CRUK/Pathological Society of Great Britain and Ireland pre-doctoral research bursary short report by Dr Alice Westwood

**Summary of work: 02/09/2017 – 30/03/2018**

My research time was focused on one project with 3 workstreams with the common theme of molecular testing in colorectal cancer:

1. Comparison of mismatch repair (MMR) protein expression between biopsies and resections in advanced colon cancer in the Fluoropyrimidine Oxaliplatin and Targeted Receptor Pre-Operative therapy (FOxTROT) trial. Loss of MSH6 expression has been well documented following chemoradiation in rectal cancer, but this has never been explored in colon cancer. I analysed and compared the mismatch repair status between biopsies and resections in the phase II (n=150) group of the FOxTROT trial to identify possible changes following chemotherapy.
2. Comparison of mutational status in the EGFR pathway across four different platforms in the FOxTROT trial. The optimal method for RAS analysis is currently unclear, therefore I looked at the concordance in mutational calls across the EGFR pathway in the phase II group of FOxTROT using NGS, Fluidigm Access Arrays, Affymetrix and pyrosequencing.
3. Exploration of cases of unusual total/clonal “null” phenotype of MMR protein expression from the Yorkshire and Humber Lynch Screening Programme. Between May 2017 & March 2018, four cases of null phenotype were found (829 screened). In these cases, multiple blocks were requested and further immunohistochemistry was carried out.

*Findings of all three projects were presented at the Pathological Society of Great Britain and Ireland / BDIAP Summer meeting in Maastricht, 2018.*

**Findings**

1. Comparison of MMR in the FOxTROT trial

* 100 cases were identified with matched pre-operative biopsies and resections. Of these, 24% of cases showed deficient MMR with the majority showing loss of MLH1/PMS2.
* There was agreement in MMR status between the biopsy and the resection in 98% of cases. The two discordant cases showed unusual patterns of sub-clonal protein loss not entirely consistent across the biopsy and resection.
* There is no evidence that pre-operative chemotherapy in colon cancer induces a change in MMR status unlike the loss of MSH6 described following radiotherapy in rectal cancer.

1. Comparison of mutational status in the EGFR pathway in FOxTROT

* Both pyrosequencing and Affymetrix showed excellent concordance with NGS.
* Fluidigm was sub-optimal due to frequent failure and inconsistent results.
* Further testing of the discordant cases is ongoing.

1. Unusual “null” phenotype of MMR expression in Lynch Syndrome Screening

* All four cases of null phenotype showed complete loss of MLH1/PMS2 with BRAF codon 600 mutation, supporting the hypothesis that these are most likely sporadic mutations. The co-existent clonal loss of MSH2 and MSH6 is most likely due to further sporadic double hit mutations. Sequencing of different clonal areas for MMR gene mutations is currently being performed to confirm our suspicions that these are further somatic events.

**Future plans**

This pre-doctoral research bursary has allowed me to spend dedicated research time in a research area that really interests me. It has also given me the opportunity to develop valuable pilot data that will be used to put forward a competitive PhD application at the end of this year.