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Integrated molecular pathology: the Belfast model

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The success of Cancer Research UK's new vision for the next 20 years will be measured by the increase in the percentage of cancer survivors, accepting the inevitability of linking scientific programmes with clinical outcomes. This vision calls for the transformation of our methods of cancer discovery and treatment, as well as a change in biomarker discovery and validation, including: the practical quality of the research output; an improvement on technical approaches; understanding the pathway for commercialisation; and the assembly of the right teams to execute validations. This scenario demands an integrated model, pursued by the Northern Ireland Molecular Pathology Laboratory, aiming to address the last stages of biomarker discovery, validation and test design with be best guarantees of success.

Clinical outcomes are the measure of quality of science

Earlier this year, Cancer Research UK (CRUK), the world's largest independent cancer charity, revealed its 20 year vision: 'to move the percentage of cancer survivors from half (today) to three quarters'. The charity's vision is to promote better science with quality measured by metrics such as impact factor of published work or number of related patents (http://www.cancerresearchuk.org/about-us/our-organisation/beating-cancer-sooner-our-strategy). Needless to say, CRUK will continue spending much of its budget over the foreseeable future on good quality research – so what has changed? In our opinion it is something fundamental: the measure of good cancer research becomes a clinical one and science will be judged on its impact on cancer patient survival and quality of life. This should not serve as a deterrent for 'basic scientists', because everyone recognises that good basic science is at the heart of every major practical discovery. It is, however, a reminder that science *per se* is necessary but not sufficient, and science with a purpose is perhaps the best way to serve patients.

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The failure of biomarker discovery and validation

According to Scott Kern's calculations [1], less than 1% of published cancer biomarkers enter clinical practice. 'A new cancer biomarker under development is likely to have already encountered one or more of the following fatal features encountered by prior markers: lack of clinical significance, hidden structure in the source data, a technically inadequate assay, inappropriate statistical methods, unmanageable domination of the data by normal variation, implausibility, deficiencies in the studied population or in the investigator system, and its disproof or abandonment for cause by others' [1]. This situation ('reality filters in biomarker discovery') is depicted in Fig. 1. It would appear that many of the filters that explain the failure of most research to translate to the clinic are related to the way we deal with samples, technology or the overall design of those studies. They are, therefore, related directly or indirectly to the way we understand and deliver molecular pathology.

The unsustainability of cancer discovery and cancer treatment

The seminal work led by Richard Sullivan for *Lancet Oncology* in 2011 [2] is still a key reminder of the unsustainable nature of our current paradigms for cancer discovery and cancer healthcare delivery. The exponential increase in cost is due to the 'huge development costs for cancer medicines', as well as other cost drivers like 'over-use, rapid expansion, and shortening life cycles of cancer technologies [...] and the lack of suitable clinical research and integrated health economic studies' [2]. It is therefore clear that a translational research or biomarker validation study should start with very careful planning so that, once fully executed, the results mean that we are as close as possible to answering the following questions.

- How was the biomarker selected and, in particular, what will be: (i) the clinical utility; (ii) the technical quality of the generated data; and (iii) the competition of other established biomarkers in that space?

