

**LH GRAY MEMORIAL TRUST AND CLINICAL AND TRANSLATIONAL RADIOTHERAPY
RESEARCH WORKING GROUP (CTRAD)**

**Biomarkers for Future RT Trials
Monday April 23rd 2012**

**Frank & Katherine May Lecture Theatre
Henry Wellcome Building
University of Leicester**

Workshop summary report

Organisers

G Don Jones (Leicester; lead organiser), Thomas Brunner (Oxford) & Kaye Williams (Manchester)

Aim and objectives of the workshop

To deliver the positive findings of basic radiobiology research more quickly and efficiently into clinical trials, and ultimately from there to deliver improved medical practice, there is the need to introduce of top quality translational research (TR) into phase I, II & III radiotherapy (RT) clinical trials. This strategy has been generally recognised by the EORTC and the NCI in the development of new of new agent-based treatments and it is a policy they are aggressively pursuing. Therefore, to be both equally credible in attempting to deliver greater numbers of improved RT-based treatments, UK-based organizations (for example CTRad) should have a similar policy and programme in place with respect to its own supported/accredited RT clinical trials.

To better understand, judge and potentially improve the therapeutic gain of newly developed RT-based treatments, one of the most appealing and profitable types of translational research to be undertaken entails the development and use of biomarkers; notably for tumour and normal tissue predisposition and response. When looking specifically at drug action in combination with RT, this could also include drug pharmacokinetic & dynamic markers. In such a setting, valid biomarkers will be able to inform as to why a particular treatment is successful (or failing/falling short), either in its own right or in a particular group of patients.

Inclusion of Biomarker-based TR within radiotherapy trials that are reviewed & supported/accredited by CTRad will be driven & overseen by the "Biomarker Network". The "Network" will be consist of members of the various CTRad workstreams plus additional outside experts (who will be consulted on an *ad-hoc* basis).

The workshop was held to discuss and promote the inclusion of biomarker-based TR within future CTRad accredited clinical trials.

The Objectives of the workshop were:

- To review current and future biomarkers pertinent to RT and any novel associated technologies
 - Specifically:
 - What potential biomarkers are currently available/near-available?
 - Are they fit for purpose?
 - What can and should be delivered?
- To address the issues/barriers that govern the implementation of valid biomarker research

- Through 'case studies', demonstrate the benefit/added value of biomarker-based TR within RT trials
- To gain knowledge of biomarker-specific funding opportunities

Topics covered and/or attach workshop agenda

The sessions of the workshop were structured/themed about the key issues pertaining to RT treatment and specifically covered the biomarkers available for assessing exposure and repair, normal tissue toxicity, hypoxia, cell death and proliferation. Finally there were sessions dedicated to trial design & support and funding.

An agenda can be found in Appendix 1.

Attendees of the workshop

Make-up of the delegates: around 65% basic science 35% clinician/clinical scientists
1 Consumer member.

Specific / significant discussion comments captured on the day

Keynote presentation:

The workshop was opened with a thought provoking presentation by **Andrew Hughes**, AstraZeneca head of early phase I/II trials in oncology and The University of Manchester, Manchester Cancer Research Centre Chair in Translational Research. He focused on the use of pharmacodynamic and predictive biomarkers as go-no go decision making tools in clinical drug development. He stressed the importance of thorough validation to ensure that interpretation of the biomarker was effectively fool-proof. In the predictive setting, an additional consideration is the prevalence of the biomarker in the target population, which would impact on the patient numbers for clinical trial. Further, to understand whether a biomarker is prognostic of predictive, control arms to investigate whether the biomarker identifies enhanced treatment to the agent under trial, or better response to any therapy.

