Workshop Report: Potential and Pitfalls of TMA: Quality data or just adding to the noise?

Meeting summary

- 76 delegates from a variety of specialties attended this one-day workshop in Nottingham, organised by the NCRI Biomarkers and Imaging CSG.
- Chaired by Prof. Mohammad Ilyas (University of Nottingham) and Dr Heike Grabsch (University of Leeds), the workshop aimed to pool knowledge and provide practical advice to members of the research community on the design, construction and scoring of TMAs and methods to handle the data generated.
- Key points from the talks are summarised below. The slides from the day are available as PDFs from http://ncrndev.org.uk/index.php?option=com_content&task=view&id=134&Itemid=272
- It is the intention that a NCRI Biomarkers and Imaging CSG-badged guidance document will be produced to convey the agreed set of best practice recommendations emerging from the workshop.
- Participants were also encouraged to make use of the expertise on the Biomarkers and Imaging CSG to help develop translational components of late phase clinical trial protocols, and to provide suggestions for future workshop topics.

Introduction

On 17th May 2012, the National Cancer Research Institute (NCRI) Biomarkers and Imaging Clinical Studies Group (BI CSG) hosted a tissue microarray (TMA)-focused workshop at the Postgraduate Multidisciplinary Education Centre, Queens Medical Centre Campus, Nottingham. Aimed at members of the research community currently using or wishing to incorporate TMAs into their research, the workshop was attended by 76 delegates from a variety of backgrounds. The main objective of the day was to debate issues surrounding TMA-based research and to agree a set of best practice recommendations.

Prof. Mohammad Ilyas (University of Nottingham) and Dr Heike Grabsch (University of Leeds) opened the meeting, welcomed the delegates and explained the aims and objectives for the day.

Workshop General Introduction

Utility of TMAs: An academic perspective

The first speaker, Prof. Ian Ellis (University of Nottingham), addressed the use of TMAs in academia, discussing both the advantages of TMAs over traditional immunohistochemical. Prof. Ellis presented examples of TMA use from his research in breast cancer where arrays were used to test for 24 biomarkers in 1076 cases. The results were broadly comparable to cDNA microarray studies and showed significant associations with patient outcomes. Prof. Ellis concluded TMA technology to be an efficient and reliable tool for use in high throughput studies (such as the investigation of phenotypic characterisation of large cohorts of breast cancer) and may be valuable in the characterisation and treatment of breast cancer in the future.

Utility of TMAs: An industry perspective

Prof. Chris Womack (AstraZeneca) summarised the use of TMAs in an industrial setting, where they are used to determine biomarker expression and relevance. Providing information on the bioinformatics used at AstraZeneca to deliver statistical analysis, visualisation and automated reporting of samples, Prof. Womack then outlined examples of where TMAs had been used to validate biomarkers in different cancer types. Finally, the challenges of using TMAs to validate biomarkers were discussed.

Utility of TMAs: The funders' perspective

Prof. Bob Brown (Imperial College London) addressed the audience as the Chair of the NCRI BI CSG. Prof. Brown began by stating the remit of the BI CSG, emphasising the group's availability as a resource for researchers wanting to embark on translational studies. The different types of biomarker studies were discussed and their importance in addressing clinically important biological questions based on an understanding of the basis of the disease improving clinical outcomes for cancer patients and improving clinical trial design was addressed. To conclude, Prof. Brown provided some personal views of why some biomarker studies fail to receive funding in the UK.

Workshop Session I: TMA Construction and Design

How to construct a TMA

As an introduction to the physical construction of TMAs, Prof. Manuel Salto-Tellez (Queen's University Belfast) addressed the important factors in TMA planning, building TMAs and cutting sections from arrayed blocks highlighting the dependency of TMA construction upon array type, arrayer type and study type. The variables in the construction of a TMA and their effect on TMA block integrity (for example, number of cases, size and density of punches etc) were discussed and examples of TMA sets used in general biomarker screening and predicting clinical prognosis were outlined. Prof. Salto-Tellez concluded his talk with an overview of Translational Research Arrays (TRARESA), a tissue microarray centred, hospital based, translational research conceptual framework for both validation and/or discovery of novel biomarkers.

TMA disasters and how to avoid them

Dr Heike Grabsch (University of Leeds) reported on personal experiences of TMA disasters and how they might be prevented in the future. She gave practical tips on how to avoid the problems which may arise during the production of TMA blocks, block sectioning, section placement on slides and on the methods which could be used for staying orientated within the block. The concept of TMA quality control by an experienced histopathologist was introduced.

TMA design and how to avoid statistical disasters

Mr Martin Jenkins (AstraZeneca) discussed the statistical considerations around design of a TMA-based study. He indicated that it was statistically inappropriate to use pre-built blocks to answer specific research questions and demonstrated how sample number requirements (in order to sufficiently power a study) varied in accordance with the research questions. A suite of TMAs containing a variety of sample numbers to allow appropriate array selection to answer a specific research question with statistical certainty was advised.