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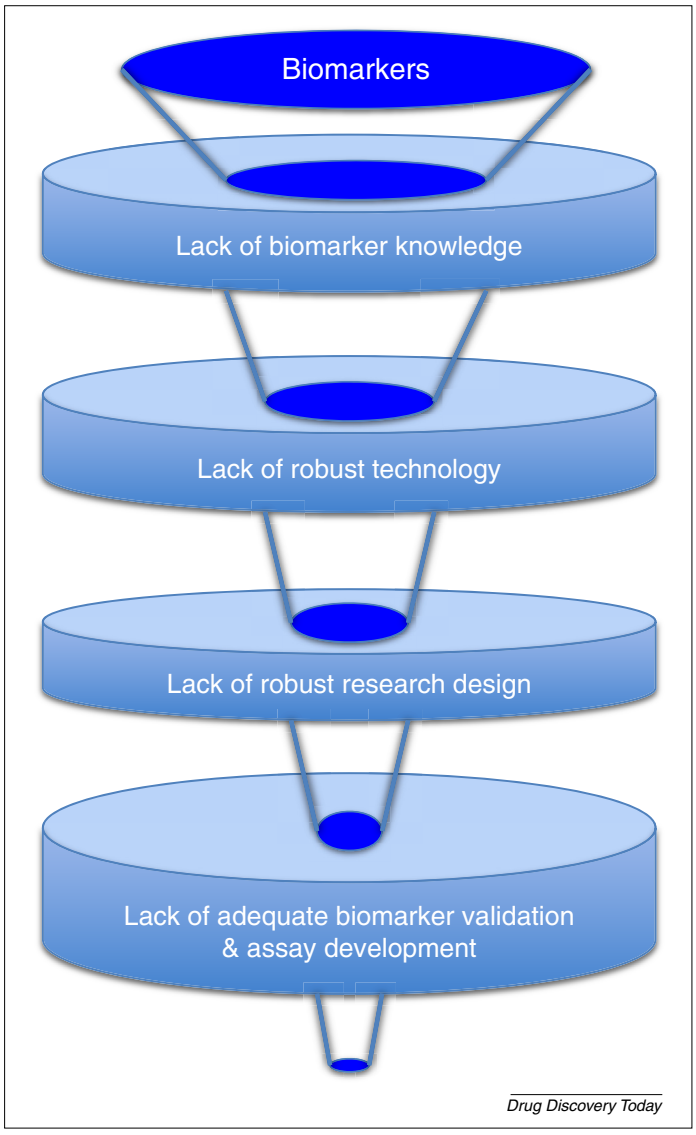

FIGURE 1

Reality filters in biomarker discovery. Adapted and modified from [14].

- What are the technical aspects of the biomarker development, focusing on: (i) is it established on validated technology; (ii) has it generated the design of standard operating procedures (SOPs) and new laboratory processes; (iii) is there an assessment of predictive and/or prognostic performance; (iv) is there an assessment of analytical qualities; (v) are there agreed specifications with the relevant agencies [such as the FDA, European Medicines Agency (EMA) or National Institute for Health and Care Excellence (NICE)] for a successful adoption; (vi) is there an established reference laboratory for the assay?
- What is the pathway to commercialisation, is it: (i) out-license to kit manufacturer for CE- and/or FDA-approved kit; (ii) out-license to service provider for Clinical Laboratory Improvement Amendments (CLIA)- and/or FDA-approved delivery; or (iii) deliver from an individual laboratory as a spinout company?

A professional delivery of all the necessary aspects of biomarker validation as indicated above would require:

- an expert panel of academic players, individuals with industrial knowhow, bioinformaticians, statisticians and economists to assess the work on a given biomarker to date;
- a development team that would include: (i) pathology expertise; (ii) molecular biology expertise; (iii) competent technicians; (iv) experts in regulatory matters; (v) economists; (vi) statisticians; and (vii) bioinformatics.

This model of planning and execution (the Belfast biomarker validation model) is shown in Fig. 2. Some of the steps of this model were included in the reporting recommendations for tumour marker prognostic studies (REMARK) criteria [3] produced by the National Cancer Institute – European Organisation for Research and Treatment of Cancer working group on cancer diagnostics, which might be pertinent to recall ten years after the criteria were originally set up.

The need for integration

From the analysis stated above, it is clear that a successful biomarker validation programme leading to test design would require a significant degree of technical and intellectual integration at many levels.

Technical integration

It is not unusual to see laboratories with a flagship technology and, hence, with a need to accommodate biomarker validation to their technology rather than choosing among a broad technical armamentarium to make the most of a specific biomarker. As such, laboratories should provide the full scope of main diagnostic laboratory technologies that are validated, maintained and used under strict standards, as well as incorporating other key aspects of sample procurement and data analysis.