Exposure and Repair:

Kai Rothkamm followed with an overview of techniques initially developed to monitor accidental radiation exposure that are finding increased use in the clinical biomarker setting. Examples were the use of the dicentric assay to analyse chromosome damage following ex vivo irradiation of peripheral blood lymphocytes (PBLs) to identify breast cancer patients that would have severe acute over reactions to radiotherapy. The utility of analysing residual DNA-damage using the γ H2AX assay towards this goal was also put forward. The single cell electrophoresis COMET assay was the focus of the presentation of **Victoria Spanswick** who took the audience through the steps required to validate this marker for clinical trial from studies prior to trial to establish intra and inter-assay variation, sample stability, handling and storage, production of internal standards, generation of SOPs and appropriate laboratory agreement through to clinical issues of chain of custody with respect of samples, results analysis, quality control, data storage and archiving and reporting. **Chris Parris** built on one of the themes introduced by Kai Rothkamm and presented a relatively high throughput approach for evaluating residual γ H2AX foci in ex vivo irradiated PBLs using imaging flow cytometry to identify over-reacting radiotherapy patients.

Normal tissue toxicity:

Chris Talbot introduced the concept of evaluating genetic determinants of radiation response to enable personalisation of radiotherapy treatment. He discussed a number of ongoing studies

from the analysis of candidate genes identified from initial studies that may relate to clinical normal tissue response (for example TGF β and fibrosis; XRCC1 and telangiectasia) to genome-wide association projects that are likely to generate further putative candidates. He also showed data indicating that breast size is a major predictor of fibrosis following breast-radiotherapy.

Gillian Barnett then followed with a presentation of the preliminary results from the RAPPER study (radiogenomics: assessment of polymorphisms for predicting the effects of radiotherapy) that aims to recruit 6000 and establish that genetic variation accounts for the differences in late toxicity between individuals. Gillian stressed that to be able to confirm a genetic component, dose-, treatment- and patient-related factors must all be accounted for. Initially the genetic studies focused on the analysis of candidate single nucleotide polymorphisms (SNPs) that had been reported to be associated with toxicity. However the predictive nature of these SNPs was not confirmed in the RAPPER cohort and a Genome Wide Association Scan (GWAS) study was progressed. First indications suggest that the GWAS has identified true associations with toxicity, particularly for the pelvic radiotherapy in prostate cancer patients, with SNPs emerging that associated with bladder function.

Hypoxia:

Catharine West opened the hypoxia session with a description of the development and validation of a gene signature as a biomarker for hypoxia in clinical tumours. She presented validation data for a 25 gene hypoxia signature using a Taqman Low Density Array Platform. The assay was sensitive, showed excellent reproducibility with intra-tumour variability (23.2%) much lower than for pimonidazole (67.2) or for single gene measurements (40-60%) based on 4 biopsies per tumour. Increased variability was noted for RNA extracted from formalin fixed versus snap frozen tumours with a two log change in dynamic range between the two types of source material. However this did not negate its use in archived formalin fixed samples. Samples are now being taken from the BCON and ARCON trials to investigate links between signature and outcome. **Philippe Lambin** introduced means of monitoring tumour hypoxia non-invasively in tumours using positron emission tomography (PET). He presented data showing that tumour regions that relapsed following radiotherapy frequently take up more of the radiotracer ^{18}F FDG in pretreatment scans. A trial is ongoing to effectively boost the radiation given to these regions to increase response. He then progressed to more direct imaging measure of hypoxia, focusing on a new hypoxia-PET tracer, ^{18}F -HX4 in preclinical and clinical studies. HX4 showed good co-registration with pimonidazole, and more rapid tumour accumulation than ^{18}F -misonidazole. The theme of imaging was continued with **Roberto Alonzi** who presented the use of magnetic resonance (MR)-based imaging to evaluate tumour associated characteristics such as perfusion, vessel permeability, cellular density, hypoxia and metabolic state. He focused on the use of MR in combination studies and stressed the importance of the technique used. He particularly emphasised that an MR biomarker that has utility in monitoring the effects of a specific drug on its own, may not have similar use when the drug is combined with radiotherapy. He exemplified this with combretastatin, whereby the dynamic contrast enhanced MR-derived biomarker Ktrans could mark drug changes alone, but when combined with radiotherapy intrinsic susceptibility-weighted MR (blood oxygen level dependent [BOLD] technique) was more suitable to monitor combined effects.

