda DG, Willett CG, Sahani DV, Zhu AX, Loeffler JS *et al*, Biomarkers of response and resistance to antiangiogenic therapy, *Nat Rev Clin Oncol* 6(6): pp327-338, 2009
11. Tofts PS, Brix G, Buckley DL, Evelhoch JL, Henderson E, Knopp MV *et al*, Estimating kinetic parameters from dynamic contrast-enhanced T1-weighted MRI of a diffusable tracer: Standardized quantities and symbols, *Journal of Magnetic Resonance Imaging* 10(3): pp223-232, 1999
12. Padhani AR, Liu G, Mu-Koh D, Chenevert TL, Thoeny HC, Takahara T *et al*, Diffusion-weighted magnetic resonance imaging as a cancer biomarker: consensus and recommendations, *Neoplasia* 11(2): pp102-125, 2009
13. Frank R, Quantitative Imaging Biomarkers Alliance FDG-PET/CT Working Group report, *Mol Imaging Biol* 10(6): p305, 2008