- Biobanking
- Traditional tissue and cellular phenotypic analysis
- Tissue-based hybridisation techniques: tissue microarray construction, immunohistochemistry, *in situ* hybridisation, immunofluorescence, automated staining and single-slide multiplexed hybridisations
- Digital pathology
- Nucleic acid extractions for different purposes
- Low-throughput, PCR based technologies such as Sanger sequencing, pyrosequencing, quantitative (q)PCR, *etc.*
- High-throughput technologies such as next-generation sequencing (NGS), high-throughput gene expression arrays, high-throughput methylation, gene copy number variation, high-throughput proteomics and metabolomics, *etc.*
- Database construction and analysis
- Bioinformatics

Functional integration

Molecular diagnostic laboratories and molecular pathology translational research laboratories share samples (because the same clinical samples are used for diagnostics and discovery), most technologies (particularly now that high-throughput technologies are part of the diagnostic activity) and technical knowhow. Until recently, we accepted that the stringency and rigor of both types of laboratories should be different. However, it is likely that the lack

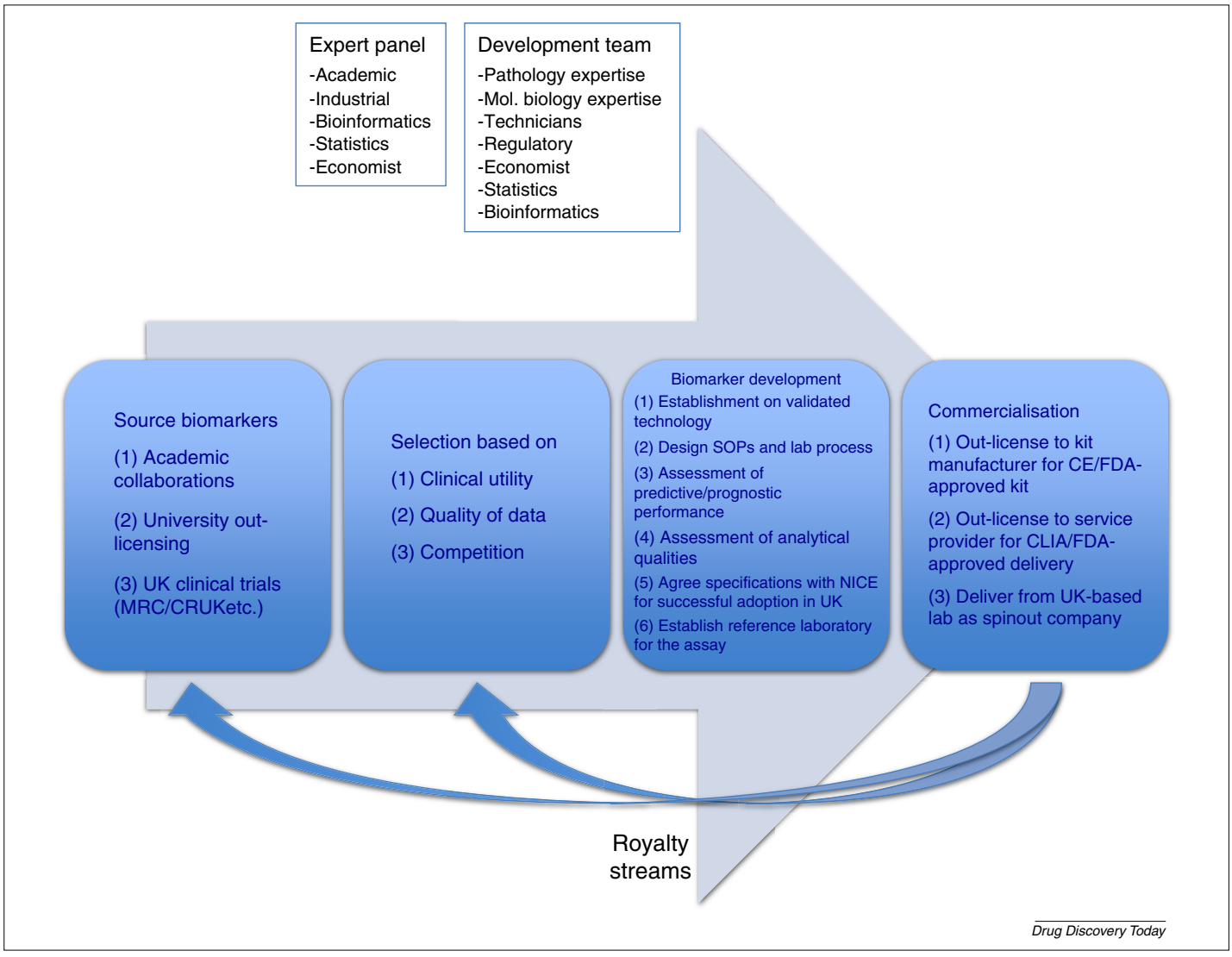

FIGURE 2

The Belfast biomarker validation model.