Cell Death and Proliferation:

Ged Brady opened this session, focusing on the use of blood borne biomarkers in early phase trials. Cancer patients have tumour-derived cells, protein, RNA and DNA circulating within their blood stream, all of which can be used to derive biomarkers that may be used in the prognostic, predictive or pharmacodynamic setting. The benefit of blood borne biomarkers is the ease of sampling and the opportunity for time-course studies with multiple samples. The technical challenges are however great with tumour cell numbers being incredibly low and circulating nucleic acids in nanogram amounts per ml of blood. However the power of the techniques is that in addition to measuring the amounts of tumour derived material within the blood as a biomarker of tumour burden or treatment response, it is also possible to analyse specific tumour

characteristics such as mutation status that could aid patient selection. **Kalena Marti** described the DREAMtherapy trial which is a single centre dual phase I study to determine the appropriate dose of AZD2171 and AZD6244 (VEGFR and MEK inhibitors, respectively) in combination with chemo-radiotherapy in rectal cancer patients. The trial design optimises the use of natural time intervals to evaluate toxicity measures to enable two drugs to be progressed simultaneously. The trial is currently recruiting and has been designed to incorporate a broad programme of translational research, ranging across blood borne, tissue and imaging biomarkers studies, using biomarkers tailored towards the mechanism of action of the drug used. Clinical data to date has been encouraging with good tolerance and response.

Trial Design and Support:

Cindy Billingham introduced the concept of exploratory trials whereby biomarker data is collected as part of the trial versus the situation where a validated biomarker is an integral part of the trial design and used to stratify patients. The validation process is crucial whereby biomarker data is mathematically modelled to prove how good a predictive tool it is. Cut-off points have to be defined that predict best and most accurately and the classifier used needs to be quick, cheap, reliable, reproducible, sensitive and specific. Different designs for phase III studies were introduced that can be used to evaluate a biomarker classifier in clinical practice which included stratified design, marker-based strategy design and also adaptive trial design which allows the use of interim trial data to progress biomarker research, effectively allowing development and validation in one trial. **Kevin Harrington** presented first-hand experience of the pros and cons of different trial designs using novel molecular targeted therapies in combination with radiotherapy. He highlighted that commonly used phase 0 “window” trials can be useful for biomarker validation/discovery for the novel drug alone, but do not offer a the chance of biomarker evaluation in the drug + IR setting. Studies in the palliative setting can bridge this gap for PD biomarker development/validation. However there are caveats. Palliative IR is short course with unconventional dose per fraction and heterogeneity of sites makes toxicity assessment challenging. Further from a company perspective, drug registration will not be possible on the back of data obtained in such trials. Within the curative setting, trials are often conservative in design for fear of acute effects, whilst having little, if any consideration for late tissue effects. They need to incorporate chemo-IR rather than IR alone as standard of care. Flip-flop designs (as for the DREAMtherapy trial) were again highlighted as a means to expedite progression of novel therapies through early stage clinical development and a modified continual reassessment trial design was presented for a trial progressing olaparib (inhibitor of the DNA-repair enzyme PARP) with radiotherapy. Mechanisms for monitoring acute effects were also presented using a dose-response curve based assessment of toxicity which could be incorporated alongside standard RTOG reporting. **Ruth Plummer** focused her presentation around the use of DNA-repair inhibitors in combination with radiotherapy. She highlighted the issue that inhibiting DNA-repair has a risk of enhancing normal tissue response and as such there is a pressing need for toxicity assessments and particularly increased evaluation of normal tissue effects in the preclinical setting. She also discussed that many of the potential biomarkers that have arisen for DNA-repair function require that a DNA-damage insult has been placed on the cells. A window trial design with a single IR dose and biopsies pre and post IR may be suitable to define patients with tumours that could be particularly sensitive to DNA-repair inhibitors. **Paul Marsden** introduced the NCRI PET research network that has established a network of accredited PET centres throughout the UK to participate in multicentre clinical trials. Centres are accredited via the centralised NCRI core lab at St Thomas’ hospital in London. A PET methodology expert panel has been established to develop guidance, QA standards and new technology to support the UKs ability to undertake high quality clinical trials using PET. Support from this network is available for those researchers wishing to undertake IR trials including PET biomarker research.

