**Dr. Seán Hynes**

**Richard Steevens Scholarship Holder**

**Report on:**

**Fellowship in Molecular Pathology,**

**Training site:**

**Northern Ireland Molecular Pathology Laboratory,**

**Centre for Cancer Research and Cell Biology,**

**Queens University Belfast & Belfast Health and Social Care Trust,**

**The United Kingdom.**

**Lead trainer:**

**Professor Manuel Salto-Tellez**

**Training co-ordinators:**

**Dr. Jackie James**

**Dr. Perry Maxwell**

**Dr. Stephen McQuaid**

**Introduction**

The Centre for Cancer Research and Cell Biology at Queen’s University Belfast is a state of the art 5,000m2 purpose built facility with GLP-standard core facilities. Within the CCRCB is the Northern Ireland Molecular Pathology Laboratory(NIMPL) is a hybrid facility with both Clinical/Trust as well as Academic/University input.

A molecular pathology programme was established with the NIMPL at the CCRCB under the leadership of Professor Manuel Salto-Tellez and is composed of four main pillars. These include 1) Molecular diagnostics, 2) Molecular pathology translational, research, 3) the Northern Irish Biobank facility and 4) Bioimaging and analysis.

The NIMPL operates with consolidated manpower from both Queens University and the Belfast Trust. Current staffing includes 3 clinical academic consultant pathologists, 2 full time clinical scientists, 2 post-doctoral fellows, 1 biomedical scientist and 6 research technicians, although this is currently expanding. Histopathological analysis is also provided through support from 6 part-time Cancer Research UK-funded practicing histopathologists as well as the staff of the Northern Ireland Biobank. As a consequence of its continued expansion the NIMPL is currently moving to a new larger facility (target date September 2016).

The laboratory has capabilities to provide tissue processing, tissue embedding, tissue microarrays, manual and automated immunohistochemistry and in situ hybridization, image scanning, digital image analysis, Sanger sequencing, Cobas real-time based PCR, and amplicon based next generation sequencing. The laboratory has CPA accreditation (UK) and is aiming for CLIA accrediation (US).

The aim of the NIMPL is to cut across technologies an infrastructures to provide molecular diagnostic testing in an accredited environment whilst engaging with meaningful translational research.

The current repertoire for accredited molecular diagnostic testing in the NIMPL includes EGFR mutation analysis, ALK/EML4 rearrangements and ALK overexpression, KRAS mutations, BRAF mutations, Gastric Her2 by immunohistochemistry and dual dichromogenic in situ hybridization (DDISH), and microsatellite instability analysis. In addition, validations are ongoing into RT-QPCR for sarcoma translocations and ion torrent next generation sequencing for multiple mutation analysis.

**Fellowship year at the NIMPL**

**Induction and Core Competencies**

Training in Molecular Pathology at the NIMPL consists of personal academic development concerning the fundamentals and basis of molecular diagnostics, clinical application of molecular diagnostic testing and research/clinical trial application of molecular diagnostic testing. Prior to starting the training module I completed an induction module. This included becoming familiar with NIMPL health and safety procedures. In addition, I completed pre-PCR training which allowed me to work in the pre-PCR environment where cells are harvested and DNA is extracted. As part of the induction process I also carried out training in the Human and Tissue Act 2004 for England, Wales and Northern Ireland.

The next phase of training involved a series of seminars co-ordinated and delivered by trainers. I completed a seminar series in the following areas tumour annotation a morphomolecular approach, PCR primer design, Sanger sequencing, validation of current PCR based tests, UK NEQAS scheme and general QA/QC issues, molecular laboratory accreditation, In situ hybridization technology, IHC validations, HER2 scoring, tissue microarray construction and design, NGS and high throughput technologies, molecular haematology, biobanking, ethics and governance, slide scanning and the use of digital/remote pathology, and integration of molecular results into the laboratory information system.

I then acquired several core competencies these included annotation, tumour content assessment, macrodissection, DNA extraction, mutational analysis using COBAS rreatime based PCR, Sanger sequencing, Her2 ISH procedures, therapeutic IHC procedures and entry of results onto laboratory information management system. Thereafter, specialist elements were added whereby I was familiarised with next generation sequencing, and optimsiation of IHC and bioinformatic aspects.

**Routine Molecular Diagnostics and Next Generation Sequencing**

These were applied in daily routine molecular diagnostics. Specimens passing through the laboratory were annotated and tumour content was estimated. This was double screened by clinical scientific staff. Sanger sequencing and cobas results were reviewed in conjunction with scientific and medical staff. DDISH results were estimated blindly and compared with both scientific and medical staff estimates. ALK overexpression was estimated and compared with the reporting teams results. FISH results were interpreted in conjunction with scientific staff.

I took part in NGS validation studies whereby three different gene panels, and two chemistries/platforms were used to validate results. This will form the basis of two papers one a review into clinical application of NGS in a universal healthcare environment and the second a clinical validation paper. Sarcoma annotation was routinely carried out with tumour percentage estimation for translocation assessment. Microarray construction and assessment was carried out for breast and prostate cancer cohorts which will form the basis of two research papers.

Whilst expanding my molecular testing repertoire with all state of the art molecular diagnostic testing I also maintained my histological diagnostic skills with the application of a morphomolecular approach to colorectal carcinomas which will form the basis of another paper. I also carried out an audit of comparing molecular results for small volume lung samples both histology and cytology.

**Other aspects of the Fellowship year**

To supplement my training throughout the year I received online training in DDISH analysis, introduction to bioinformatics, data protection, and freedom of information. I also attended the Pathological Society Winter meeting on molecular pathology, London, January 2016. I also delivered two talks to trainees on the application of NGS in GI pathology and NGS applications in clinical trials and diagnostics at Galway University Hospital. I also attended Bioinformatics training in London in July 2016. With careful time management I also contributed to projects concerning the morphomolecular approach in diagnostics for breast, prostate and colon cancer which are anticipated to result in high impact publications in the near future.

**Conclusions**

Overall, the experience I have gained from the NIMPL has been invaluable in developing my training in state of the art and cutting edge molecular diagnostics. The opportunity afforded to me by the Steevens scholarship has provided me with a unique experience in the European context and is equivalent to any training programme available globally.

**Acknowledgements**

I would like to thank the HSE for the training opportunity afforded to me by the Steevens scholarship and I am very grateful to Prof Salto-Tellez, Dr. James and all the staff at the NIMPL for their help and guidance throughout the year there.

**Notes**

Reference information above is derived from the NIMPL training manual and log.

I have completed a training log with the NIMPL, which is available for review on request.