of accreditation or stringency in activities such as the last stages of translational research discovery, biomarker validation and test development (which are traditionally linked with research rather than diagnostic laboratories) could be part of the 'filter effect' shown in Fig. 1. Another important component in the field of biomarker development, clinical-trial-related testing (for upfront patient stratification of for subsequent discovery), is traditionally a research endeavour, whereas *de facto* it is closer to a diagnostic exercise. Therefore, it would appear that the purpose, organisation and quality required from laboratories in the last stages of research and in diagnostics is essentially the same.

Financial integration

The technical and functional integration stated above could also lead to a relevant financial integration. Here, we do not advocate the return of past models where diagnostics procedures were subsidising research, research was supporting diagnostics or, even worse, there was no clear understanding of the overall cost of research and diagnostics. We feel, however, that if technology, technical knowhow and laboratory accreditation schemes are common this could have a positive effect in avoiding duplication, having similar purchasing pathways and making the most of existing resources.

Integration of talent

We live in a world where the differentiation between activities is blurred, and research requires the rigor of diagnostics as much as diagnostics improves with the dynamism of research. This has a clear effect on the profile of existing and future practitioners in this field: pathologists understanding the small details of clinical trials; oncologists with significant experience in biomarker validation; bioinformaticians understanding the clinical relevance of their analyses. It is likely that the training of future leaders in research and diagnostics needs to pay more attention to the scientific interfaces of today.

The model

In 2011 we had the chance of designing a new molecular pathology programme in Belfast. Based on the analysis provided in this article, we decided to move into an integrated model for the

provision of molecular pathology [4]. It is centered in Northern Ireland-Molecular Pathology Laboratory (NI-MPL), with important industry ties and a programme for training in MP. In the same physical location and management structure, it includes:

- full technological integration, including low- and high-throughput technologies (NGS, high-throughput gene expression and high-throughput methylation) [5,6] and digital pathology [7];
- information and bioinformatics integration provided by the Pathology Integromics for Cancer (PICan) system [8];
- academia-healthcare-industry integration – every year NI-MPL performs more than 1000 clinical and more than 5000 research tests and collaborates with important biomarker companies in the UK (Randox, Almac and Path XL, among others);
- integration through accreditation as NI-MPL is fully accredited by Clinical Pathology Accreditation (CPA) and follows the UK National External Quality Assessment Service (UK NEQAS) quality assurance/quality control (QA/QC) – this diagnostic rigor percolates into SOP-driven, high-quality translational research;
- integration through training, by championing UK training in molecular pathology at two levels – (i) histopathologists [9]; and (ii) clinical scientists, by leading the tissue molecular component of the new Fellowship scheme of the Royal College of Pathologists;
- integration within the cancer research community–participating in the past three years in more than 40 high-impact-factor publications, such as the generation of a signature to predict chemotherapy response in breast cancer [10], the role of BRCA1-mRNA splicing in genomic instability [11], the characterisation of fibroblast growth factor receptor (FGFR)4 [12] or AXL [13] in colorectal cancer.

Concluding remarks

Biomarker development is not a single discipline within medicine or science. Indeed, the pathway from the laboratory to the clinic should involve numerous different specialties working closely together in a stepwise fashion. Molecular pathology represents a common core that contributes to every step of the process. A high-quality molecular pathology operation is indispensible if the pipeline is to run smoothly. In an age of complex technology, fading boundaries and a strong need to consolidate scientific and clinical talent, an integrated approach to the provision of molecular pathology could better serve CRUK's latest challenge: to encourage longer and better lives for cancer patients.

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