Discussion

The morning discussion session was chaired by Dr Dan Berney (Bart's and the London School of Medicine and Dentistry) and Prof. Dean Fennell (University of Leicester). As well as the issues raised during the talks, problems in obtaining sample blocks from hospitals were identified. Suggestions of engaging pathologists earlier in the pathway, involving pathologists in the research project and giving them the deserved research recognition were suggested as ways to overcome these issues. The inclusion of pathology funding in grant submissions and variation amongst pathologists when scoring TMAs were also discussed. Issues on storage of cut sections (to preserve antigenicity) and the use of the tape transfer method to reduce wastage for accurate sample placement on slides were debated.

<u>Workshop Session II: TMA Scoring</u>

Facts, artifacts and interpretative differences

Prof. Göran Landberg (University of Manchester) opened the second workshop session with a talk addressing the issues affecting the interpretation of TMA sections. He began by addressing the various different artefacts which can occur such as distortion of part or the whole of the section resulting in loss of orientation or partial or complete loss of individual cores. This was followed by considerations regarding the actual scoring of the TMAs (including factors which affect the intensity of staining) and the role of the automated image analysis.

Quantum dots in TMAs

Dr Byers (University of Manchester) gave an overview of how quantum dots (Qdots) conjugated to specific oligonucleotides or antibodies have been used to perform *in-situ* hybridisation and immunohistochemistry on paraffin embedded tissue. Qdots can be synthesised to emit fluorescence over a range of wavelengths and this, with appropriate multi-spectral imaging, allows easy multiplexing

without compromising the fidelity of expression levels. Dr Byers explained how Qdots do not undergo photobleaching in the same way as other fluorescent reporters. Examples of where Qdots have been used to look at multiple genes by TMA were discussed.

Automated imaging analysis

Beginning his talk with the advantages of automated image analysis, Dr Darren Treanor (University of Leeds) then warned of the potential pitfalls. The presentation of a proposed pipeline for validating image analysis was recommended as strategy to improve robustness and reproducibility. Furthermore, minimum standards for data handling were suggested as a way to deal with the amount of complex data produced by automated image analysis.

Discussion

The discussion following workshop session II was chaired by Prof. Mohammad Ilays (University of Nottingham) and Prof. Chris Womack (AstraZeneca). Scoring of TMAs was a hot topic of interest particularly how many cores to score when core dropout is seen, whether continuous scales were better than categorical scales and whether an average should be taken in cases where more than one core is scored. In addition, the advantages and disadvantages of scoring by pathologist vs. machines and the use of positive controls were debated. Furthermore, the topics of double staining of TMAs using chromogens vs. flourophore and problems with staining intensity were addressed, noting that many of the delegates reported stronger intensity seen (i) in TMAs compared to whole tissue sections and (ii) on computer monitors compared to microscopes. This also raised the issue of consistency of scoring across sites.

Workshop Session III: Bioinformatics and Data Handling

Scanning and databasing

Dr Andy Green (University of Nottingham) began his presentation by emphasising the current problems with TMA assessment and manual scoring and followed by outlining the advantages and disadvantages of automated scoring and databasing. Examples of digital scanners currently on the market and software used for slide viewing and analysis were discussed and the issues of the storage of TMA digital images were addressed.

Data analysis

To demonstrate the power of the data obtained through TMAs, Prof. Graham Ball (Nottingham Trent University) presented a case study of the remodelling of the Nottingham Prognostic Index (NPI). The NPI is used to assess risk of death based on the pathological features of a breast tumour. However by adding high quality biomarker data to the NPI, further classification of patients into more risk categories can be achieved. As the modelling has evolved, there are indications that it may be possible to accurately predict the likelihood of death with a specific NPI score. Prof. Ball finished by discussing the future directions of artificial neural networks, data mining and data modelling using biomarker data from TMAs.

Open access data

Prof. Peter Hamilton (Queen's University of Belfast) outlined the idea of open access TMA databases, citing both the benefits and challenges with the approach. One major problem reported was the standardisation of data and considerations for data standardisation were addressed. Prof. Hamilton suggested the use of the TMA data exchange specification, a community-based, open-access tool for sharing TMA data, and concluded by discussing the benefits of defining standardised clinical minimum information for TMA.

Discussion

The final workshop of the day ended with a discussion chaired by Prof. David Harrison (University of St. Andrews). Questions began with the consideration of databases' archiving policies, as databases continue to grow in size, and whether a retrospective national database would be worthwhile when quality assurance has not been performed in parallel. A topic of contention was whether an open-source database of TMA data alone would be useful and arguments for the provision of tissue alongside the data for validation and reanalysis were presented. Discussing data mining, points were raised regarding crediting TMA researchers contributing to the databases and the handling of intellectual property in cases where, for example, drug response markers were identified from open-source data.

Recommendations on Minimum Information for TMA Experiments

The day ended with a general discussion intended to establish a set of consensus recommendations for the performing TMA based analysis. This discussion has informed a NCRI BI CSG-badged guidance document currently being prepared which will detail an agreed set of best practice recommendations for the use of TMA experiments. On the whole, the workshop was well received by the delegates, speakers and chairs and constructive discussions were had.