Funding:

Nigel Blackburn's presentation highlighted the current changes within CRUK and modifications to the relevant funding streams. The focus of the presentation was the drug development office, which can help progress therapies from lead optimisation onwards. The new agents committee can deal with phase I first in man studies with biomarkers plus/minus therapeutic. However, there was little clarity in positioning of biomarker focused trials in a radiotherapy context.

Intended outputs or measure of impact

The deliverables of the Workshop were:

- To introduce and establish the CTRad Biomarker Network
- Identify expert Biomarker 'Champions' in clinical trials who would be the 'go-to' people for specific advice about a particular markers/techniques/methodologies
- To seek feedback from the Workshop participants as how should the network function to facilitate biomarkers in radiotherapy trials
- Provide an editorial and report of meeting and effective network collaborations

The ultimate aim of the above being to enable/empower both scientists & clinicians to address the barriers associated with the implementation of valid biomarker research in future RT trials, to engender high priority for biomarker-based TR in future RT trials, to foster fresh collaborations and to stimulate the genesis and submission of new high quality RT trial proposals for consideration by CTRad and future funding.

Appendix 1: Workshop agenda

09.15-10.00 Registration & Welcome. Tim Illidge & G Don Jones

10.00-10.30 KEYNOTE PRESENTATION. Chairs: Kaye Williams, G Don Jones,

Andrew Hughes: “Predictive and pharmacodynamic biomarkers in industry decision making: Help, hype or hope”

10.30-11.30 EXPOSURE & REPAIR. Chairs: Peter Farmer, Simon Reed

10.30-10.50 **Kai Rothkamm:** “Biological measures of radiation exposure and their potential as predictive or prognostic biomarkers in radiotherapy trials”

10.50-11.10 **John Hartley:** “Use of the comet assay in clinical trials” (*Vicky Spanswick on day*)

11.10-11.30 **Chris Parris:** “DNA damage measures predict normal tissue toxicity in radiotherapy patients”

11.30-12.10 NORMAL TISSUE TOXICITY. Chairs: Paul Symonds, Neil Burnet

11.30-11.50 **Chris Talbot:** “Finding the genetic determinants of adverse reactions to radiotherapy: the LeND project”

11.50-12.10 **Gillian Barnett:** “The RAPPER study: Preliminary results”

12.10-12:30 Discussion (discussion leader Catharine West)

12.30-1:10 Lunch

1.10-2.10 HYPOXIA. Chairs: Ester Hammond, Ian Stratford (*replaced by Kaye Williams*)

1.10-1.30 **Catharine West:** “Measuring hypoxia in clinical trials”

1.30-1.50 **Philippe Lambin:** “Hypoxia imaging in patients: Does it work? What for?”

1.50-2.10 **Roberto Alonsi:** “Magnetic resonance imaging biomarkers for prostate radiotherapy trials”

2.10-2.50 CELL DEATH & PROLIFERATION. Chairs: Thomas Brunner, Laura Kenny

2.10-2.30 **Ged Brady:** “Biomarker development for drug radiation combination trials”

2.30-2.50 **Kalena Marti:** “Translational aspects of DREAMtherapy trial”

2.50-3.10 Coffee

3.10-4.20 TRIAL DESIGN & SUPPORT. Chairs: Anne Thomas, Paul Marsden

3.10-3.25 **Lucinda Billingham:** “Innovative methodology for radiotherapy trials incorporating biomarkers”

3.25-3.40 **Kevin Harrington:** “Taking RT combinations in to clinical trial: designing early and late trials to suit”

3.40-3.55 **Ruth Plummer:** “Novel agent/radiotherapy trial design – challenges compared to systemic therapy combinations”

3.25-4.10 **Lucy Pike:** “Supporting PET QA in clinical trials: The NCRI PET Core Lab”

4.10-4.30 Discussion (discussion leader Ian Stratford; *replaced by Tim Illidge*)

4.30-5.00 FUNDING. Chairs: Tim Illidge, Susan Short

4.30-4.50 **Louise Jones:** CR-UK BIDD Committee: “Navigating the biomarker roadmap” (*covered by Nigel Blackburn on day*)

4.50-5.10 **Nigel Blackburn:** CR-UK DDO: “Biomarkers in DDO trials”

5.10-5.25 Final Comments & Meeting Close. Tim Illidge, G Don Jones